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REVIEW ARTICLE

A Review on Transdermal Drug Delivery System

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ABSTRACT

Transdermal drug delivery systems (TDDS), additionally called "Transdermal patches," square measure dose forms designed to deliver a therapeutically effective quantity of drug across a patient's skin. so as to deliver therapeutic agents through the human skin for general effects, the great morphological, biophysical and chemistry properties of the skin square measure to be thought-about. transcutaneous delivery provides a number one edge over injectables and oral routes by increasing patient compliance and avoiding initial pass metabolism severally. transcutaneous delivery not solely provides controlled, constant administration of the drug, however additionally permits continuous input of medicine with short biological half-lives and eliminates periodical entry into circulation, which frequently causes undesirable side effects. The TDDS review articles offer valuable info relating to the transcutaneous drug delivery systems, styles of Trandermal patches, factors affecting and its analysis method details as a prepared reference for the analysis human United Nations agency is concerned in TDDS. With the advancement in technology company industries have trendified all its resources. Earlier we have a tendency to use convectional dose type however currently we have a tendency to use novel drug delivery system. one in all greatest innovation of novel drug delivery is pad. The advantage of transdermal drug delivery system is that it's painless technique of administration of medicine.

Keywords: Transdermal drug delivery system, skin anatomy, polymers used in TDDS types, evaluation parameters.

INTRODUCTION

Novel drug delivery system (NDDS) is capable of controlling the speed of drug delivery, sustained the duration of action and targeting the diseased tissue, thereby resulting in better therapeutic effects with minimum side effects (1). Two aspects most important to drug delivery:-

Spatial Drug Delivery

In this, Drug is targeted to particular organ or tissue.

Temporal Drug Delivery

The rate of the drug delivery to the target is cotrolled. The main area for the research and development for NDDS are as follors:

- Liposomes
- Niosomes
- Nanoparticles
- Transdermal drug delivery
- Implants
- Oral system
- Micro encapsulation / Microcapsules
- Polymer in drug delivery

Novel drug delivery system can be divided into two main classes.

1. Sustained release drug delivery system.
2. Controlled release drug delivery system.

Sustained release drug delivery system

It is a pharmaceutical dosage from formulated to retard the release of a therapeutic effect such that the systemic circulation is delayed and/ or prolonged and the plasma profile is sustained in duration .The onset of its pharmaceutical action is usually slow, and therefore the duration of its therapeutic effect is sustained (2).

- Increase duration of action of drug
- Reduced dose frequency
- Once daily oral preparations.
- Long lasting depot injections (e.g. contraceptives, hormone replacements, antipsychotic drugs) (3).

Controlled release drug delivery system

This system has a meaning that goes beyond the scope of sustained drug action. It manifests a predictability and reproducibility within the drug release kinetics. The release of drug substances from a controlled release drug delivery system gains at a rate profile that is not only predictable kinetically but also reproduced from one unit to another (4).

They are classified as follows:-

I. Site-Targeting drug delivery system
II. Activation – Modulated drug delivery system
III. Feed – Back Regulated drug delivery system
IV. Rate programmed drug delivery system (5)

Merits of drug delivery system

1. Better treatment of many chronic illnesses. eg. Cancer,Asthma, Arthritis.
2. Increased Bio- availability.
3. Reduction within the occurrence and badness of untoward systemic side effects associated with high blood plasma drug concentration.
4. Sustenance of the entire amount of drug administered over the amount dose periods.
5. Reduction in the total amount drug administered over the period of drug treatment which reduce occurance of systemic and local side effects.
6. Prevention from first pass metabolism and gastrointestinal tract degradation (6).

Limitations

Physiological factors such as gastro intestinal enzyme, activates pH /gastric and intestinal transit rates, food and disease which often influence drug bioavailability from conventional dosage forms may interfere with the accuracy of control release and absorption of drug from the system. The products which remain intact may become accommodates at some sites results slow release of drug from the dosage form may produce a high localized concentration of drug which produces local irritation (7). Drugs with half- life of 1hr or less are difficult to be formulated as sustained release formulation. The high rate of elimination of such drugs from the body requires an highly large maintenance dose which provides 8-12 hrs of continuous release (8). Since these products contain an outsized amount of drug. There is a chance of unsafe over dosage, if the product is improperly made and the total drug contained there is released at one time or over too short time of interval.

It's difficult to cease the therapy once after administration could also be for reasons of toxicity or the other It's going to be not suitable to encompass potent drugs in such system. Transdermal drug delivery system (TDDS) has been an increased interest in the drug administration via the skin for both local therapeutic effects on diseased skin (topical delivery) as well as for systemic delivery of drugs (9). The skin as a site of drug delivery features a number of significant advantages over many other routes of drug administration, including the power to avoid problems of gastric irritation, pH and emptying rate effects,(10) avoid hepatic first-pass metabolism thereby increasing the bioavailability of drug, reduce the chance of systemic side effects by minimizing plasma concentrations compared to oral therapy, provide a sustained release of drug at the location of application; rapid termination of therapy by removal of the device or formulation,(11) the reduction of fluctuations in plasma levels of drugs, and avoid pain associated with injections. The transdermal delivery also can eliminate pulsed entry into the circulation, which could often cause undesirable side effects (12).

Transdermal drug delivery systems (TDDSs) are often denied as self-contained discrete dosage forms which, when applied to the intact skin, deliver the drug(s) through the skin portal at a predetermined and reproducible rate into the systemic circulation over a prolonged period. The goal of dosage design for transdermal products is to maximise the ux through the skin into the circulation and simultaneously minimize the retention and metabolism of the drug in the skin. Transdermal delivery provides a number one edge over injectable and oral routes by increasing patient compliance and avoiding first-pass metabolism, respectively. The transdermal route is so desirable, however there's one little obstacle: whereas the perform of the GI tract is to render ingested material appropriate for absorption, the skin's function is to keep things out of the body. The major barrier within the skin is that the stratum corneum, the highest layer of the epidermis. The corneum consists of keratinized, flattened remnants of once actively dividing epidermal cells. Hygroscopic, but impermeable to water, it behaves as a troublesome, flexible membrane. The intercellular space is rich in lipids. The stratum corneum is regarding 10 microns thick, but on the palms and soles it ranges up to 600 microns in thickness .Although the stratum corneum is an efficient barrier, some chemical substances are ready to penetrate it and to achieve the underlying tissues and blood vessels. These "successful" substances are characterised by low molecular weight (≤500 Da), lipophilicity, and effectiveness at low dosage. The largest daily dose of drug in patch kind is that of nicotine: twenty-one milligrams transdermic absorption happens

through a slow method of diffusion driven by the gradient between the high concentration within the delivery system and therefore the zero concentration prevailing within the skin. Thus, the delivery system should be unbroken in continuous contact with the skin for a substantial time (hours to days).

TDDS essentially consists of adhesive drug-containing devices of defined area that delivers a preset quantity of drug to the intact skin at a pre-programmed rate, which is in a position to penetrate through totally different layers of skin to achieve the systemic circulation. Currently, the transdermic route, together with oral treatment, ranks because the most productive innovative research space in drug delivery. Backing layer, drug containing layer, rate controlling membrane, adhesive and release liner are the components of TDDS though all layers may not be available in all types of TDDS as there are several types of transdermal patches (1). There are single layer drug in adhesive, multilayer drug in adhesive, vapour patch, reservoir system and matrix system. Similarly natural polymers, synthetic polymers, synthetic elastomers and biopolymers are utilized in TDDS (14). The biological properties of drug for preparing transdermal patch should be of short half-life, shouldnot produce allergic response and therefore the drug ought to be potent with a daily dose of the order of some mg/day. Substances that quickly diminish the impermeability of the skin are called permeation enhancers. As the cuticle is that the main barrier for penetration of the drug, many chemical enhancers like sulphoxide, alcohols, fatty acids, polyols, ureas and physical enhancers like sonophorosis, electroporation, iontophorosis, magnetophorosis have been used in TDDS (15).

TRANSDERMAL PATCH

DEFINATION

A transdermal patch or skin patch is a medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and into the bloodstream (16).

ADVANTAGES

- Topical patches are a painless, non-invasive way to deliver substances directly into the body.
- Topical patches are a better way to deliver substances that are broken down by the stomach acids, not well-absorbed from the gut, or extensively degraded by the liver.
- Topical patches over a controlled, steady delivery of medication over long periods of time.
- Topical patches have fewer side effects than oral medications or supplements.
- Topical patches are easier to use and remember.
- Topical patches over an alternative to people who cannot, or prefer not to take medications or supplements orally.
- Topical patches are cost-effective.
- People prefer topical patches (17).

DISADVANTAGES OF TDDS

- The drug that requires high blood levels cannot be administered and may even cause irritation or sensitization of the skin.
- The adhesives may not adhere well to all types of skin and may be uncomfortable to wear.
- High cost of the product is also a major drawback for the wide acceptance of this products. (18)
- Physical movement and perfuse sweating can lead to detachment of patch.
- Either the drug, adhesive or any other excipients of the patch formulation can cause erythema, itching, and local oedema.
- Hydrophilic drugs with potent therapeutic action diffuse slowly as the skin favor the permeation of lipophilic drugs.

- The thickness and the barrier function may vary from one site to another within a person and person to person (inter and intra individual variation) (19).

Limitation

- TDDS cannot deliver ionic drugs.
- TDDS cannot achieve high drug levels in blood/plasma.
- It cannot develop for drugs of large molecular size.
- TDDS cannot deliver drugs in a pulsatile fashion.
- TDDS cannot develop if drug or formulation causes irritation to skin.

SKIN

The skin is the largest organ of the human body which covers a surface area of approximately 2 sq.m. and receives about one third of the blood circulation through the body. It serves as a permeability barrier against the transdermal absorption of various chemical and biological agents. It is one of the most readily available organs of the body with a thickness of few millimeters (2.97 0.28 mm) which, (20)

Separates the underlying blood circulation network from the outside environment.
Serves as a barrier against physical, chemical and microbiological attacks.
Acts as a thermostat in maintaining body temperature.
Plays role in the regulation of blood pressure.
Protects against the penetration of UV rays. (21)

Skin may be a major consider determining the varied drug delivery aspects like permeation and absorption of drug across the derma. The diffusional resistance of the skin is greatly dependent on its anatomy and ultrastructure (22).

Anatomy and physiology of skin

Human skin comprises of three distinct but mutually dependent tissues: The stratified, vascular, cellular called as “epidermis” Underlying dermis of connective tissues, Hypodermis (23)

Epidermis

The multilayered epidermis varies in thickness, depending on cell size and number of cell layers of epidermis, ranging from 0.8 mm on palms and soles down to 0.06 mm on the eyelids. This is the outermost layer of skin also called as Stratum corneum. It is approximately 10 mm thick when dry but swells to several times this thicknes swhen fully hydrated. It contains 10 to 25 layers of dead, keratinized cells called corneocytes. It is flexible but relatively impermeable. The horny layer is the principal barrier for penetration of drug. The architecture of horny layer may be modeled as a wall like structure. In this model, the keratinized cells function as protein “bricks” embedded in lipid “mortar.” (24) The lipids are arranged in multiple bilayers. There is sufficient amphiphilic material in the lipid fraction, such as polar free fatty acids and cholesterol, to maintain a bilayer form. Viable epidermis is situated beneath the stratum corneum and varies in thickness from 0.06 mm on the eyelids to 0.8 mm on the palms. Going inwards, it consists of various layers as stratum lucidum, stratum granulosum, stratum spinosumand the stratum basal (25). In the basal layer, mitosis of the cells constantly renews the epidermis and this proliferation compensates the loss of dead horney cells from the skin surface. As the cells produced by the basal layer move outward (26), they alter morphologically and histochemically, undergoing keratinization to form the outermost layer of stratum corneum (27).

Dermis

Dermis is 3 to 5 millimeter thick layer and consists of a matrix of connective tissue, that contains blood vessels, lymph vessels and nerves. The cutaneous blood supply has essential function in regulation of body temperature. It additionally provides nutrients and oxygen to the skin whereas removing toxins and waste products. Capillaries reach to within 0.2 millimeter of skin surface and provide sink conditions for many molecules penetrating the skin barrier. The blood supply thus keeps the dermal concentration of a permeate very low and the resulting concentration difference across the epidermis provides essential concentration gradient for transdermal permeation (28).

Hypodermis

The layer or subcutaneous fat tissue supports the derma and epidermis. It serves as a fat storage area. This layer helps to regulate temperature, provides nutritional support and mechanically protection. It carries principal blood vessels and nerves to skin and should contain sensory pressure organs. For transdermal drug delivery, drug has to penetrate through all these three layers and reach into systemic circulation while in case of topical drug delivery only penetration through stratum corneum is important then retention of drug in skin layers is desired (29).

Skin appendages

Hair and hair follicles, secretory ducts, sweat glands (sebaceous, eccrine and apocrine) and nails are the principle skin appendages (30). On an average, 40-70 hair follicles and 200-250 sweat ducts/cm2 area are present in a healthy human body. About 0.1% of overall skin surface is covered by appendages. The eccrine glands produces sweat having a pH ranging from 4.0-6.8. Sweat glands are capable of secreting amino acids, proteins and antibodies. On an average 400 glands/cm2 are located in the palms and soles. Sebum, an oily material secreted by sebaceous glands located on the surface of face, ears, nose, forehead and anogenital region. The size of the glands varies from 200 2000μm in diameter. The main constituent elements of the sebum are glycerides, free fatty acids, cholesterol, cholesterol esters and squalene (31). The pH of the outer surface of the SC is slightly acidic which is about pH-5 due to presence of sebaceous glands. The key role of sebaceous glands is lubrication and plasticizer effects on lipids of SC (32).

Basic Principle of Transdermal permeation

Transdermal permeation is based on passive diffusion. Skin is the most intensive and readily accessible organ of the body as only a fraction of millimeter of tissue separates its surface from the underlying capillary network. The release of a therapeutic agent from a formulation applied to the skin surface and its transport to the systemic circulation is a multistep process, which includes

1) Diffusion of drug from drug to the rate controlling membrane.
2) Dissolution within and release from the formulation.
3) Sorption by stratum corneum and penetration through viable epidermis.
4) Uptake of drug by capillary network in the dermal papillary layer.
5) Effect on the target organ.
6) Partitioning into the skin's outermost layer, the stratum corneum.
7) Diffusion through the stratum corneum, principal via a lipidic intercellular pathway (33).

Basic Components for selecting Transdermal Drug Delivery Systems

A. Polymer matrix or matrices.
B. The drug
C. Permeation enhancers
D. Other excipients (34)

A.POLYMER MATRIX

The polymer controls the discharge of the drug from the device. The following criteria should be satisfied for a polymer to be employed in transdermal patches.

a)Molecular weight, chemical functionality of the polymer should be specified the precise drug diffuses properly and gets free through it.

b)The polymer should be stable.

c)The polymer should be nontoxic

d)The polymer should be easily of manufactured

e)The polymer should be inexpensive

f) The polymer and its degradation product should be non-toxic or non-antagonistic to the host.

g)Large amounts of the active agent are incorporated into it (35).

The Polymer controls the release of the drug from the device. Possible useful polymers for transdermal devices are:

(i).Natural Polymers

E.g., cellulose derivatives,
Zein,
Gelatin,
Shellac,
Waxes,
Proteins,
Gums and their derivatives, Natural rubber, Starch etc (36).

(ii).Synthetic Elastomers

E.g., Polybutadieine,
Hydrin rubber,
Polysiloxane,
Silicone rubber,
Nitrile,
Acrylonitrile,
Butyl rubber,
Styrenebutadieine rubber,
Neoprene etc (37).

(iii).Synthetic Polymers

E.g., polyvinyl alcohol,
Polyvinyl chloride,
Polyethylene,
Polypropylene,
Polyacrylate,
Polyamide,
Polyurea,
Polyvinyl pyrrolidone,
Polymethylmethacrylate,
Epoxy etc (38).

B. DRUG

For successfully developing a transdermal drug delivery system, the drug should be chosen with great care. The following are some of the desirable properties of a drug for transdermal delivery.

Physicochemical properties

- The drug should have a molecular weight less than approximately 1000 Daltons.
- The drug should have affinity for both lipophilic and hydrophilic phases. Extreme partitioning characteristics are not conducive to successful drug delivery via the skin.
- The drug should have low melting point.
- Along with these properties the drug should be potent, having short half-life and be non-irritating.
- Aqueous solubility : >1mg/ml

Biological properties

a)The drug should be potent with a daily dose of the order of a few mg/day.
b) The half-life (t½) of the drug should be short.
c)The drug must not produce allergic response.
d)Tolerance to the drug must not develop under the near zero-order release profile of transdermal patches (39)**.**

DRUG SELECTION CRITERIA

- Dose is less than 10mg per day
- Molecular weight <1000 daltons
- Aqueous solubility >1mg/ml
- Oil solubility>1mg/ml
- Drug should not be irritant to skin
- Drug should not stimulate an immune response in the skin
- Drug should not be potent and have short half-life (40).

C. PERMEATION ENHANCERS

Substances which temporarily diminish the impermeability of the skin are known as permeation enhancers These are compounds which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant.. As the epidermis is the main barrier for penetration of the drug.These may conveniently be classified under the following main headings (41):-

Chemical enhancer

(i).Solvents

These compounds increase penetration possibly by swallowing the polar pathway and/or by fluidizing lipids (42).
Examples include water alcohols – methanol and ethanol;
alkyl methyl sulfoxides – dimethyl sulfoxide (DMSO)
alkyl homologs of methyl sulfoxide dimethyl acetamide and dimethyl formamide;
pyrrolidones- 2 pyrrolidone,
N-methyl, 2-purrolidone;laurocapram (Azone) (43),

(ii).Surfactants

These compounds are proposed to enhance polar pathway transport, especially of hydrophilic drugs. The ability of a surfactant to alter penetration is a function of the polar head group and the hydrocarbon chain length. The effect of surfactant action upon the skin may change the physical state of water in the skin in such a way to permit greater freedom to the passage of charged, hydrophilic substances. These can accelerate and increase transdermal

permeation and percutaneous absorption. Their permeation promoting activity is due to the decrease in surface tension, to improve the wetting of the skin and to enhance the distribution of the drugs, among the three types of surface-active agents. Anionic laurate ions have the greatest penetration and strongest permeation promotion action (44).

Anionic Surfactants

E.g. Dicotylsulpho succinate, Sodium lauryl sulphate, Decodecymethylsulphoxide etc.

Nonionic Surfactants

E.g. Pluronic F127, Pluronic F68, etc.

Bile Salts

E.g. Sodium mstaurocholate, Sodium deoxycholate, Sodium tauroglycocholate.

Binary system

These systems apparently open up the heterogeneous multilaminate pathway as well as the continuous pathways. E.g. Propylene glycol-oleic acid and 1, 4-butane diollinoleic acid.

(iii). Miscellaneous chemicals

In an attempt to reduce reversibility barrier function of the stratum corneum, which is the major obstacle administration of therapeutic agents, new types of penetration enhancers like naturally occurring cyclic terpenes. These include urea, a hydrating and keratolytic agent; N, N-dimethyl- m-toluamide; calcium thioglycolate; anticholinergic agents. Some potential permeation enhancers have recently been described but the available data on their effectiveness sparse. These include eucalyptol, di-o-methyl-ß- cyclodextrin and soyabean casein, solventspropylene glycol, glycerol, silicone fluids, isopropyl palmitate (45).

Physical enhancers

Sonophorosis

Sonophorosis is the enhancement of migration of drug molecules through the skin by ultrasonic energy. Sonophorosis occurs between the sound waves stimulate micro-vibrations within the skin epidermis and increase the overall kinetic energy of the molecule. When the sound is emitted from the particular frequency, the sound wave disrupt the lipid bilayers. The higher the frequency, the more dispersed the transmission (46).

Electroporation

The method involves generating the aqueous pores deep into the lipid bilayer on application of high voltage pulsed current (~100V/cm) to the skin (47). Thus formed transitory pores are able to deliver the therapeutic agents in a controlled manner across the skin. Other parameters that affect drug diffusion include pulsative properties such as waveform, pulse rate and pulse number. The technique is successful in delivering both micro and macromolecules as the transient pores allow direct penetration in horny layer. Examples include hormones like insulin, estradiol, neurotensin, calcitonin and Luteinizing hormone - releasing hormone (LHRH). Many of the researchers have tried the combination strategy with sonophoresis to enhance the diffusion of drug across the skin (48).

Iontophorosis

Iontophorosis is the method where the movements of the ions across a membrane enhanced using an externally applied potential difference. It is a technique in which the drug is driven by utilizing physiologically acceptable level of current (≤0.5mA/cm2) across the skin. When the membrane under consideration is skin, the method is called transdermal iontophorosis. The principle barrier to the transport of tge molecules into an across the skin is stratum corneum (SC), this is the uppermost layer of the epidermis witha thickness of between 10-100 micrometer. The possible pathway of drug permeation from the dosage form is either by electroosmosis or electrorepulsion.

Electrorepulsion improves the permeation of charged ionic moieties across the skin by utilizing electrode bearing a similar charge as that of the drug. On the contrary, bulk transport of the drug (uncharged) occurs in case of electroosmosis. Iontophoresis is much more effective than passive permeation of charged molecules to elicit pharmacological response beneath the subdermal tissues (49).

Magnetophorosis

The flux across the skin can be enhanced on the application of magnetic field across the skin barrier. The skin resistance for delivery of a drug molecule can be reduced by increasing the strength of magnetic field. Drug can be delivered in a controlled and pulsed fashion by varying the magnetic field (50).

D. OTHER EXCIPIENTS

a).Adhesives

The fastening of all transdermal devices to the skin has so far been done by using a pressure sensitive adhesive which can be positioned on the face of the device and in the back of the device and extendingperipherally. Both adhesive systems should fulfill the following criteria:-

- Should adhere to the skin sharply, should be easily removed.
- Should not leave associate unwashable residue on the skin.
- Should not irritate or sensitize the skin.
- The face adhesive system should conjointly fulfill the subsequent criteria;
- Physical and chemical compatibility with the drug, excipients and enhancers of the device of which it is a part.
- Permeation of drug should not be affected.
- The delivery of simple or emulsified permeation enhancers should not be affected (35,39).

b)Backing membrane

Backing membranes are flexible and they provide a good bond to the drug reservoir, prevent drug from leaving the dosage form through the top, and accept printing. It is impermeable substance that protects the product during use on the skin e.g. metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate (aluminium foil), adhesive foam pad (flexible polyurethane) with occlusive base plate (aluminium foil disc) etc.

Linear: - Protect the patch during storage. The linear is removed prior to use.
Backing: - Protect the patch from the outer environment(34,39).

The application of the transdermal patch and the flow of the active drug constituent from the patch to the circulatory system via skin occur through various methods (51).

Iontophoresis

Iontophoresis passes a few milliamperes of current to a few square centimeters of skin through the electrode placed in contact with the formulation, which facilitates drug delivery across the barrier. Mainly used of pilocarpine delivery to induce sweating as part of cystic fibrosis diagnostic test. Iontophoretic delivery of lidocaine appears to be a promising approach for rapid onset of anesthesia (52).

Electroporation

Electroporation is a method of application of short, high-voltage electrical pulses to the skin. After electroporation, the permeability of the skin for diffusion of drugs is increased by 4 orders of magnitude. The electrical pulses are believed to form transient aqueous pores in the stratum corneum, through which drug transport occurs. It is safe and the electrical pulses can be administered painlessly using closely spaced electrodes to constrain the electric field within the nerve-free stratum corneum (53).

Application by ultrasound

Application of ultrasound, particularly low frequency ultrasound, has been shown to enhance transdermal transport of various drugs including macromolecules. It is also known as sonophoresis (54).

Use of microscopic projection

Transdermal patches with microscopic projections called micro-needles were used to facilitate transdermal drug transport. Needles ranging from approximately 10-100 µm in length are arranged in arrays. When pressed into the skin, the arrays make microscopic punctures that are large enough to deliver macromolecules, but small enough that the patient does not feel the penetration or pain. The drug is surface coated on the micro-needles to aid in rapid absorption. They are used in development of cutaneous vaccines for tetanus and influenza. Various other methods are also used for the application of the transdermal patches like thermalporation, magnetophoresis, and photomechanical waves. However, these methods are in their early stage of development and required further detail studying (55).

TYPES OF TRANSDERMAL PATCH

Single-layer Drug-in-Adhesive

The adhesive layer of this technique also contains the drug. In this type of patch the adhesive layer not solely serves to adhere the varied layers together, beside the complete system to the skin, but is additionally responsible for the releasing of the drug. The adhesive layer is surrounded by a temporary liner and a backing (16).

Multi-layer Drug-in-Adhesive

The multi-layer drug-in adhesive patch is analogous to the single-layer system in this each adhesive layers are responsible for the releasing of the drug. The multi-layer system is completely different but that it adds another layer of drug-in-adhesive, sometimes separated by a membrane (but not altogether cases). This patch conjointly features a temporary liner-layer and a permanent backing (56).

Reservoir

Unlike the Single-layer and Multi-layer Drug-in adhesive systems the reservoir transcutaneous system has a separate drug layer. The drug layer is a liquid compartment containing a drug solution or suspension separated by the adhesive layer. This patch is also backed by the backing layer. During this type of system the speed of release is zero order. Hypoallergenic adhesive polymer may be applied as outer surface polymeric membrane that is compatible with drug (16).

Matrix

The Matrix system encompasses a drug layer of a semisolid matrix containing a drug resolution or suspension. The adhesive layer in this patch surrounds the drug layer partly overlaying it.

Drug-in-adhesive system

In this type the drug reservoir is made by dispersing the drug in an adhesive polymer so spreading the medicated adhesive polymer by solvent casting or melting (in the case of hot-melt adhesives) on an impermeable backing layer. On top of the reservoir, immediate adhesive polymer layers are applied for defense purpose.

Matrix-dispersion system

In this sort the drug is spread homogenously in an exceedingly hydrophilic or oleophilic polymer matrix. This drug containing chemical compound disk is mounted on to AN occlusive base plate in an exceedingly compartment made-up from a drug impermeable backing layer. Instead of applying the adhesive on the face of the drug reservoir, it is spread along with the circumference to form a strip of adhesive rim (57).

Micro-resevior system

In this type the drug delivery system is a combination of reservoir and matrix-dispersion system. The drug reservoir is made by first suspending the drug in an solution of water soluble polymer and so dispersing the solution homogeneously in a lipophilic polymer to form thousands of unreached microscopic spheres of drug reservoirs. This thermodynamically unstable dispersion is stable quickly by instantly cross-linking the compound in situ by using cross linking agents (58).

IDEAL MOLECULAR PROPERTIES FOR TRANSDERMAL DRUG DELIVERY

From the above considerations we can conclude with some observations that can termed as ideal molecular properties for drug penetration. They are as follows.

- An adequate solubility in lipid and water is necessary for better penetration of drug (1mg/ml).
- Optimum partition coefficient is required for good therapeutic action.
- Low melting point of drug is desired (<200°C).
- The pH of the saturated solution should be in between 5 to 9**(25)**.

FACTORS AFFECTING TRANSDERMAL BIOAVAILABILITY

Two major factors affect the bioavailability of the drug via transdermal routes

A. Physico-chemical Factors

Skin hydration

In contact with water the permeability of skin will increase considerably. Hydration is most vital issue increasing the permeation of skin. So use of matter is finished in percutaneous delivery.

Temperature and pH

The permeation of drug increase 10 folds with temperature variation. The diffusion coefficient decreases as temperature falls. Weak acids and weak bases dissociate depending on the pH and pKa or pKb values. The proportion of unionized drug determines the drug concentration in skin. Thus temperature and pH are vital factors moving drug penetration (59).

Diffusion constant

Penetration of drug depends on diffusion coefficient of drug. At a constant temperature, the diffusion coefficient of drug depends on properties of drug, diffusion medium and interaction between them.

Drug concentration

The flux is proportional to the concentration gradient across the barrier and concentration gradient will be higher if the concentration of will be more across the barrier.

Partition coefficient

The optimal partition coefficient (K) is needed for good action. Drugs with high K don't seem to be able to leave the lipoid portion of skin. Also, medication with low K won't be permeated.

Molecularsize and shape

Drug absorption is reciprocally associated with molecular weight, small molecules (60).

Biological Factors

Skin condition

Acids and alkalis, many solvents like chloroform, methanol damage the skin cells and promotes penetration. Diseased state of patient alters the skin conditions. The intact skin is best barrier however the above mentioned conditions have an effect on penetration.

Skin age

The young skin is more permeable than older. Children's are more sensitive for skin absorption of toxins. Thus, skin age is one of the factors moving penetration of drug in TDDS.

Blood flow

Changes in peripheral circulation can affect transdermal absorption. Regional skin sites Thickness of skin, nature of stratum corneum and density of appendages vary site to site. These factors affect significantly penetration. Skin metabolism Skin metabolizes steroids, hormones, chemical carcinogens and some drugs. So skin metabolism determines efficacy of drug permeated through the skin

Species differences:

The skin thickness, density of appendages and keratinization of skin vary species to species, so affects the penetration (31).

Environmental factors

Sunlight

Due to Sunlight the walls of blood vessels become thinner leading to bruising with only minor trauma in sun-exposed areas. Also pigmentation: The most noticeable sun-induced pigment change is a freckle or solar lentigo.

Cold Season

Often result in itchy, dry skin. Skin responds by increasing oil production to make amends for the weather's drying effects. A good moisturizer will help ease symptoms of dry skin. Also, drinking lots of water can keep your skin hydrated and looking radiant.

Air Pollution

Dust can clog pores and increase bacteria on the face and surface of skin, both of which lead to acne or spots. This affects drug delivery through the skin. Invisible chemical pollutants within the air can interfere with skin's natural protection system, breaking down the natural skin's oils that normally entice moisture in skin and keep it supple.

Effect of Heat on Transdermal patch

Heat induced high absorption of transdermal delivered drugs. Patient should be advised to avoid exposing the patch application site to external heat source like heated water bags, hot water bottles. Even high body temperature may increase the transdermally delivered medicine. In this case the patch should be removed immediately. Transdermal drug patches are hold on in their original packing and detain a cool, dry place till they're able to used (61).

VARIOUS METHODS FOR PREPARATION TDDS

Asymmetric TPX membrane method

An example patch may be fabricated for this a heat sealable polyester film (type 1009, 3m) with a concave of 1cm diameter are used as the backing membrane. Drug sample is distributed into the concave membrane, coated by a TPX asymmetric membrane, and sealed by an adhesive.

(Asymmetric TPX membrane preparation)

These are fabricated by using the dry/wet inversion method. TPX is dissolved in a mixture of solvent (cyclohexane) and nonsolvent additives at 60°c to make a polymer solution. The polymer solution is kept at 40°C for twenty-four hrs and cast on a glass plate to a pre-determined thickness with a gardner knife. After that the casting film is evaporated at 50°C for thirty sec, then the glass plate is to be immersed now in coagulation bath [maintained the temperature at 25°C]. After ten minutes of immersion, the membrane may be removed, air dry in a very circulation kitchen appliance at 50°C for 12 hrs] (62).

Circular teflon mould method

Solutions containing polymers in varied ratios are utilized in an organic solvent. Calculated quantity of drug is dissolved in half the amount of same organic solvent. Enhancers in numeruos concentrations are dissolved within the other half of the organic solvent then additional. Di-N-butylphthalate is additional as a plasticizer into drug chemical compound resolution. The total contents are to be stirred for 12 hrs then poured into a circular Teflon mould. The moulds are to be placed on a leveled surface and coated with inverted funnel to control solvent vaporization in a very streamline flow hood model with an air speed of 0.5 m/s. The solvent is allowed to evaporate for 24 hrs. The dried films are to be hold on for an additional 24 hrs at 25±0.5°C in a desiccators containing silica gel before evaluation to eliminate aging effects. The type films are to be evaluated among one week of their preparation (63).

Mercury substrate method

In this method drug is dissolved in polymer solution along with plasticizer. The above solution is to be stirred for 10-15 minutes to produce a homogenous dispersion and poured in to a leveled mercury surface, covered with inverted funnel to control solvent evaporation.

By using "IPM membranes" method

In this methodology drug is dissolved in polymer solution in conjunction with plasticizer. The above solution is to be stirred for 10- 15 minutes to supply a consistent dispersion and poured in to a leveled mercury surface, lined with inverted funnel to control solvent evaporation (64).

By using "EVAC membranes" method

In order to prepare the target transdermal therapeutic system, I Chronicles carbopol reservoir gel, polyethelene (PE), ethylene vinyl acetate polymer (EVAC) membranes are often used as rate control membranes. If the drug isn't soluble in water, propylene glycol is employed for the preparation of gel. Drug is dissolved in propylene glycol, carbopol resin are added to the on top of solution and neutralised by using 5-hitter w/w sodium hydroxide resolution. The drug (in gel form) is placed on a sheet of backing layer covering the desired space. A rate dominant membrane are placed over the gel and therefore the edges are sealed by heat to get a leak proof device (65).

Aluminium backed adhesive film method

Transdermal drug delivery system could manufacture unstable matrices if the loading dose is bigger than 10 mg.Aluminium backed adhesive film technique could be a appropriate one for preparation of same, chloroform is selection of solvent, as a result of most of the drugs as well as adhesive are soluble in chloroform. The drug is dissolved in chloroform and adhesive material are added to the drug solution and dissolved. A custammade aluminium former is lined with aluminum foil and therefore the ends blanked off with tight-fitting cork blocks (62).

Preparation of TDDS by using Proliposomes

The proliposomes are prepared by carrier technique using film deposition technique. From the earlier reference drug and lecithin within the quantitative relation of 0.1:2.0 may be used as an optimized one. The proliposomes are ready by taking 5mg of mannitol powder in a 100 ml spherical bottom flask that is kept at 60-70°c temperature and therefore the flask is revolved at 80-90 rate and dried the mannitol at vacuum for half-hour. After drying, the

temperature of the water bath is adjusted to 20-30°C. Drug and lecithin are dissolved in an appropriate organic solvent mixture, a 0.5ml aliquot of the organic solution is introduced into the spherical flat-bottom flask at 37°C, after complete drying second aliquots (0.5ml) of the solution is to be additional. After the last loading, the flask containing proliposomes are connected in a lyophilizer and subsequently drug loaded mannitol powders (proliposomes) are placed in a desiccator overnight and then sieved through 100 mesh. The collected powder is transferred into a glass bottle and keep at the freeze temperature till characterization (66).

By using free film method

Free film of cellulose acetate is prepared by casting on mercury surface. A polymer solution two w/w is to be ready by using chloroform (67). Plasticizers are to be incorporated at a concentration of 400th w/w of polymer weight (68). Five milliliter of polymer solution was poured in a very glass ring that is placed over the mercury surface in a very glass Petri dish (62). The rate of evaporation of the solvent is controlled by placing an inverted funnel over the petri dish. The film formation is noted by observing the mercury surface once complete evaporation of the solvent.The dry film are separated out and keep between the sheets of paper in a desiccator till use. Free films of various thickness is prepared by ever-changing the amount of the polymer solution (69).

EVALUATION OF TRANSDERMAL PATCH

[1] Physicochemical evaluation
[2] *In vitro* evaluation
[3] *In vivo* evaluation

Physicochemical evaluation

Thickness

The thickness of the drug loaded patch is measured in different points by using a digital micrometer and determines the average thickness and standard deviation for the same to ensure the thickness of the prepared patch (70).

Uniformity of weight

The prepared patches are to be dried at 60°c for 4hrs before testing. A specified area of patch is to be cut in different parts of the patch and weigh in digital balance. The average weight and standard deviation values are to be calculated from the individual weights (70).

Folding endurance

A strip of specific are is to be cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance (70).

Percentage of Moisture content

The prepared films are to be weighed individually and to be kept in a desiccator containing fused calcium chloride at room temperature for 24 hrs. After 24 hrs the films are to be reweighed and determine the percentage moisture content from the below mentioned formula (70).

Percentage moisture content = [Initial weight- Final weight/ Final weight] ×100.

Percentage Moisture uptake

The weighed films are to be kept in a desiccator at room temperature for 24 hrs containing saturated solution of potassium chloride in order to maintain 84% RH. After 24 hrs the films are to be reweighed and determine the percentage moisture uptake from the below mentioned formula (70).

Percentage moisture uptake = [Final weight- Initial weight/ initial weight] ×100.

Drug content

A specified area of patch is to be dissolved in a suitable solvent in specific volume. Then the solution is to be filtered through a filter medium and analyse the drug contain with the suitable method (UV or HPLC technique). Each value represents average of three different samples (71).

Shear Adhesion test

This test is to be performed for the measurement of the cohesive strength of an adhesive polymer. It can be influenced by the molecular weight, the degree of cross linking and the composition of polymer, type and the amount of tackifier added. An adhesive coated tape is applied onto a stainless steel plate; a specified weight is hung from the tape, to affect it pulling in a direction parallel to the plate. Shear adhesion strength is determined by measuring the time it takes to pull the tape off the plate. The longer the time take for removal, greater is the shear strength (72).

Peel Adhesion test

In this test, the force required to remove an adhesive coating form a test substrate is referred to as peel adhesion. Molecular weight of adhesive polymer, the type and amount of additives are the variables that determined the peel adhesion properties. A single tape is applied to a stainless steel plate or a backing membrane of choice and then tape is pulled from the substrate at a 180º angle, and the force required for tape removed is measured (72).

Thumb tack test

It is a qualitative test applied for tack property determination of adhesive. The thumb is simply pressed on the adhesive and the relative tack property is detected (72).

Rolling ball tack test

This test measures the softness of a polymer that relates to talk. In this test, stainless steel ball of 7/16 inches in diameter is released on an inclined track so that it rolls down and comes into contact with horizontal, upward facing adhesive. The distance the ball travels along the adhesive provides the measurement of tack, which is expressed in inch (73).

Quick Stick (peel-tack) test

In this test, the tape is pulled away from the substrate at 90ºC at a speed of 12 inches/min. The peel force required to break the bond between adhesive and substrate is measured and recorded as tack value, which is expressed in ounces or grams per inch width (73).

Probe Tack test

In this test, the tip of a clean probe with a defined surface roughness is brought into contact with adhesive, and when a bond is formed between probe and adhesive. The subsequent removal of the probe mechanically breaks it. The force required to pull the probe away from the adhesive at fixed rate is recorded as tack and it is expressed in grams (73).

***IN VITRO* RELEASE STUDIES**

Drug release mechanisms and kinetics are two characteristics of the dosage forms which play an important role in describing the drug dissolution profile from a controlled release dosage forms and hence their in vivo performance. A number of mathematical model have been developed to describe the drug dissolution kinetics from controlled release drug delivery system there are various methods available for determination of drug release rate of TDDS (74).

The Paddle over Disc: - (USP apparatus 5)

- This method is used for testing the release of the drug from transdermal products.
- The apparatus consistsof a sample holderor disc assembly that hold the product.

- The entire preparation is placed in a dissolution flask filled with specified medium maintained at 32^{0}C. The paddle is placed directly over the disc assembly.
- The disc assembly holds the system flat and is positioned such that release surface area is placed parallel with the bottom of the paddle blade. Vessel is covered to minimize evaporation during test.
- Sample are drawn midway between the surface of the dissolution medium and the top of the paddle blade at specified times
- This method is identical to the USP paddle dissolution apparatus, except that the transdermal system is attached to a disc or cell resting at the bottom of the vessel which contains medium at 32 ±5°C (75).

The Cylinder modified USP Basket: (USP apparatus 6)
This method is similar to the USP basket type dissolution apparatus, except that the system is attached to the surface of a hollow cylinder immersed in medium at 32 ±5°C. The amount of drug available for absorption to the systemic pool is greatly dependent on drug released from the polymeric transdermal films. The drug reached at skin surface is then passed to the dermal microcirculation by penetration through cells of epidermis, between the cells of epidermis through skin appendages (76).

The reciprocating disc: (USP apparatus 7)
In this method patches attached to holders areoscillated in small volumes of medium, allowing the apparatus to be useful for systems delivering low concentration of drug. In addition paddle over extraction cell method may be used (77).

[3] *IN-VIVO* RELEASE STUDIES

Preparation of skin for permeation studies

An in vitro permeation study can be carried out by diffusion cell. Full thickness abdominal skin of male Westar rats weighing 200 to 250g. Hair from the abdominal region is to be removed carefully by using a electric clipper; the dermal side of the skin is thoroughly cleaned with distilled water to remove any adhering tissues or blood vessels, equilibrated for an hour in dissolution medium or phosphate buffer pH 7.4 before starting the experiment and is placed on a magnetic stirrer with a small magnetic needle for uniform distribution of the diffusant. The temperature of the cell is maintained at 32 ± 0.5°C using a thermostatically controlled heater. The isolated rat skin piece is to be mounted between the compartments of the diffusion cell, with the epidermis facing upward into the donor compartment. Sample volume of definite volume is to be removed from the receptor compartment at regular intervals, and an equal volume of fresh medium is to be replaced. Samples are to be filtered through filtering medium and can be analyzedspectrophotometrically or HPLC. Flux can be determined directly as the slope of the curve between the steady-state values of the amount of drug permeated (mg cm-2) vs. time in hours and permeability coefficients were deduced by dividing the flux by the initial drug load (mg cm-2) (16).

Horizontal-type skin permeation system
This has been widely used for the evaluation of drug permeation across skin. The cell is divided in receptor and donor compartments with a low solution volume (3.5ml) for each compartment and a small membrane area (0.64cm^2). They are continuously stirred by matched set of star-head \magnets,which are rotated at a speed of 600rpm. The system is controlled by thermostated water through a water jacket surrounding the two compartments (78).

Franz diffusion cell
The cell is composed of two compartments: donor and receptor. The receptor compartment has a volume of 5-12ml and effective surface area of 1-5 cm^2. The diffusion buffer is continuously stirred at 600rpm by a magnetic bar. The temperature in the bulk of the solution is maintained by circulating thermostated water through a water jacket that surrounds the receptor compartment (78).

Flow-through diffusion cell

Flow through diffusion cells have the advantage that they can be used when the drug has lower solubility in the receptor compartment. This cell can be fully automated and connected directly to HPLC. They have large capacity donor chamber to aloe appropriate loading of the applied compound and a low volume (0.3ml) receiving chamber that ensures rapid removal of penetrant at relatively low pumping rates (78).

CONCLUSION

This article provide an valuable information regarding the transdermal drug delivery systems and its evaluation process details as a ready reference for the research scientist who are involved in TDDS. The foregoing shows that TDDS have great potentials, being able to use for both hydrophobic and hydrophilic active substance into promising deliverable drugs. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and polymer are required. TDDS a realistic practical application as the next generation of drug delivery system.

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Table.1 Drugs used in the Transdermal Patch

Brand Name	Drug	Manufacturer	Indications
Nicotinell^{R}, Nicoderm^{R}	Nicotine	Novartis	Pharmacological smoking cessation
Matrifen^{R}	Fentanyl	Nycomed	Pain relief patch
Ort**hoEvra**^{TM}	Norelgostromin/ Ethinyldiethylamine	ORTHO-McNEIL	Post menstrual syndrome
N**u**Patc**h 100**	Diclofenacdiethylamie	ZydusCadila	Anti-inflammatory
Alora	Estradiol	There Tech/Proctol and Gamble	Post menstrual syndrome
Estraderm	Estradiol	Alza/Novartis	Post menstrual syndrome
Climara	Estradiol	3M pharmaceutical/Berlex Labs	Post menstrual syndrome
Androderm	Testosterone	There Tech/GlaxoSmithKline	Hypognadism in males
Nitrodisc	Nitroglycerine	Roberts Pharmaceutical	Angina Pectoris

Transderm-Scop[R]	Scopolamine	Alza/Novartis	Motion sickness
Nuvelle TS	Estrogen/Progesterone	Ethical Holding/Schering	Hormone replacement theraphy
Deponit	Nitroglycerine	Schwarz-Pharma	Angina Pectoris
Nitrodur	Nitroglycerine	Key Pharmaceuticals	Angina Pectoris
Catapres TTD[R]	Clonidine	Alza/BoehingerIngelheim	Hypertension
FemPatch	Estradiol	Parke-Davis	Post menstrual syndrome
Minitran Climaderm	Nitroglycerine Estradiol	3M pharmaceuticals Ethical Holding/Wyeth-Ayerest	Angina Pectoris Post menstrual syndrome
Duragesic[R]	Fentanyl	Alza/Janssen Pharmaceutical	Moderate/severe pain
Transderm-Nitro[R]	Nitroglycerine	Alza/Novartis	Angina Pectoris
Testoderm TTS[R]	Testosterone	Alza	Hypognadism in males
Oxytrol[R]	Oxybutynin	Watson Pharma	Overactive bladder
Prostep	Nicotine	Elan Corp/Lederle Labs	Smoking cessation

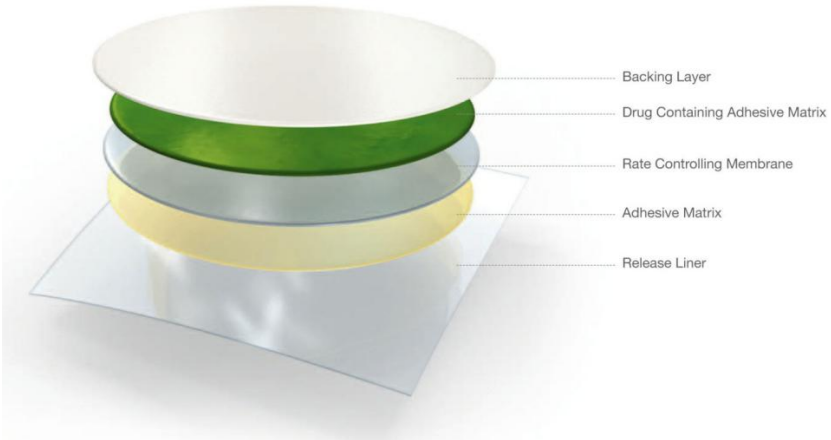

Figure No: 1: Basis Components of Transdermal patch

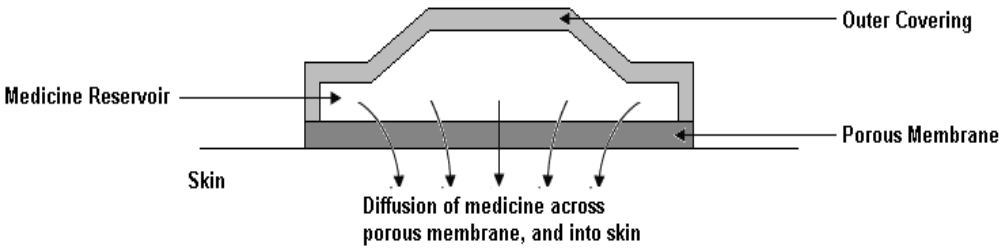

Figure No: 2. Mechanism of Action of Transdermal Patch

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RESEARCH ARTICLE

Screening of *In vitro* Antidiabetic Activity of Coconut Shell and Pericarp by Alpha Amylase Inhibitory Assay

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ABSTRACT

Diabetes mellitus is a group of metabolic disease that affects half of the world population. It is characterized by hypoglycemia resulting from defects in insulin secretion, insulin action, or both. In India 40 million people with diabetes and by 2025 this number will raise up to 70 million. The current work aims to develop a drug from *Cocos nucifera*, which has the ability to control diabetes up to a level. For that, the collected *Cocos nucifera* samples were extracted with different solvents like petroleum ether, methanol, and water using a different method of extraction. Then the extracts are evaluated for anti-diabetic activity using the *In-vitro* alpha-amylase inhibitory method. The results conclude that the water extract of the pericarp and the decoction of the shell shows a percentage inhibition of 69.5% and 69.7% respectively. The extracts are then subjected to phytochemical analysis which reveals the presence of phenolic compounds. This work attempts to summarize and evaluate *Cocos nucifera* as an anti-diabetic agent.

Keywords: *Cocos nucifera,* Diabetes mellitus, Anti-diabetic activity, Alpha-amylase, Phenolic compounds

INTRODUCTION

Diabetes mellitus is a disease in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. The root and causes of diabetes are complex. Most cases begin with mainly two processes metabolic and autoimmune. Metabolic factorsinclude lifestyle factors such as overeating, physical inactivity, and obesity leads to inefficiency of the body to use insulin (insulin resistance) [1]. Metabolic forms of diabetes include type 2 and gestational diabetes. Type 2 diabetes accounts for 90-95% of diabetic cases. Autoimmune diabetes includes type 1 diabetes and latent

autoimmune diabetes of adulthood. Among that type 1 diabetes also known as juvenile diabetes, this develops in children and young adults. Latent autoimmune diabetes of adulthood develops later in life. Individuals with this type of diabetes who overeat, are sedentary, gain weight, or have certain genes can, like people with metabolic forms of diabetes, develop insulin resistance. This state is known as double diabetes [2]. Diabetes mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) and in the next 10 years, it may affect about five times more people than it does now (WHO/Acadia, 1992; ADA, 1997). In India, The prevalence rate of diabetes is estimated to be 1-5% [3].

Diabetes involves hyperglycemia, hypertension, hyperinsulinemia, and hyperlipidemia. All of these factors contribute to the long term complications of diabetes which include: Vascular disease, atherosclerosis, heart conditions, and stroke, kidney disease, eye disease, nerve damage, impaired thinking, infections and wounds, cancer, musculoskeletal disease, pregnancy complications, emotional difficulties, insulin shock, diabetic ketoacidosis, hyperosmolar hyperglycemic nonketotic state [4]. Patients who are diagnosed with diabetes usually require regular monitoring by various health care providers to manage their conditions and reduce the risk of complications. Type 2 diabetes may be reversed with lifestyle changes, especially losing weight with exercise and by eating healthier foods. Some causes of type 2 diabetes can also be improved with weight loss surgery. And there is no cure for type1 diabetes. Treating either type1 or type 2 diabetes include medicines, diet, and exercise to control blood sugar level. Keeping ideal body weight and an active lifestyle may prevent or delay the start of type 2 diabetes. Many patients are prescribed, anti-diabetic agents. The U.S Food and Drug Administration has approved oral diabetes drugs only to treat type 2 diabetes, But physicians sometimes use them to treat other conditions including prediabetes, insulin resistance, and polycystic ovarian syndrome.

Most of the commercially available anti-diabetic drugs are single targeted. That is the reason commercial drugs cannot fulfill the overall improvement. Allopathic drugs have failed to control the disease effectively[5,6]. The main side effects of the currently available medicines include weight gain, increased risk of hypoglycemia, increased risk of gastrointestinal problems, lactic acidosis, kidney disease, shock, increased risk of vitamin B12 deficiency, metallic taste, gastrointestinal problems, increased risk of heart failure, edema, higher risk of anemia, increased LDL level, increased risk of bladder cancer and also cost of the drug. Considering these side effects of this present therapy of DM there is a need of developing a medicament that will overcome the limitations [7,8].

Herbal medicines have received a great deal of attention as alternative medicines used for DM. To overcome the limitations of current medication of diabetes, herbal remedies and dietary treatments are very useful. Considering the side effects of allopathic medications we are preparing a drug that has the ability to control diabetes to a level and has fewer side effects and cost-effectiveness when compared to the allopathic system. Coconut (*Cocos nucifera* L.) belonging to the family Arecaceae and is the only species in the genus *Cocos L.* is a tropical tree species which once was the first major estate crop, extending over large uniform areas, but now mainly grown and harvested by small farmers. The plant is originally from Southeast Asia and islands between the Indian and Pacific oceans.

Cocos nucifera has a lot of use including that it also have certain pharmacological activities like Anti-inflammatory, anti-bacterial, anti-diabetic, anti-oxidant, anti-parasitic, anti-leishmania, anti-neoplastic, anti-malarial, anti-thrombotic, anti-atherosclerotic, anti- cholecystitis effect, anti-protozoal, antidermatophytic activity, anti-caries activity, hypolipidemic, depressant and anti-convulsant, as an electrolyte, cardioprotective effect, immunostimulatory, hepatoprotective effect, analgesic, antibacterial, anti-fungal, antiviral, renal protective, hepatoprotective, disinfectant, hormonal effect, insect repellent, eco-friendly biodiesel and so on [9]. A lot of health benefits are there in coconut, coconut oil, coconut milk, and coconut water. Coconut is called a wonder food since it is almost a perfect meal. In the Vedas, it has been called "Kalpavriksha" meaning the tree which provides all necessities of life. It is used in many rituals and ceremonies apart from its use as food. Coconut is incredibly useful and the variety of products it makes apart from as an ingredient. *Cocos nucifera* is a widely dispersed plant that has important pharmacological activities with low toxicity. The medicinal uses of *Cocos nucifera* have an environmental

appeal since this plant is widely used in the food industry and the use of discarded plant parts will reduce waste and pollution [10,11].

MATERIALS AND METHODS

Materials Required: Coconut shell, Coconut pericarp, Petroleum ether, Methanol, Water, Alpha-amylase, Starch solution, Phosphate buffer.

METHODOLOGY

Collection and processing of coconut shell and pericarp

The mature coconut was collected and sun-dried to separate the coconut kernel portion from the shell. The kernel portion is then sun-dried and the brownish part (pericarp) surrounding the white kernel portion is separated by means of a peeler and dried in shade. It is then crushed using hands and made into a size suitable in packing into the thimble [12].

Extraction of coconut shell and pericarp [13,14]

The empty coconut shells and pericarp which is made into a suitable size were taken in equal quantities (25 gmeach) and packed into the thimble. About 50 ml of solvent is added to the round bottom flask and the condenser is fixed. The pack is soaked in the extraction tube with the solvent for one night and the extraction is started from the next day. The temperature is fixed to 50ºC initially, but as the boiling continues the temperature is lowered to 20 º C. One pack is extracted for 12 hours in petroleum ether followed by methanol and water. The extracts are collected in separate beakers and made crude by using the distillation apparatus and the solvent is reused. Total collected shells and pericarps are packed for 12 packs. Finally, the whole different extracts are combined, distilled, and made into crude.

Extraction of coconut shell alone

The soxhlet apparatus is set and the crushed shells (50 gm) are packed in the thimble and extracted in the same way as above for 24 hours.

Extraction of pericarp alone

50 gm of the pericarp is taken and packed in the thimble and extracted with petroleum ether, methanol, and water in the same way as mentioned earlier.

The decoction of coconut shell alone [15]

50gm of the crushed shells are taken in a beaker, 100 ml water is added and soaked for one day. The contents were boiled for 15 minutes continuously and cooled and strained using filter paper.

The decoction of pericarp alone

25 gm of the pericarp is taken in the beaker and 100 ml of water is added and boiled for 15 minutes. Cooled and strained.

In-vitro antidiabetic activity of extracts [16, 17, 18]

The different extracts collected are evaluated for antidiabetic activity using the alpha-amylase inhibitory assay. Different concentrations of 10 mg/ml sample and make up to 100µl using 25mM phosphate buffer pH 6.9, containing 25µl of porcine α amylase at a concentration of 0.5 mg/ml were incubated at 25°c for 10 min. After pre-incubation, 25µl of 0.5% starch solution in 25mM phosphate buffer pH 6.9 was added. The reaction mixtures were then incubated at 25°c for 10 min. The reaction was stopped with 50µl of 96mM 3, 5 dinitrosalicylic acid color reagent. The microplate was then incubated in a boiling water bath for 5 min and cooled to room temperature. Absorbance was

measured at 540nm using a micro plate reader (Erba, Lisa scan).

Phytochemical analysis [19]

All the extracts were subjected to qualitative tests for the identification of the presence of different constituents like alkaloids, amino acids, carbohydrates, fixed oils and fats, glycosides, phenolic compounds, tannins, saponins, gums and mucilage, and flavonoids.

RESULTS AND DISCUSSION

The extraction of coconut shells and pericarp is done for different batches using petroleum ether, methanol, and water (Table 1). The extraction with the solvent petroleum ether was found to be more effective than the methanol and water. The highest yield was obtained for the petroleum ether extract of shell and pericarp (8.34±2). These extracts of various concentrations (125, 250, 500, and 1000 µg/ml) were evaluated for antidiabetic studies using alpha amylase (Table 2). All the extracts exhibits antidiabetic activity. The percentage inhibition is compared with the standard drug acarbose. An increase in percentage inhibition is seen as the concentration of each sample increases.

Comparison of petroleum ether extracts

The alpha-amylase inhibitory activity of petroleum ether extracts of coconut shell and pericarp is compared with the standard drug acarbose and control (petroleum ether) (Figure 1). Four different concentrations (125, 250, 500, and 1000µg/ml) of the extracts and the control was analyzed.The percentage inhibition increases slightly with increase in concentration but the change in percentage inhibition is not significant. However, the percentage inhibition exhibited by the petroleum ether extract of coconut shell and pericarp combination is lower than that of the petroleum ether control and the standard drug acarbose at each concentration. The three samples of extracts at 1000 µg/ml concentration shows the highest inhibitory activity. On comparing the various concentrations of petroleum ether extracts 1000 µg/ml concentration is the most effective (50.88%). This result was not satisfactory as the standard drug gives a higher percentage inhibition than the extract.

Comparison of methanolic extracts

Methanolic extract of shell and pericarp, shell alone, and pericarp alone is compared with the standard drug acarbose and methanol (control) (Figure 2). Themethanolic extract of the shell and pericarp combination shows the best actvity among various extracts of methanolicextratcs. The methanolic extract of shell and pericarp at the lowest concentration (125µg/ml) gives a percentage inhibition of 49.88% which was higher than that of methanolic extract of the shell (22.02%) and methanolic extract of the pericarp alone (12.37%). An exponential increase in percentage inhibition is seen with the increase in the concentration of the sample. The methanolic extract of the combination of shell and pericarp at 1000 µg/ml concentration shows the highest percentage inhibition (79.16%). The methanolic extract of the shell and pericarp (500 µg/ml) gives a percentage inhibition (73.79%) similar to that of the standard drug of 1000 µg/ml concentration (73.82%).The methanol solvent used as the control gives the least percentage inhibition than the various extracts and the standard drug.

The methanolic extract of the shell and pericarp combination (79.16%) was determined as the best extract among the methanolic extracts since it produces a result better than that of the standard drug acarbose (73.82%). Even though after adjusting the value of control in the percentage inhibition of shell and pericarp the inhibitory activity is negligible in comparison with the standard acarbose.

Comparison of aqueous extracts

Aqueous extracts of shell and pericarp combination, shell alone, and pericarp alone is compared with the standard drug acarbose (Figure 3). The alpha amylase inhibitory activity of aqueous extract of pericarp increases proportionally with increase in concentration. The aqueous extract of pericarp shows a significant increase in alpha

amylase inhibitory activity with respect to increase in concentration of the extract. The highest activity is exhibited by 1000 µg/ml concentration of aqueous extract of pericarp. The aqueous extract of the shell also exhibit a significant increase in the percentage inhibition with respect to concentration. The aqueous extract of pericarp (1000 µg/ml) shows no significant difference compared with the standard.The comparison of various aqueous extracts at 1000 µg/ml concentration shows the following order of percentage inhibition, aqueous extract of pericarp > aqueous extract of shell and pericarp > aqueous extract of shell.

Comparison of decoctions

Water decoctions of four different concentrations of coconut shell and pericarp were prepared and their alpha amylase inhibitory activity was compared (Figure 4). The decoction of the shell and decoction of pericarp at 1000 µg/ml displays a promising alpha amylase inhibitory activity (69.7% and 50.7% respectively). The change in percentage inhibitory activity of decoction of shell and decoction of pericarp is highly significant. At the highest concentration the decoction of shell shows no significant difference with the standard. The results were compared to that of the standard drug and reveals the advantage sample preparation as decoction. Since the samples are decoctions they do not have to compare and analyze their activity with the control. Both the decoctions of shell and pericarp shows increasing percentage inhibition of alpha amylase as the concentration increases.

The samples showing the best alpha amylase inhibitory activity from each group of extracts and decoctions are compared with the standard and their control (Figure 5). The methanolic extract of the shell and pericarp combination at 1000 µg/ml shows the highest percentage inhibition of 79.16%. Although this extract is not considered as the best since it may have some residual methanol which may be responsible for the activity because the methanol control shows a percentage inhibiton of 37.48%. This residual effect is also applicable for petroleum ether extract which shows a percentage inhibition of 50.88%. So the best extracts are considered to be the aqueous extract of the pericarp and water decoction of the shell which gives a similar percentage inhibition (69.5% and 69.7% respectively).

Phytochemical analysis

All the extracts exhibit the anti-diabetic activity using alpha-amylase inhibitory assay and that has proceeded with phytochemical analysis for different constituents. And the extracts show positive results in molish test, lead acetate test and ferric chloride test.So the extracts shows the presence of carbohydrates and phenolic compounds. The extract of shell and pericarp combination and the shell alone also shows the presence of some proteins.

DISCUSSION

Cocus nucifera commonly known as coconut is an important fruit tree recognized mainly for its nutritional and medicinal values. In India, it is known as the tree of life and its applications were documented about 4000 years ago. The whole part of the coconut tree is used in one way or the other [20]. Several researchers have been studying different parts of coconut for different medicinal and nutritional properties. From husk to coconut water everything has been subjected for both in vitro and in vivo antidiabetic studies. K. A. Adekola et al. studied the anti-oxidative and anti-hyperglycemic properties of brown testas of mature coconut and beans seed coats. The total phenolic and flavonoid contents, the antioxidant potentials, and the α-amylase and α-glucosidase inhibitory activities of the crude extracts were studied in vitro. The extracts of coconut testa and red kidney bean seed coat displayed higher α-glucosidase inhibition and α-amylase inhibition than the other extracts. These two extracts showed higher antioxidant capacities owing to their high phenolic and flavonoid contents [21].

Mani Priya and Subha Ranjani conducted a comparative study on the antidiabetic potential of three different plants in both in vivo and in vitro conditions. *Syzygium cumini*, *Aegle marmelos*, and *Cocos nucifera* were selected and their aqueous and methanolic extracts were studied for their antidiabetic potential. In the preliminary phytochemical analysis and Paper Chromatography, different types of phytocompounds responsible for regulating the pancreatic

hormone for the synthesis of insulin had been found in the extracts. In the in vitro antidiabetic analysis, among all the three plant extracts, an aqueous extract of *Cocos nucifera* has high sugar reducing capacity. HPLC analysis also reveals the hypoglycemic effect of coconut and it was then taken for in vivo evaluation [22]. The antidiabetic potential of the aqueous and ethanolic extract of coconut endocarp is studied by NidhiTyagi et al. The phytochemical analysis reveals that both the extracts were rich in phenolic and flavonoid contents. Also in vivo evaluation results that the ethanolic extracts possess higher antidiabetic potential than the aqueous extracts [23]. Several other in vivo researchers investigating the antidiabetic potential of coconut water [24], coconut husk [25], and coconut flowers [26] have also been done. In summary, each and every part of the *Cocos nucifera* has been evaluated for medicinal as well as nutritional properties.

The results of the above literature were compromising with the results we obtained. The phytochemical analysis reveals the presence of phenolic compounds in the coconut shell and pericarp extracts that are responsible for the hypoglycemic effect. The amylase inhibitory assay results also show that the extracts of the shell and pericarp are having anti-diabetic potential. The study also discusses the hypoglycemic effect of the coconut shell and pericarp irrespective of the solvent in which it is extracted.

CONCLUSION

This study aimed to identify the antidiabetic potential of the shell and the pericarp of the coconut. The study also defines the difference of antidiabetic potential exhibited by hydroalcoholic and aqueous extract of the coconut shell and the pericarp. Meanwhile, the phytoconstituents present in the extract and responsible for the activity are also identified. The hydroalcoholic extraction of coconut shells and the pericarp is done using the soxhlet apparatus. The highest yield obtained for the hydroalcoholic extraction of coconut shell and pericarp is by using petroleum ether as the solvent (8.34±2) than the methanolic extraction (1.06±1.5) and water extraction (0.4.±0.66). In the decoction method of extraction, the higher yield is shown by the shell alone when compared to the decoction method using pericarp. Soxhlet extraction samples of pet ether, methanol, and water are evaluated for anti-diabetic studies using the alpha-amylase inhibitory assay.

Comparing the assay values, organic solvent extracts give a higher percentage inhibition but their activity can be said to be average, considering the values of their control. This problem is not encountered in the case of aqueous extracts and decoctions. The water extract of the pericarp (figure 3) at the highest concentration gives a percentage inhibition of 69.5% which was similar to the standard acarbose (73.82%). On the contrary, the water decoction of the shell gives a percentage inhibition of 69.7% at the highest concentration (figure 4). So the conclusion can be made such that the water extracts and decoctions of coconut shell and pericarp show the best activity. The phytochemical analysis reveals the presence of phenolic compounds in the coconut shell and pericarp extracts that are responsible for the hypoglycemic effect.

Cocos nucifera is a widely distributed plant that has so many important pharmacological effects that differ according to the plant part used. As the diabetic population is increasing day by day, the current study reveals the importance of developing an anti-diabetic drug. And the development of nutraceuticals from the *Cocos nucifera* is also wealth promising. Some limitations of the studies on *Cocos nucifera* must be admitted. The studies conducted on the *Cocos nucifera* are only based on the activities not based on the mechanisms and the formulations developed from the *Cocos nucifera* must be developed on the basis of clinical trials. The main goals of the studies should be, isolate the compounds producing activities, and clarify the mechanism behind the activities.

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Conflict of Interest

Authors declare that there was no conflict of interest.

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Table: 1 Total practical yield of extracts

Methodology	Type of extract	Total yield (gm)
Extraction of coconut shell and pericarp	Petroleum ether extract of shell and pericarp	8.34±2
	Methanolic extract of shell and pericarp	1.06±1.5
	Aqueous extract of shell and pericarp	0.40±0.66
Extraction of coconut shell alone	Petroleum ether extract of shell	3.50±1.44
	Methanolic extract of shell	3.50±4.94
	Aqueous extract of shell	0.255±0.22
Extraction of pericarp alone	Petroleum ether extract pericarp	3.21±3.46
	Methanolic extract of the pericarp	0.25±0.023
	Aqueous extract of the pericarp	0.216±0.45
A decoction of coconut shell alone	Water decoction of shell	4.16±0.144
A decoction of pericarp alone	Water decoction of the pericarp	3.54±0.034

Table 2: Alpha-amylase inhibitory activity

	Standard	Control		Shell and pericarp Extract in			Shell Extract in			Pericarp Extract in		
Sample concentration (µg/ml)	Acarbose drug	Pet.ether	Methanol	Pet ether	Methanol	Water	Methanol	Water	Water decoction	Methanol	Water	Water decoction
125	55.85	48.3	11.91	36.02	49.88	36.76	22.02	15.2	4.86	12.37	12.6	13.82
250	58.61	54.23	20.32	40.89	59.71	39.09	31.68	32.64	34.49	37.64	20	31.62
500	60	59.41	29.28	46.06	73.79	57.67	43.03	48.17	55.44	42.17	67.64	34.41
1000	73.82	60.17	35.19	50.88	79.16	64.26	55.45	60.32	69.7	62.59	69.5	50.7

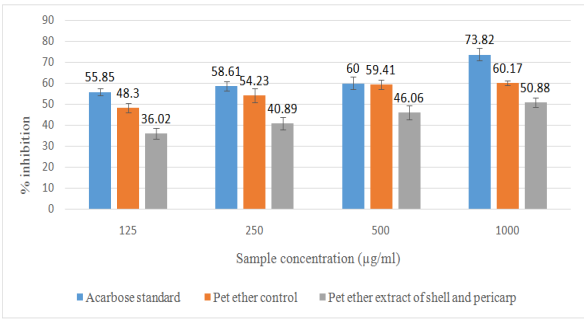

Figure 1 Comparison of petroleum ether extracts of *Cocos nucifera*

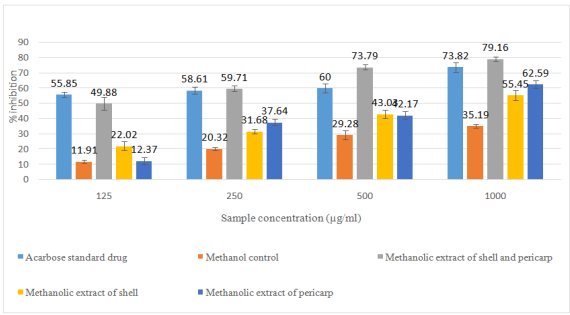

Figure 2 Comparison graph of *Cocos nucifera* Methanolic extracts with standard and control

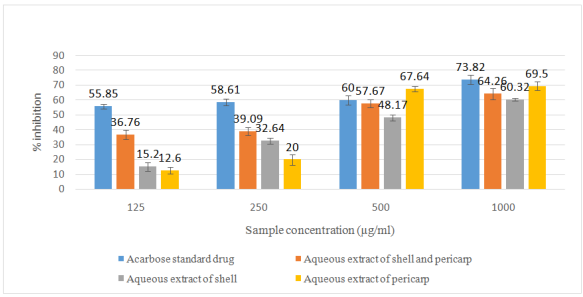

Figure 3 Comparison of *Cocos nucifera* Aqueous extracts with standard

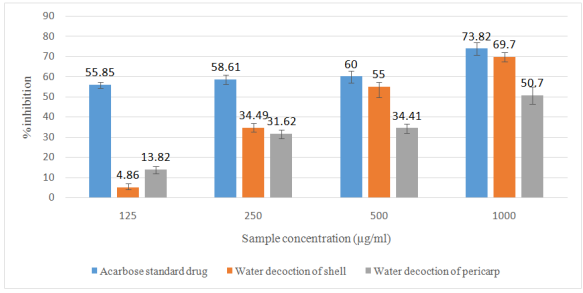

Figure 4 Comparison of *Cocos nucifera* water decoctions with standard

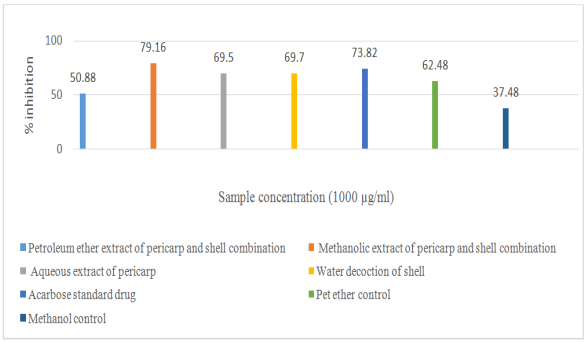

Figure 5 Comparison graph of best of all the extracts with standard acarbose drug

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REVIEW ARTICLE

A Review: Vaccine Drug Delivery System

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ABSTRACT

A vaccine is a biological preparation which enhance the immunity to a particular disease. Vaccine is a material that induces an immunological mediated resistance to a disease but not necessarily an infection. The term vaccine was elucidate by Edward Jenner's 1796 use of cow pox (Latin variola vaccinia, taken from the Latin vaccīn-us, from vacca, cow), to inoculate humans, providing them protection against smallpox Vaccine are generally composed of killed or attenuated organisms. Vaccine drug delivery improvements may include the use of novel routes of delivery including intradermal, intranasal,edible vaccine, Transdermal vaccine delivery, DNA delivery vaccine, mucosal delivery, single shot vaccine delivery. This review represents the different delivery system.

Keywords: Vaccine, Antigen, Drug delivery system, Types of vaccine**.**

INTRODUCTION

A vaccine is biological preparation which enhances the immunity to a particular disease. A vaccine is typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. This agent stimulates the body immune system to identify the agent as foreign, destroy it, and keep a record that it later encounters. Vaccines may be therapeutic (means vaccines against cancer are also being investigated; see cancer vaccine) or prophylactic (means to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), The term vaccine was elucidate by Edward Jenner's 1796 use of cow pox (Latin variola vaccinia, taken from the Latin vaccīn-us, from vacca, cow), to inoculate humans, providing them protection against smallpox[1]. Vaccine is material that induces an immunologically mediated resistance to a disease but not necessarily an infection. Vaccines area unit usually composed of killed or attenuated organisms or subunits of organisms or DNA secret writing substance proteins of pathogens. Sub-unit vaccines

though exceptionally selective and specific in reacting with antibodies often fail to show such reactions in circumstances such as shifts in epitopic identification center of antibody and are poorly immunogenic. However, the selectivity and specificity of sub-units of the causative organism like proteins, carbohydrates can be exploited for producing strong and prolonged immune responses by catering them to the immune system in such the simplest way that a selected and robust immunologic response is induced . These epitopes may also allow the generation of vaccines not only against infectious diseases, but also against chronic diseases such as hepatitis C or cancer[2].

In order to induce an effective protective immunity, these vaccines requires a boosting with agents called "Adjuvants." Adjuvants are believed, to act by forming a complexes with the agent to be delivered from which immunogens are slowly released. Vaccines square measure outlined as "the preparations given to patients to induce immune responses that results in the assembly of antibodies (humoral) or cell-mediated responses that may facilitate in combating with ''infectious agents or non-infectious conditions such as malignancies". The WHO's policy recommended universal immunisation of all kids to scale back kid mortality below its distended Programme of immunisation (EPI). Immunization is an effective tool for controlling and even eradicating disease. Our country has contributes to one-fourth of global under five mortality with a significant number of deaths which can be prevented by vaccines. Immunization needs to be brought closer to the communities for proper coverage. Innovative methods and practices are needed for the better immunization. Most vaccines areavailable in the developed countries, now are available in India. Newer delivery systems square measure the necessity of the hour and so square measure being extensively researched. Some of the explanations for the necessity of latest immunogen delivery systems square measure - horrifying safety profile of live vaccines, weak immunogenicity of sub-unit vaccines and poor patient compliance to booster doses. Carriers help in sustained release and accurate targeting and are being used in the developing of the new vaccine delivery systems. Amongst others, development of the "needle free technologies", to assist administration of the vaccines through completely different routes into the anatomy, is generating worldwide interest[3].

TYPES OF VACCINE [4,5]

1. Killed
2. Attenuated
3. Toxoid
4. Subunit
5. Conjugate
6. Experimental
7. Valence

Killed (inactivated) Vaccines

Killed (inactivated) Vaccines are prepared when safe live vaccine are not available. Some vaccines contain killed, but previously virulent, micro-organisms that have been destroyed with chemical, heat, radioactivity or antibiotics. Examples are the influenza vaccine, cholera vaccine, hepatitis A vaccine, etc.

Attenuated

Attenuated vaccine are viral, some are bacterial in nature. Example are viral disease yellow fever, measles etc. The live Mycobacterium Tuberculosis vaccine are developed by Calmette and Guerin it is not made up of contagious strain, but contain a virulent modified strain called "BCG" induce an immune response to the vaccine. The live attenuated vaccine containing strain Yersinia pestis EV is used for plague immunization

Toxoid

Toxoid vaccines are made from inactivated toxin compounds that cause illness rather than the micro-organisms. Like Tetanus and Diphtheria. Toxoids vaccines are known for their efficacy. For example, *Crotalus* atrox toxoid is used to vaccinate dogs against Rattlesnake bites.

Subunits

Protein subunits- rather than introducing, inactivated or attenuated micro-organisms to an immune system (which would constitute a "whole-agent" vaccine)[4]Example include the subunit vaccine against Hepatitis B virus that composed of only the surface proteins of the virus (previously extracted from the blood serum of chronically infected patients, but now produce by recombination of the viral gene into yeast, the virus-like particle (VLP) vaccine against human papilloma virus (HPV) are composed of the viral major capsid protein, and the hemagglutinin and neuraminidase subunits of the influenza virus. Subunit vaccine is being used for plague immunization.

Conjugate

Certain bacteria have polysaccharide outer coats area unit poorly immunogenic. By linking these outer coats to proteins (e.g. toxins), the immune system may be led to recognize the polysaccharide as if it were a protein antigen. This approach were used in the haemophilic influenza type B vaccine.

Valence

Valence may be monovalent (also called univalent) or multivalent (also called polyvalent).A monovalent vaccine is designed to immunize against a single antigen or single microorganism. A multivalent or polyvalent vaccine are designed to immunize against two or additional strains of same microorganism, or against two or more microorganisms. The valence of a multivalent vaccine could be denoted with a Greek or Latin prefix (e.g., tetravalent or quadrivalent). In certain cases, monovalent vaccine may be preferable for the rapidly developing a strong immune response.

Vaccine Drug Delivery System

The Delivery of antigens from the oil-based adjuvants such as Freunds adjuvant lead to a reduction in the number of doses of vaccine to be administered but due to the toxicity causes inductions of granulomas at the site of injection, such adjuvants are not widely used. FDA approved adjuvants for human uses are aluminium hydroxide and aluminium phosphate in the form of alum. Hence, the search for safer and potent adjuvants results in the formulation of antigen into the delivery systems that administer antigen in particulate form rather than the solution form. Other reasons that driving the development of vaccines as controlled drug delivery systems are as follows:

- Immunization failure with the conventional immunization regimen involving prime doses and booster doses, as patients neglect the latter.

Vaccines delivery systems on the other hand:

- Allows the incorporation of doses of antigens so that booster doses are no longer necessary as antigens are released slowly in a controlled manner.
- Control the occupying space and relating to time presentation of antigens to the immune system there by promoting their targeting direct to the immune cells[7].

Vaccine delivery systems can be classified as follows

Solid particulates: Solid particulate systems such as microspheres and lipospheres are being exploited for vaccine delivery based on the fact that intestine is an imperfect barrier to small particulates. Antigens are entrapped in such particulates when taken up by the M-cells can generate immunity. Methods such as light microscopy, confocal microscopy, electron microscopy, extraction of polymer from tissue followed by quantification by gel permeation chromatography, flow cytometry indicated that micro particulates of <10 μm in diameter can enter gut associated lymphoid tissue (GALT) within 1 h of oral administration and can be used as antigen carriers for controlled release vaccine applications. *Particle size* is an important consideration while formulating micro particulate systems as it influences their uptake and release and hence immune responses. Small (<10 μm) microspheres due to their large surface to mass ratio, are capable of facilitating extracellular delivery of antigen to the phagocytic accessor cells leading to faster release and increased antigen processing. Larger particles could not be phagocytised by

macrophages until they have disintegrated into smaller debris. A combination of larger and smaller particles might produce a pulsatile pattern for antigen release thus mimicking an immunization process involving prime and booster shots[8].

Polymers in solid particulate vaccine delivery

Biodegradable polymers such as PLGA, previously used as surgical implant and suture material is now being exploited for matrix antigen delivery. PLGA microspheres that are rapidly taken up by the M-cells and translocated towards the underlying lymphatic tissue within 1 h. Shi *et al*., developed spray dried PLGA microspheres loaded with recombinant tuberculosis (TB) antigen, TB10.4-Ag85B for pulmonary administration against tuberculosis infection. Particles were of 3.3 μm in size and, hence, were respirable. Results have shown initial burst release of antigens followed by a sustained release up to 10 days. Interleukin-2 secretion in a T-lymphocyte assay due to the microspheres was found to be stronger than antigen solutions.

However, use of PLGA can be limited by acid hydrolytic degradation products that cause harm to the entrapped protein and loss of immunogenicity on storage. Also organic solvents used to load the antigen onto the polymer can be detrimental to the antigen[9]. Domb *et al* entrapped a recombinant malaria antigen, R32NS1, derived from the circumsporozoite protein of *Plasmodium falciparum* in biodegradable polymers like polylactide (PLD) or polycaprolactone (PCL) in the absence or presence of lipid A as an adjuvant. PCL lipospheres without immunomodulators have been shown a superior sustained immunogenic response over PLD lipospheres, the reason being in the different biodegradation rate of polymers[10]. Chitosan, a mucoadhesive linear polysaccharide derived from partial de-acetylation of chitin is safer over PLGA as there is no need to use organic solvents because of the ability of positively charged chitosan that bind with negatively charged immunogenic DNA. Dinesh kumar *et al*., prepared chitosan microspheres (1%, 2%, 3%) loaded with tetanus toxoid which constituted 1%, 2%, 3% of chitosan microspheres. *In vitro*studies have shown cumulative percentage release of tetanus toxoid from microspheres as 74.09%, 89.31%, and 80.23%, respectively, for 50 days.

Poly (phosphazenes) are class of polymers with a simple –P = N– backbone with physicochemical properties strongly influenced by side chain attachments to phosphorous atom. Antigen were entrapped into such polymers in aqueous state at reduced temperatures. The system is then rendered insoluble by the addition of crosslinking agents such as calcium thus promoting sustained release from the precipitated solid. Further control were obtained by coating the solid surface with cationic polymer poly (lysine) and this approach has been used in the release of antibacterial drugs in CR string made from Ca Alginate designed for impaction into dental periodontal cavities. Polyanhydrides such as poly (fumaric-co-sebacic) anhydride fabricated into microspheres of 0.5-5 μm in diameter were seen as early as 1 h post feeding and were observed in the Peyer's patch at 3, 6, 12, following oral administration[11].

EDIBLE VACCINE

Subunit vaccines contain specific macromolecules, i.e., one specific epitope from many epitopes present on the antigen. Subunit vaccines are thus safer over conventional vaccines as they eliminate the use of live viruses or microbes to stimulate immunity. But subunit vaccines involve expensive manufacturing procedures and are thermo labile necessitating cold chain storage from point of manufacture until vaccination which aggravates the expenses in providing costlier facilities like refrigeration to render stability to the preparation.

Production of vaccines in “plants” offer attractive benefits and overcome many of the above-mentioned limitations.

- Plant vaccines serve as an inexpensive means of processing and expressing proteins that can be quite complex to handle as plants require only sunlight, water, and minerals to carry out the process.
- Avoidance of contamination with animal pathogens, improved stability of heat labile vaccine components and oral delivery of resulting vaccines are few of many advantages obtained when plants are used for the expression of vaccines.
- Both mucosal and systemic immune responses can be produced by the mucosal administration of a plant derived vaccine[12].

DNA VACCINES

DNA vaccines consist of bacterial plasmids into which the specific sequences are incorporated. Gene expression is promoted by the cytomegalovirus promoter and its adjacent intron A sequence (ensures high transcription efficiency) and elements like a transcription termination signal and a prokaryotic antibiotic resistance gene[13]. DNA inserted in the plasmid stimulates immunity by acting as a pathogen-associated molecular pattern (PAMP) which has high affinity for Toll-like receptors (TLRs). TLRs are "pattern recognition receptors" with an ability to identify the conserved molecular patterns of the DNA associated with pathogens. One such sequence that is common in bacterial DNA but rare in mammalian DNA is the hypomethylated CpG dinucleotide that mainly binds to TLR-9. Stimulation of a range of TLR9-expressing cells, including B cells and dendritic cells (DC) leads to a cascade of activation, proliferation and differentiation of natural killer cells, T cells and monocytes/macrophages. Attempts are now being made by the industry to use synthetic CpG phosphorothioate oligonucleotides as adjuvants for a range of different vaccines. However one reason for which DNA vaccines may not be effective for human application is that, TLR9 is not expressed by myeloid dendritic cells but only on plasmacytoid dendritic cells of the mammals. Interestingly, it was found that DNA vaccines perform well in *Tlr9*–mice, which indicates that there are alternate pathways apart from TLR-9 stimulation for inducing immune responses[14].

A DNA fusion vaccine designed to activate immunity against B-cell Lymphoma

Genes encoding for variable regions (Vh, Vl) of tumour specific antigens (Idiotypic determinants) expressed by B-cell lymphoma were assembled as single chain Fv (scFv). But this fragment is weakly immunogenic. The fusion of 3′ position of scFv with a gene encoding the fragment C portion of tetanus toxin gave a DNA fusion vaccine and lead to the amplification of the immune response and suppression of lymphoma growth. Polyclonal and monoclonal anti-Id antibodies showed clinical effects but raising patient-specific antibodies is practically difficult. DNA vaccines avoid this problem as Id determinants can be expressed by using the variable region genes, either as whole or as single chain Fv (scFv) fused with a sequence derived from tetanus toxin (fragment C (FrC)) to the scFv[15].

Enhancement of DNA vaccine potency by different approaches

During the last decade, DNA-based immunization has been promoted as a new approach to prime specific humoral and cellular immune responses to protein antigens. In mouse models, DNA vaccines have been successfully directed against a wide variety of tumours, almost exclusively by driving strong cellular immune responses in an antigen-specific fashion. However, there is still a need to improve the delivery of DNA vaccines and to increase the immunogenicity of antigens expressed from the plasmids . For example, tumour burden has been decreased by novel DNA vaccine strategies that deliver cytokines as plasmids directly into tumours in both mouse and human models[16]. Altogether, the selected trials for DNA vaccines have shown that immune responses can be generated in humans, but they also highlight the need for increased potency if this vaccine technology is to be effective. The reasons for the failure of the DNA is vaccines to induce potent immune responses in humans have not been elucidated. However, it is reasonable to assume that the low levels of the antigen production, inefficient cellular delivery of DNA plasmids and insufficient stimulation of the innate immune system have roles in low potency of DNA vaccine. Therefore, with further optimization of the DNA vaccine strategies can be improved, with significant effects on the outcome of immunization. Generally, the methods of delivering a DNA plasmid are divided into:

Physical approaches including

- Tattooing
- Gene gun
- Ultrasound
- Electroporation
- Laser

Viral and non-viral delivery systems (Non-physical delivery methods) including

- Biological gene delivery systems (viral vectors)
- o Non-biological gene delivery systems (non-viral vectors) such as:
- o Cationic lipids/liposomes
- o Polysaccharides and cationic polymers
- o Micro-/Nano-particles
- o Cationic peptides/Cell-penetrating peptides (CPP)

Tattooing

Tattooing has been recently delineate as a physical delivery technology for deoxyribonucleic acid injection to skin cells. This approach, which is similar to the effective smallpox-vaccination technique, seems to decrease the time that is required for the induction of potent immune responses and protective immunity. This result can be associated with the fast and extremely transient nature of matter production once vaccination. Gene expression once deoxyribonucleic acid tattooing has been shown to be beyond that once injection and factor gun delivery. As compared to shot, DNA delivery by tattooing seems to produce different gene expression patterns. One study showed that tattooing of 20 μg DNA results at least ten times lower peak values of gene expression than intramuscular injection of 100 μg DNA in mouse model. Gene expression after tattooing showed a peak after six hours that it disappeared over the next four days. On the contrary, the intramuscular injection of DNA resulted in high levels of gene expression with a peak after one week that it was detectable up to one month. Despite the lower dose of DNA and decreased gene expression, DNA delivered by tattoo induced higher antigen-specific cellular as well as humoral immune responses than that by intramuscular DNA injection.

Furthermore, the effect of two adjuvants, cardiotoxin and plasmid DNA carrying the mouse granulocyte macrophage colony-stimulating factor (GM-CSF) has been evaluated on the efficacy of a DNA vaccine delivered either by tattoo or intramuscular needle injection[17]. In this study, a codon modified gene encoding the L1 major capsid protein of the human papillomavirus type 16 (HPV16) was used as a model antigen. The results indicated that molecular adjuvants substantially enhance the efficiency of the HPV16 L1 DNA vaccine when administered intramuscularly. Also, the delivery of the HPV16 L1 deoxyribonucleic acid within the absence of adjuvants employing a tattoo device induced abundant stronger and a lot of fast body substance and cellular immune responses than intramuscular needle delivery together with molecular adjuvants. However, the tattoo delivery of deoxyribonucleic acid may be a cost-efficient technique that will be utilized in laboratory conditions once a lot of fast and a lot of strong immune responses ar needed. Indeed, the tattoo procedure causes several minor mechanical injuries followed by trauma, necrosis, inflammation, and regeneration of the skin and so non-specifically stimulates the system[18].

Gene gun

The particle-mediated or gene gun technology has been developed as a non-viral method for gene transfer into various mammalian tissues. A broad vary of vegetative cell varieties, including primary cultures and established cell lines, has been successfully transfected ex vivo or in vitro by gene gun technology, either as suspension oradherent cultures. The gene gun is a biolistic device that enables delivered DNA to directly transfect keratinocytes and epidermal Langerhans cells. These events stimulate DC maturation and migration to the local lymphoid tissue, where DCs prime T cells for antigen specific immune responses. Recently, factor gun mediate transgene delivery system has been used for skin vaccination against malignant melanoma mistreatment tumour-associated substance (TAA) human gpl00 and newsman factor assays as experimental systems[19]. High expression of epidermal growth factor receptor (EGFR) protein was observed in several types of cancer including breast, bladder, colon and lung carcinomas. In a study in mouse, the immunological and antitumor responses was evaluated by administration of the plasmid DNA encoding extracellular domain of human EGFR through three different methods: needle intramuscular administration, gene gun administration using gold-coated DNA and gene gun administration using

non-coating DNA. Among these methods, gene Gun administration using non-coating plasmid DNAinduced the strongest cytotoxic T lymphocyte activity and best anti-tumour effects in lung cancer animal model, which may provide the basis for the design of DNA immunizing agent in human trial within the future. Altogether, route of DNA immunization and its formulation could represent an important element in the design of EGFR DNA vaccine against EGFR-positive tumour. Furthermore, the effect of the CpG motif was observed to switch the Th2-type cytokine microenvironment produced by gene-gun bombardment in draining lymph nodes. The results showed that the addition of the CpG motif will increase IL-12 ribonucleic acid expression in debilitating humour nodes whether or not induced by injection, intramuscular injection or gene-gun bombardment. These data suggest that delivery of the CpG motif induces a Th1-biased microenvironment in draining lymph nodes.

Taken together, the CpG motif can act as a 'danger signal' ANd an foil of Th1 immune reaction in polymer vaccination The delivery of HPV polymer immunizing agents mistreatment intracutaneous administration through factor gun was shown to be the foremost economical methodology of vaccine administration as compared with routine injection. Recently, factor gun has been indicated to be able to deliver non carrier naked polymer beneath a unaggressive system. Non-carrier naked therapeutic HPV polymer immunizing agent considerably resulted in less native skin harm than gold particle- coated polymer vaccination. This approach was also able to enhance HPV antigen-specific T cell immunity and anti-tumour effects as compared to the gold particle coated therapeutic HPV DNA vaccine. Recently, a HPV16 polymer immunizing agent secret writing a symbol sequence connected to AN attenuated style of HPV16 E7 (E7 detox) and consolidated to heat shock protein seventy [(Sig/ E7detox/HSP70)] has been used in clinical trials. In a previous study, the immunologic and anti-tumour responses have been evaluated by the pNGVL4a-Sig/E7 (detox)/HSP70 vaccine administered using three different delivery methods including needle intramuscular, Biojector and gene gun. According to obtained results, polymer immunizing agent administered via factor gun generated the very best variety of E7-specific CD8+ T cells as compared to needle intramuscular and biojector administration with mice model[20].

Ultrasound

Ultrasound (US) can be used to transiently disrupt cell membranes to enable the incorporation of DNA into cells. In addition, the combination of therapeutic US and micro bubble echo contrast agents could enhance gene transfection efficiency. In this technique, DNA is effectively and directly transferred into the cytosol. This system has been applied to deliver proteins into cells, but not yet to deliver antigens into DCs for cancer immunotherapy. In vitro and in vivo studies have revealed that the technique of ultrasound can aid in the transduction of naked plasmid DNA into colon carcinoma cells. Furthermore, the intra-tumoral injection of naked plasmid DNA followed by ultrasound in a mouse squamous cell carcinoma model resulted in enhanced DNA delivery and gene expression. Currently, ultrasound has been applied in a clinical trial. A phase II study of repeated intranodal injection of Memgen's cancer vaccine was done using Adenovirus- CD 154 (Ad-ISF35) delivered by ultrasound, in subjects with chronic lymphocytic leukaemia/small lymphocytic Lymphoma[21].

Electroporation

Electroporation (EP) technology has remained a reliable laboratory tool for the delivery of nucleic acid molecules into target cells. This approach uses brief electrical pulses that create transient "pores" in the cell membrane, thus allowing large molecules such as DNA or RNA to enter the cell's cytoplasm. Immediately following surcease of the electrical field, these pores would close and the molecules would be trapped in the cytoplasm without causing cell death. Typically, milli- and time unit pulses are used for electroporation. Recently, the employment of unit of time electrical pulses (10-300 ns) at terribly high magnitudes (10-300 kV/cm) has been studied for direct DNA transfer to the nucleus in vitro. In addition to the increased permeability of target cells, EP may also enhance immune responses through increased protein expression, secretion of inflammatory chemokines and cytokines, and recruitment of antigen presenting cells (i.e., macrophages, dendritic cells) at the EP site. As a result, both antigen-specific humoral and cellular immune responses are increased by EP mediated delivery of plasmid DNA in comparison with levels achieved by intramuscular injection of DNA alone. Indeed, the addition of in vivo EP has been associated with an

even enhancement of cell-mediated and body substance immune responses in little and enormous animals, supporting its use in humans. Subsequently, a comparison of ultrasound versus electroportation (EP) demonstrated that EP can significantly enhance the transfection efficiency of naked plasmid DNA into skeletal muscle against ultrasound. Recently, EP-mediated delivery of inclusion DNA has been shown to be effective as a boosting immunogen in mice fit with DNA alone, possibly owing to the high level of antigen production obtained by the EP-booster vaccine. Interestingly, this regimen was more effective than the one consisting of two doses of DNA with EP. Actually, this approach might be very attractive because it would eliminate the need for two different types of vaccine. For example, the use of a DNA vaccine expressing the CTL epitope AH1 from colon carcinoma CT26 indicated that effective priming and tumour protection in mice are highly dependent on vaccine dose and volume. Indeed, electroporation throughout priming with the optimum vaccination protocol didn't improveAH1-specific CD8+ T cell responses. In contrast, electroporation during boosting strikingly improved vaccine efficiency. Consequently, prime/boost with naked DNA by electroporation dramatically increased T-cell mediated immunity as well as antibody response disease DNA vaccine to be delivered in human using electroporation-based DNA delivery[22].

Laser

Electroporation (EP) technology has remained a reliable laboratory tool for the delivery of nucleic acid molecules into target cells. This approach uses brief electrical pulses that create transient “pores” in the cell membrane, thus allowing large molecules such as DNA or RNA to enter the cell’s cytoplasm. Immediately following cessation of the electrical field, these pores would close and the molecules would be trapped in the cytoplasm without causing cell death. Typically, milli- and time unit pulses are used for electroporation. Recently, the utilization of time unit electrical pulses (10-300 ns) at terribly high magnitudes (10-300 kV/cm) has been studied for direct polymer transfer to the nucleus in vitro. In addition to the increased permeability of target cells, EP may also enhance immune responses through increased protein expression, secretion of inflammatory chemokines and cytokines, and recruitment of antigen presenting cells (i.e., macrophages, dendritic cells) at the EP site. As a result, both antigen-specific humoral and cellular immune responses are increased by EP mediated delivery of plasmid DNA in comparison with levels achieved by intramuscular injection of DNA alone.

Indeed, the addition of in vivo EP has been associated with a homogenous improvement of cell-mediated and body substance immune responses in tiny and huge animals, supporting its use in humans. Subsequently, a comparison of ultrasound versus electroportation (EP) demonstrated that EP can significantly enhance the transfection efficiency of naked plasmid DNA into skeletal muscle against ultrasound. Recently, EP-mediated delivery of plasmid dna has been shown to be effective as a boosting immunogen in mice set with dna alone, possibly owing to the high level of antigen production obtained by the EP-booster vaccine. Interestingly, this regimen was more effective than the one consisting of two doses of DNA with EP. Actually, this approach might be very attractive because it would eliminate the need for two different types of vaccine. For example, the use of a DNA vaccine expressing the CTL epitope AH1 from colon carcinoma CT26 indicated that effective priming and tumour protection in mice are highly dependent on vaccine dose and volume. Indeed, electroporation throughout priming with the best vaccination protocol didn't improveAH1-specific CD8+ T cell responses. In contrast, electroporation during boosting strikingly improved vaccine efficiency. Consequently, prime/boost with naked DNA by electroporation dramatically increased T-cell mediated immunity as well as antibody response disease DNA vaccine to be delivered in human using electroporation-based DNA delivery[22].

Viral and non-viral delivery systems

Over the past forty years, DNA delivery has become a strong analysis tool for elucidating gene structure, regulation and performance. Transfection efficaciousness relies on each the potency of DNA delivery into the nucleus and DNA expression, as well. Although higher expression can usually be achieved with strong promoters and enhancers (e.g., human cytomegalovirus: hCMV), improvements in the efficiency of DNA delivery per second have been difficult to

achieve. Therefore, most DNA delivery systems operate at three general levels: DNA condensation, endocytosis and nuclear targeting.

Biological gene delivery systems (viral vectors)

The design of economical vectors for immunogen development and cancer gene medical care is a part of intensive analysis. Live vectors (attenuated or non-pathogenic live virus or bacteria) such as vaccine virus and other poxviruses, adenovirus and BCG have been evolved specifically to deliver DNA into cells and are the foremost common sequence delivery tools employed in sequence medical care. The major advantage of live vectors is that they produce the antigen in its native conformation, which is important for generating neutralizing antibodies and can facilitate antigen entry into the MHC category I process pathway for the induction of CD8+ CTL. The most effective immunization protocol may involve priming with one type of immunogen and boosting with another17. This method may be useful because:

1) One methodology may be more effective in priming naïve cells, while another modality may be more effective in enhancing memory cell function;
2) Two different arms of the immune system may be enhanced by using two different modalities (i.e., CD4+ and then CD8+ T cells); and,
3) Some of the most effective methods of immunization, like the use of recombinant vaccinia virus or adenoviruses, can be applied for only a limited number of times because of host anti-vector responses. These vectors may be most effective when used aspriming agents, followed by boosting with other agents. The very deep knowledge acquired on the genetics and molecular biology of herpes simplex virus (HSV) as major human pathogen will surely expand different ideas on the development of potential vectors for many applications to be used in human aid. These applications include:

a) Delivery of human genes to cells of the nervous system,
b) Selective destruction of cancer cells,
c) Prophylaxis against infection with HSV or other infectious diseases and
d) Targeted infection of specific tissues or organs.

Viruses represent ideal nanoparticles due to their regular geometries, well characterized surface properties and Nano scale dimensions. Molecules can be incorporated onto the viral surface with control over their spacing and orientation, and this can be used to add reactivity to specific sites of the capsid. Recombinant adenoviruses (Ads) have enormous potential forgene therapy because they are extremely efficient at delivering DNA to target cells, can infect both dividing andquiescent cells, have a large capacity for incorporation of DNA expression cassettes, and have a low potential for oncogenesis because they do not insert their genome into the host DNA. At present, the engineering of“smart” nanoparticles are based upon recombinant adenovirus vectors. Due to the standard nature of the Adcapsid, multiple therapeutic or diagnostic modalities, such as the addition of magnetic resonance imagingcontrast agents, radiation sensitizers and antigenic peptides for vaccines, can be incorporated by modifying totally different sites on the microorganism capsid. For an ideal vaccine, it is crucial to avoid vector related immune responses, have relative specificity for transducing DC, and induce high levels of transgene expression. Adenoviral (AdV) vectors can deliver high.

Antigen concentrations, promote effective processing and MHC expression, and stimulate potent cellmediated immunity. While AdV vectors have performed well in pre-clinical vaccine models, their application to patient care has limitations. Indeed, the in vivo administrationof AdV vectors is associated with both innate and adaptive host responses that result in tissue inflammation and injury, viral neutralization, and premature clearance of AdV-transduced cells. However, Adshave received extensive clinical evaluation and are used for one-quarter of all gene therapy trials.

Non-biological gene delivery systems (non-viral vectors)

Non-viral vectors must be able to tightly compact andprotect DNA, target specific cell-surface receptors, disruptthe endosomal membrane and deliver the DNAcargo to the nucleus. Generally, non-viral vectors include naked DNA, DNA-liposome complexes andDNA-polymer complexes. In other way, non-viral particulate vectors used for gene delivery are divided into microspheres, Nano spheres and liposomes. The encapsulation of plasmid DNA into micro- or Nano spheres can provide protection from the environmentprior to delivery and aid in targeting to a specific celltype for efficient delivery. Liposomes and polymershave also been utilized for the delivery of plasmid DNA,although they exhibit some toxicity in vivo. The association of DNA with lipids or polymers results in positively charged particles small enough for cell entry through receptor-mediated endocytosis. One example of the utilizationof liposomes is the intravenous delivery of the surviving promoter as a DNA-liposome complex which has been shown to be highly specific and has the ability to suppress cancer growth in vitro and in vivo.

The injection of DNA complexed to oxidized or reduced mannan-poly-L-lysin in vivo resulted in the production of antibodies with anti-tumor potential as compared to DNA alone in mice model. Formulation of plasmid DNA with a non-ionic block copolymer, poloxamer CRL1005, and the cationic surfactant benzalkonium chloride resulted in a stable complex that elicited the efficient antigen-specific cellular and humoral immune responses and is currently being evaluated in a Phase II clinical trial for melanoma.

UPTAKE OF ANTIGEN

The initial event of the immune response is the interaction of antigen with antigen reactive lymphoid cells (Davies, Leuchars, Wallis, Marchant and Elliott, 1967). Only a small number of lymphoid cells are antigen reactive and interact with antigen in order that the spleen or a lymph node may subsequently express the reactivity fully (Kennedy, Till, Siminovitch and McCulloch, 1966). Antigen-reactive cells can be fractionated into two populations which, in a density gradient, sediment differently. One of these populations comprises cells that are mitotically active, while the other consists of cells that are mitotically stable (August, Merler, Lucas and Janeway, 1970). Cells in the former class respond to antigenic stimulation by entering into division, while those in the latter take up antigen specifically without subsequent mitotic activity. These mitotically stable, antigen-reactive cells may sub serve the function postulated by Miller and Mitchell for trapping antigen (Miller and Mitchell, 1969). The experiments described herein present evidence that antigen-reactive cells contain antibody. The majority of this antibody is not found on the cell surface. Antigen, after uptake, appears to become bound to nuclear deoxyribonucleic acid (DNA), and may thereby mediate the formation of new cell products[23].

Single shot vaccines

The single-shot immunogen may be a combination product of a chief element—antigen with AN acceptable adjuvant—and a microsphere component that encapsulates substance and provides the booster immunizations by delayed unharness of the substance. To provide effective patient protection, many traditional vaccines require multiple injections, which results in costly and inconvenience regimen. These disadvantages have evoked the development of single shot vaccines that can provide protection against infection by only one injection. The microsphere component uses OctoPlus's proprietary Octo VAX microsphere technology, which is based on cross-linked modified dextran polymers. Dextrans are ideal polymers to form biocompatible hydrogels. Two major advantages of dextran microspheres as protein delivery systems are that the particles are prepared in the absence of organic solvents, and that degradation of the microspheres does not result in a pH drop. Both exposure to organic solvents and an acidic environment ar best-known to negatively have an effect on supermolecule stability. several different dextrans have been developed for hydrogel formation. One of these dextran-based polymers is derivatized with hydroxy-ethyl methacrylate (dex-HEMA, Figure 2), which introduces hydrolytically sensitive carbonate ester groups that ensure biodegradation under physiological conditions. Studies have shown that macromolecule medical specialty developed with this compound retain the activity of the encapsulated protein following encapsulation and unharness[24].

Mucosal delivery of vaccine

Mucosal surfaces are enormous surface areas that are a common site of entry for pathogenic microorganisms (Neutra, 2006). The presence of antigens, pathogens and vaccines within the body that enter through mucosal surfaces are easily detected by the adaptive immune system from those that are introduced directly into tissues or the blood by injection or injury. This clearly indicates the importance of local mucosal immune responses for protection against disease, as for example, mucosal antibodies against Vibrio cholerae bacteria and cholera toxin is associated with resistance to cholera (Levine, 2000). Mucosal immunization through oral, nasal, rectal or vaginal routes can effectively induce mucosal immune responses rather than the vaccines that are injected. Therefore the vaccines that are administered onto the mucosal surfaces have proved to be more efficient in producing mucosal immune responses than injected vaccines.

Intranasal vaccines embody those against respiratory disease A and B virus, proteosoma-influenza, adenovirus-vectored influenza, group B meningococcal native, attenuated respiratory syncytial virus and para influenza 3virus21. Needle free delivery of vaccine. Needle-free vaccination includes all ways for delivering vaccines that don't need a needle and syringe for administration. There ar variety of delivery choices for needle free
Vaccinations, ranging from nasal sprays to patches worn on the skin.

Transdermal vaccine delivery[25]

Within the epidermis and the dermis, the skin provides immunological protection to the body. The skin houses specialized cells in both the epidermis (Langerhans Cells, LC) and the dermis (dermal dendritic cells, dDC); these cells are an important component of the immune system and are not found anywhere else in the body. Collectively, these specialized cells act as sentinels, probing their surroundings for signs of immunological threats. They are able to process microbial antigens and ultimately migrate into lymphatic capillaries to lymph nodes initiating an immune response that may be both faster and stronger than that generated in response to the same amount of antigen administered *via* intramuscular injection. Although LCs account for only 2% of cells in the epidermis, they are relatively large, and their long dendrites stretch across the epidermis to form a tight network that effectively captures particulate- or macromolecule-challengers

Skin as a site for vaccine delivery

The skin has multiple barrier properties to reduce water loss from the body and stop the permeation of environmental contaminants into the body. These barriers can be considered as physical, enzymatic and immunological. Physical barrier The epidermis is in a constant state of renewal, with formation of a new cell layer of keratinocytes at the stratum basal, loss of their nucleus and other organelles to make desiccated, proteinaceous corneocytes on their journey towards desquamation, which occurs from the skin surface, at the same rate as formation, in normal skin. The outmost layer, the stratum corneum, consists of a brick wall like structure of corneocytes in a matrix of intercellular lipids, with desmosomes acting as molecular rivets between the corneocytes. The stratum corneum presents an effective physical barrier to the permeation of large molecules such as vaccines. This is the primary barrier property that has to be overcome to supply effective transcutaneous vaccinum delivery. Enzymatic barrier the skin possesses many enzymes capable of hydrolysing peptides and proteins. These are involved in the keratinocyte maturation and desquamation process, formation of natural moisturizing factor (NMF) and general homeostasis. Their potential to degrade topically applied vaccine antigens should be considered. Immunological barrier when the skin is damaged, environmental contaminants can access the epidermis to initiate an immunological response[26].

Liquid-jet injection [27-29]

Needle-free injection devices Liquid jet injectors use a high-velocity jet (typically 100 to 200 m/s) to deliver molecules through the skin into the subcutaneous or intramuscular region. Jet injectors can be broadly classified into multi-use nozzle jet injectors (MUNJIs) and disposable cartridge jet injectors (DCJIs), depending on the number of injections

carried out with a single device. Commercially available liquid jet injectors consists of a power source (compressed gas or spring), piston, drug or vaccine-loaded compartment and an application nozzle, with typical orifice size in the range of 150 to 300 μm. Upon actuation the power source pushes the piston rapidly increases the pressure within the drug-loaded compartment, thereby forcing the drug solution through the orifice as a high velocity liquid jet. When the jet impacts on the skin it creates a hole through allowing the liquid to enter the skin. The process of whole formation and liquid jet deposition occurs within microseconds. The deposited liquid can then disperse within the tissues to illicit an immune response. Applications of liquid-jet injectors have been focused on delivery of macromolecules that do not passively permeate the skin.

Commercially available devices include the Antares Vision® and Choice® (Antares, Minneapolis) that deliver a variable dose of insulin; V-Go Mini-Ject system for insulin (Valeritas, Parsippany, NJ); Biojector 2000 (Bioject, Tualatin, OR); PenJet (PenJet Corp., Santa Monica, CA) for smallpox vaccination; Injex (HNS International, Anaheim, CA) for administration of insulin and human growth hormone; Zeneo (Crossject, Paris, France). Antares Vision® jet propulsion delivery system (Antares Pharma, Mineapolis, USA). Transdermal delivery of vaccines 5 Needle-free injection has been shown to increase immune responses to both conventional and DNA-based vaccines. For example, seroconversion rates and antibody titres elicited in humans, by a hepatitis A vaccine or a trivalent influenza vaccine, were found to be increased by at least 10% when using needle-free injections compared to needle and syringe administration (Williams et al., 2000). Clinical studies have shown that the number of responders and the mean antibody response were comparable to or better as compared to needle injection, possibly due to better tissue distribution of the vaccine.

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Epidermal powder immunization

Powder injectors was first used for DNA and RNA transfection into plants. The method subsequently has been investigated for transdermal protein delivery, gene therapy and vaccination. The device design methods are same to liquid injectors, with a powder compartment and compressed carrier gas, such as helium. Upon actuation, the particles are carried by the gas, to impact the skin surface at high velocity, puncturing micron-sized holes in the

epidermis to facilitate skin deposition. Humoral and cell mediated immune response following vaccination with jet propelled particles (including influenza, hepatitis B, rabies) has been demonstrated in animal studies. Clinical studies have also been undertaken, with immune responses generated against influenza (Drape et al., 2006) and malaria. A commercial example is the Particle Mediated Epidermal Delivery (PMED®) technology, initially developed at Oxford University, U.K. and currently owned by Pfizer. PMED delivers DNA vaccines into the skin in a dry powder formulation of microscopic gold particles and is currently in development for a range of vaccines.

Topical application

In addition to the systems that bombard the skin with liquid or solid vaccines, a number of other methods have been investigated that can be applied to the skin, to reduce the stratum corneum barrier, and/or carry vaccine into the skin. Topical applications range from non-invasive formulation based approaches (e.g. colloidal carriers), energy based approaches (ultrasound or sonophoresis, and electroporation), stratum corneum ablation and minimally invasive approaches (such as microneedles). Topical adjuvants Topical administration the vaccine with adjuvants, such as cholera toxin, has been shown to induce strong systemic and mucosal immune responses.

Latest advancement in vaccines delivery [30, 31]

a) Cancer vaccines

Cancer vaccines belongs to a class of substances known as biological response modifiers. There are two broad types of cancer vaccines. Preventive (or prophylactic) vaccines and Treatment (or therapeutic) vaccines. Preventive vaccines are made to prevent cancer from developing in healthy people. FDA Approved preventive cancer vaccines in united state are Gardasil® and Cervarix®, that protect against infection by the two types of HPV - types 16 and 18 - that cause approximately 70 percent of all cases of cervical cancer worldwide. Treatment vaccines are intended to treat an existing cancer by strengthening the body's natural defenses against the cancer. In April 2010, the FDA approves the first cancer treatment vaccine. This vaccine, sipuleucel-T (Provenge®, manufactured by Dendreon), is approved for use in some men with metastatic prostate cancer

b) Swine flu vaccine

Nasovac, a vaccine for swine flu has been launched by a Pune-based firm Serum Institute of India Ltd. NASOVAC (Influenza Vaccine (Human, Live Attenuated)) Pandemic (H1N1), freeze dried is a live monovalent vaccine for administration by intranasal spray. The influenza vaccine contains Influenza virus which are cultivated on embryonated eggs. A dose of 0.5 ml is administered as 0.25 ml per nostril using a 0.5/1.0 ml syringe and a spray device. The sprayer device creates an fine spray that primarily settles the vaccine in the nose and nasopharynx. A single intranasal dose is recommended for people above 3 years of age (Serum Institute of India).

c) AIDS Vaccine

AIDSVAX is Associate in Nursing experimental HIV immunogen that was developed originally at Genentech in san francisco, California, and later tested by the VaxGen company, a Genentech offset. It contains a synthetic version of a protein called gp120, found on the outer covering of the HIV virus. The AIDSVAX is given to stimulate the assembly of neutralizing antibodies, proteins that block HIV from infecting cells. The ALVAC-HIV vaccine is made of an attenuated (weakened) canarypox virus that has been genetically altered to contain man-made copies of selected HIV genes. The vaccine is manufactured by Aventis Pasteur of Lyon, France. ALVAC-HIV (vCP1452) is given to stimulate the body's production of CTLs against HIV. Both vaccines are under clinical trial.

d) Nicotine vaccine

NicVAX® is a vaccine against nicotine. Nicotine is very small and therefore the human body is not able to make antibodies on its own against it. NicVAX is made up of many small nicotine molecules attached to a large protein. When nicotine is attached to a large protein, body is now able to see nicotine and make antibodies against it.

e) Diabetes vaccine

Diamyd, a vaccine to prevent diabetes, may be in the markets soon. It is intended for the treatment of children and adolescents with recent-onset type 1 diabetes. It is currently undergoing Phase III clinical trials in Europe (9 countries) and the US.

CONCLUSION

In the last decade vaccine are delivered by syringes and needles but in these ways major problem is to get safety. Vaccine is designed for the treatment of infectious diseases so it requires an greater safety. From the some point of view safety is bring about by delivery technologies so, improvement of the technology designed for vaccines delivery is required. Now a day number of significant advances in technologies are designed for delivery of vaccine also newer vaccines is identified for infectious diseases. Intradermal delivery designed for delivery into dermis is both easy and consistent, highly trained medical staff does to require and should improve dosing consistency and overall vaccine efficacy. The potential for this technology to reduce the required dose compared with intramuscular delivery could result in health economic benefits and increase the possibility of mass intradermal vaccination campaigns. Needle-free vaccine delivery is suitable for many reasons including improved safety, better compliance, decreased pain (especially in children), easier and faster vaccine delivery, and likely reduced costs compared to vaccines delivered by needle and syringe. These advantages are helpful in many circumstances and perhaps are most notable in the setting of mass immunizations necessary due to natural pandemics, immunization campaigns in the developing world, and bioterrorism events.

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Table.1. Excipients used in the formulation of vaccines[6]

EXCIPIENTS	USES
Antibiotics	It is used in vaccines to protect bacterial growth during production and storage.
Protein	It is used in influenza and yellow fever vaccines as they are prepared from chicken eggs
Formaldehyde	It is used to inactivate bacterial product for toxoid vaccines. Formaldehyde is also used to inactivate unwanted viruses and kill bacteria.
Aluminium	Salts and gels are used as adjuvants, they allow for a lower dose vaccine.

Table 2. Delivery of vaccines by polymeric micro particles through different routes

Antigen	Polymer	Particle size (µm)	Route of delivery
B. pertussis fimbriae(9)	PLGA	0.8-5.3	IP, PO
B.pertussis hemagluttin	PLGA	1	IN
Diphtheria toxoid(10)	PLGA	30-100	IM
Ilfluenza virus, formalinized	PLGA	2.2-10.8	SC and PO
Tetanus toxoid(11)	PLA and PLGA	10-60	SC
Vibrio cholera cell-free lysate	PLGA	1-10	PO and IT

PLGA=Poly(latic-co-glycolicacid); PLA=Poly (lactic acid); PS=Poly (styrene); PMMA=Poly (methyl methacrylate); IP=Intraperitoneal; IN=Intranasal; IM=Intramuscular; SC=Subcutaneous; PO=Peroral; IT=Intrathecal.

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RESEARCH ARTICLE

Formulation and Evaluation of Topical Gel Incorporated with Nimesulide Loaded Magnetite Nanoparticles

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ABSTRACT

Nimesulide is a COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. It is used for the treatment of acute pain, inflammation and for the symptomatic treatment of osteoarthritis. But nimesulide is banned in many countries due to chances of liver failure. Thus the present study was aimed to develop a suitable dosage form to apply topically at inflammatory conditions, without affecting internal organs. This study converts nimesulide into magnetically modulated topical gel for topical application. The magnetic field over the applied area helps to retard the movement of drugs into the deeper tissues. This results in accumulation of dosage form and delivery of drug at controlled rate in the target site. The nimesulide drug has been loaded over the magnetite particles using HPMC E5 rate controlling polymer by powder coating method. Seal coating was given to the drug loaded magnetite nanoparticles to prevent the solubility of nimesulide in the gel. The prepared drug loaded magnetite particles were converted into topical gel preparation.

Keywords: Magnetite nanoparticles, nimesulide gel, powder coating, seal coating.

INTRODUCTION

Nimesulide is a preferential COX-2 inhibitor that has been effectively used for the treatment of a variety of inflammatory and painful conditions, including osteoarthritis. Nimesulide is almost completely absorbed orally, 99%

plasma protein bound and has a half-life of 2-5 hours [1]. Nimesulide upon oral administration causes liver failure. It has a volume of distribution (Vd) of 12-27 L. Thus it is not highly distributed and remains in the systemic circulation mainly, which may be a reason for liver failure [2]. The drug has a low extraction ratio of 0.1 [2], which indicates the drug is presenting more into the eliminating organ like liver – it is again harmful. This made the situation to bypass the route of administration from oral to topical, in diseases accompanying symptoms like pain and inflammation.

The drug loaded magnetite nanoparticles can be localized at a specific targeted area by application of an external magnetic field where the drug molecules are gradually released. Thus the therapeutic efficacy of the drug is improved by lowering the toxic side effects on healthy tissues [3]. Topical delivery is a suitable means of drug delivery because of patient compliance, ease of application, accessibility to the site of action and bypass of first pass metabolism. Topical gel formulation provides a suitable delivery system for drugs because they are less greasy and can be easily removed from skin [4]. The aim of the present study was to formulate and evaluate a stable topical gel preparation of nimesulide loaded magnetite nanoparticles for inflammatory conditions.

MATERIALS AND METHODS

CHEMICALS

Nimesulide was purchased from Research lab fine chem industries, Mumbai. Ferrous sulphate and ferric chloride was purchased from Nice chemicals (P) LTD, Kochi. Chemicals like HPMC E5 and ethyl cellulose were purchased from Yarrow chem products, Mumbai.

OVERVIEW OF THE WORK

Figure 1: overview of work

Preparation of magnetite nanoparticles [5]

Magnetite nanoparticles were prepared by coprecipitation of Fe^{2+} and Fe^{3+} ions in the presence of a base. 30g of ferrous sulphate and 40g of ferric chloride were dissolved in 100ml deionized water. The solution is stirred using a mechanical stirrer and added 3M NaOH solution quickly at 300 ºC until the mixture reached a pH around 11. Kept it aside without stirring for 30min. After 30min, the mixture was heated to 80ºC for 30min.

Preparation of drug-loaded magnetite nanoparticles(DLMNPs)

The drug was loaded over the magnetite nanoparticles by Powder coating method [6]. The polymer solution is prepared by dissolving 200mg HPMC E5 in 20ml water by continuous stirring, to get a clear solution. The mixture of the drug (1g) and magnetite (1g) was loaded into the Pelletizer and heated for a few minutes to dry the particles and to ensure immediate drying during the coating process. The polymer solution is sprayed over the drug magnetite mixture at a temperature of 60ºC. The quantified volume of polymer solution HPMC E5 (200mg) is sprayed over core material to coat the core [7].

Seal coating of drug-loaded magnetite particles [8]

The DLMNPs need to be further formulated into a gel for the ease of administration. But the coated drug molecules may solubilise in the gel, thus to prevent the solubility. The DLMNPs are seal coated with ethyl cellulose in chloroform. To optimise the thickness of seal coating the DLMNPs are coated with 2, 4, 6, 8, 10% of polymer and evaluated by physical observation of seal coated nanoparticles in gel. It is sealed with Ethyl cellulose solution in chloroform.1.5g DLMNPs were loaded in the pelletizer and maintained a constant temperature. The pelletizer is rotated at 50 rpm and ethyl cellulose solution is sprayed in small quantities over the nanoparticles and allowed to dry. Seal coated magnetite particles were evaluated for the solubility of drug in the gel and suitable formulation which are stable without solubilising in gel are selected for formulation development.

Preparation of gel incorporated with drug loaded magnetite nanoparticles [9-12]

Topical gel is prepared by dispersing 1% w/w carbopol in water and continuous stirring for a period of 1 hr using a mechanical stirrer till the polymer is completely dissolved in water. Then 1ml glycerol and 0.5ml triethanolamine were added to the polymer solution with stirring to form the gel. Seal coated magnetite particles were incorporated into the gel and mixed well to form a uniformly dispersed gel.

EVALUATION TESTS

Percentage yield of magnetite particles [13,14] is calculated, the magnetite particles are evaluated for Scanning Electron Microscopy (SEM) [15], flow property of magnetite particles [13,15], The DLMNPs are evaluated for SEM analysis and solubility in the gel base. The seal coated magnetite particles are evaluated for solubility in the gel base, dissolution [16,17] in USP I apparatus using phosphate buffer of pH 7.4. The formulated gel is evaluated for homogeneity, consistency [18,19], pH, viscosity is done by Brookfield Viscometer using spindle number 64 rotated at 20 rpm [19,20], spreadability[20,21], and stability study is conducted for the gel, gel is stored at room temperature for one month [22] .

RESULTS AND DISCUSSION

Surface morphology of magnetite particles by scanning electron microscopy analysis

Figure 2: SEM image of magnetite particles. SEM analysis of magnetite particles is shown in figure 2. The electron beam, which typically has an energy range of 20kV. Secondary Electron Imaging (SEI), works on the principle that this electron beam generates a "splash" of electrons with kinetic energies much lower than the primary incident electrons, called secondary electrons. Because of their low energies and low penetration depth, the detection of secondary electrons as a function of primary beam position makes it possible to attain high magnifications (X 7,000) and high resolutions for imaging the areas of interest.The magnetite particles have mostly rounded shapes, where most of the magnetite particles have a diameter of 2µm.

Percentage yield and flow property

The percentage yield of the prepared magnetite nanoparticles was determined and percentage yield was found to be 95%. The optimum percentage yield (90-100%) indicates the process efficiency. The flow property of the magnetite particles were determined by calculating angle of repose, Hausner's ratio and Carr's index. The results are summarized in the following table. The angle of repose is found to be within the range 31-35, Hausner's ratio is within the range 1.12-1.18 and Carr's index is within the range 11-15%. So the magnetite particles have good flow property. Table 1: Flow property of magnetite particles

Surface morphology of DLMNPs by SEM

Figure 3: SEM image of drug loaded magnetite particles, SEM of drug loaded magnetite particles is shown in figure 3. The electron beam has an energy range of 20kV and magnification of X 10,000. The drug loaded magnetite particles have mostly rounded shapes, where most of the magnetite particles have a diameter of 1µm.Figure 4: DLMNPs incorporated gel

Evaluations of seal coated particles

The solubility of DLMNPs was evaluated by coating with different concentrations of ethyl cellulose (2%,4%,6%,8%,10%). Drug is coated with 2% and 4% ethyl cellulose, thickness of the coating was found to be less therefore, the drug becomes easily soluble in the gel. After coating with 6%,8% and 10%, thickness of the coating was found to be increased than 2% and 4%. So the drug was insoluble in gel. When concentration of ethyl cellulose increases thickness also increases. So, these samples (6%, 8%, 10%) were evaluated. Figure 5: Solubility of 6% seal coated magnetite particles in the gel

Dissolution studies

Figure 6: Comparison of dissolution data of Uncoated and seal coated magnetite particles. From the above figure 6 we can compare the different concentrations of ethyl cellulose such as 6%, 8% and 10% respectively. The percentage cumulative drug release data of samples and 6%, 8% and 10% dissolution data were shown in figure 6. From the percentage cumulative drug release data, sample 6% have more cumulative drug release profile. With increase in ethyl cellulose coating thickness, the rate of drug release was found to be decreased. Therefore, sample 6%, which have more drug release profile, is incorporated into the gel. The comparison of dissolution data of uncoated DLMNPs and 6% were shown in figure 6. Compared with uncoated particles, drug release was slow from the seal coated magnetite particles. But after 24hr, the drug release was almost the same and at 48hr, 97 % of the loaded drug was released from the particles. So, 6% is incorporated into the gel. Figure 7: Dissolution data of seal coated magnetite particles and DLMNPs incorporated gel.

After 48 hrs, the % cumulative drug release from the seal coated magnetite particles was found to be 97%. Therefore, drug loaded magnetite particles coated with 0.37% ethyl cellulose give an extended drug release and can incorporate into the gel.The gel has good homogeneity and consistency. The pH of the prepared gel was found to be 7.4 which lies in the normal pH range of skin and would not produce any skin irritation. The in vitro % cumulative drug release from the gel after 48 hrs was found to be 94%. So, the gel provides extended drug release.

PHYSICAL EVALUATION STUDIES

Consistency, homogeneity, pH, viscosity and spreadability of the prepared gel, validation and stability studies were determined as per the IP standard. Table 2: Physical evaluation

CONCLUSION

In this work, an attempt was made to formulate and evaluate a topical gel incorporated with nimesulide loaded magnetite nanoparticles. Drug loading over the magnetite was done by powder coating method using HPMC E5 polymer and found to have more drug loading efficiency and dissolution profile. Seal coating is given for the drug loaded magnetite particles with a hydrophobic ethyl cellulose polymer to prevent the solubility of drug in the gel. Seal coated drug loaded magnetite particles were incorporated into the gel and evaluated. Gel is found to have good homogeneity and consistency. The pH of the prepared gel is in the normal pH range of skin and would not produce any skin irritation and also it gives extended drug release.

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Table 1: Flow property of magnetite particles

Derived Properties		Flow Properties		
Bulk density (g/cc)	**Tapped density (g/cc)**	**Angle of repose**	**Carr's index**	**Hausner's ratio**
0.64±0.01	0.73±0.02	31.5±0.4	12.9±0.08	1.13±0.01

± - standard deviation, n = 3

Table 2: Physical Evaluation

Physical Evaluation	DLMNP incorporated gel	Stability study of gel
pH	7.4	7.1
Viscosity	1763.3±7.6 cp	1759.06±7.9cp
Spreadability	5.6±0.12 gcm/s	6.01±0.22 gcm/s
Consistency	Good	Good
Homogeneity	Good	Good

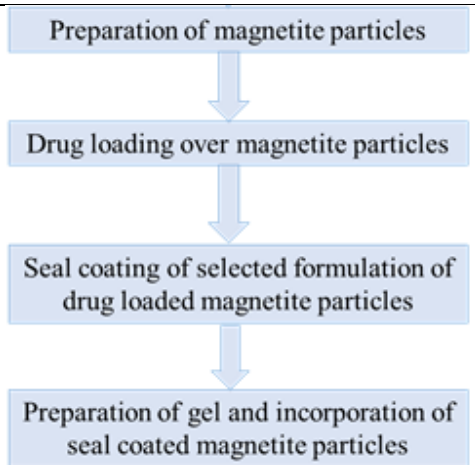


Figure 1: Overview of work

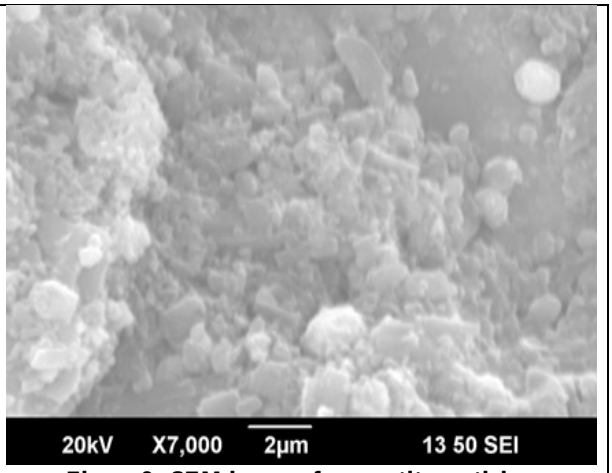


Figure 2: SEM image of magnetite particles

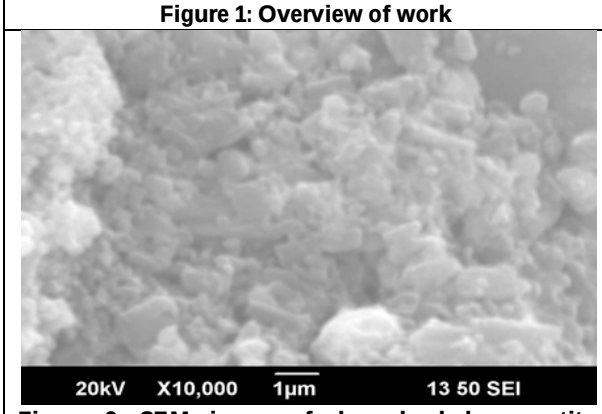


Figure 3: SEM image of drug loaded magnetite particles

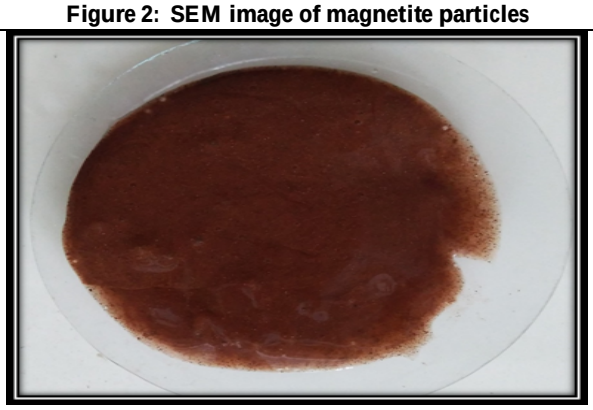
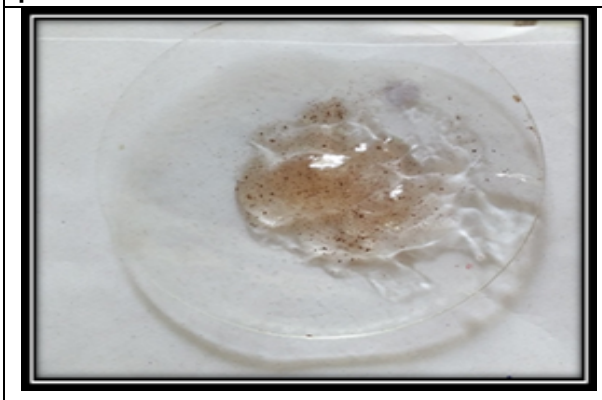


Figure 4: DLMNPs incorporated gel



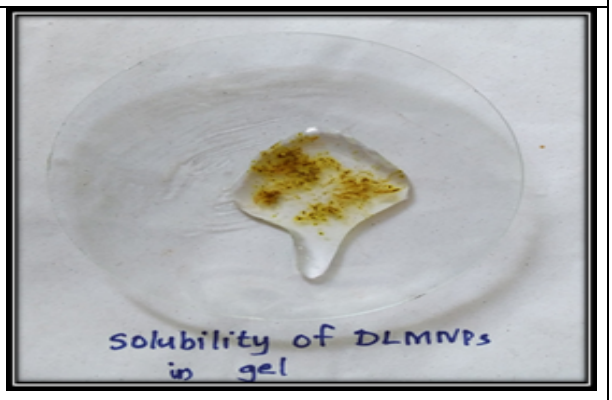


Figure 5: Solubility of 6% seal coated magnetite particles in the gel

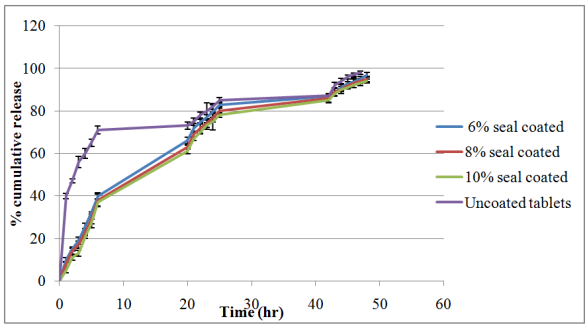


Figure 6: Comparison of dissolution data of Uncoated and seal coated magnetite particles

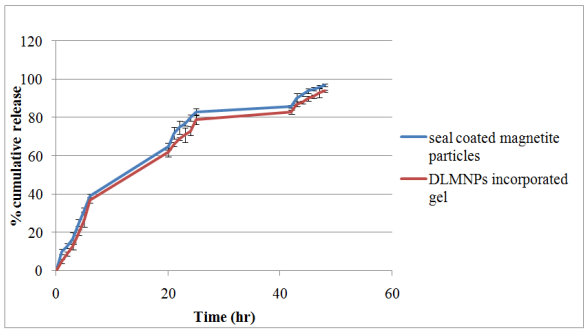


Figure 7: Dissolution data of seal coated magnetite particles and DLMNPs incorporated gel

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RESEARCH ARTICLE

Controlled Drug Delivery System

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ABSTRACT

Controlled release products are designed to maintain constant therapeutic plasma concentration of the drug within the therapeutic range of the drug over prolonged period and offer minimum side effects. This can be achieved using a variety of delivery systems .These products are designed to reduce the frequency of dosing by modifying the rate of drug absorption. The frequency of administration or the dosing interval of any drug depends upon its half-life or mean residence time (MRT).Generally controlled release products administered by any route are design such that rate of drug absorption should be equal to rate of drug elimination. Their are different types of controlled drug delivery system. The development or selection of system further depend up on the physicochemical and pharmacological properties of active pharmaceutical ingredient. One of the least complicated approaches to the manufacture of controlled release dosage forms involves the direct compression of blend of drug, retardant material and additives to formulate a tablet in which the drug is embedded in a matrix of the retardant. Matrix tablets is a promising approach for the establishment of extended release drug therapy as tablets offer the lowest cost approach to sustained and controlled release.

Keywords: controlled release, plasma concentration, frequency of dosing, Matrix tablets.

INTRODUCTION

Most conventional oral drug products, such as tablets and capsules, are formulated to release the active drug immediately after oral administration, to obtain rapid and complete systemic drug absorption. Such immediate-release products result in relatively complete systemic drug absorption and onset of accompanying pharmacodyanmic effects. However, after absorption of the drug from the dosage form is complete, plasma drug concentration decline according to the drugs pharmacokinetic profile[1]. Eventually, plasma drug concentrations fall

below the minimum effective plasma concentration (MEC), resulting in loss of therapeutic activity. Before this point is reached, another dose is usually given if a sustained therapeutic effect is desired. An alternative to administering another dose is to use a dosage from that will provide sustained drug release, and therefore maintain plasma drug concentrations, beyond what is typically seen using immediate-release dosage forms. In recent years, various modified-release drug products have been developed to control the release rate of the drug and / (or) the time for drug release [2].

Modified Drug Delivery System

Extended-Release Drug Products: A dosage form that allows at least a twofold reduction in dosage frequency as compared to that drug presented as an immediate-release (conventional) dosage form. Examples of extended-release dosage forms include controlled-release, sustained-release, and long acting drug products[3]. Delayed-Release Drug Products: A dosage form that releases a discrete portion or portions of drug at a time (or) at times other than promptly after administration, although one portion may be released promptly after administration. Enteric-coated dosage forms are the most common delayed-release products[4]. Targeted-Release Drug Products: A dosage forms that release drug at or near the intended physiologic site of action. Targeted-release dosage forms may either immediate (or) extended- release characteristics [5]. The term controlled-release drug product was previously used to describe various types of oral extended-release-rate dosage forms, including sustained-release, sustained action, long-action, slow-release, and programmed drug delivery [6].

Conventional Drug Delivery System Pharmaceutical products designed for oral delivery are mainly conventional drug delivery systems, which are designed for immediate release of drug for rapid (or) immediate absorption. Administration of the conventional dosage form by extra vascular route does not maintain the drug level in blood for an extended period of time. The short duration of action is due to the inability of conventional dosage form to control temporal delivery[7]. The conventional dosage forms like solutions, suspension, capsules, tablets and suppository etc. have some limitations such as:

- Drugs with short half-life require frequent administration, which increases chances of missing the dose of drug leading to poor patient compliance.
- A typical peak-valley plasma concentration-time profile is obtained which makes attainment of steady state condition difficult. The unavoidable fluctuations in the drug concentration may lead to under medication (or) overmedication as steady state concentration values fall (or) rise beyond the therapeutic range.
- The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index, whenever overdosing occurs.
- In order to overcome the drawbacks of conventional drug delivery systems, several technical advancements have led to the development of controlled drug delivery system that could revolutionize method of medication and provide a number of therapeutic benefits [8].

CONTROLLED RELEASE DRUG DELIVERY SYSTEMS (CRDDS)

Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. Localized drug action by spatial placement of a controlled release system adjacent to (or) in the diseased tissue. Targeted drug action by using carriers (or) chemical derivatives to deliver drug to a particular target cell type. Provide a physiologically / therapeutically based drug release system. In other words, the amount and the rate of drug release are determined by the physiological/ therapeutic needs of the body[9]. A controlled drug delivery system is usually designed to deliver the drug at particular rate. Safe and effective blood levels are maintained for a period as long as the system continues to delivery the drug. This predetermined rate of drug release is based on the desired therapeutic concentration and the drugs pharmacokinetics [10].

ADVANTAGES OF CONTROLLED RELEASE DOSAGE FORMS[11]

- Avoid patient compliance problems
- Employ less total drug
- Minimization or elimination of local or systemic side effects.
- Minimal drug accumulation on chronic usage.
- Improve efficiency of treatment.
- Cure or control the condition more promptly
- Reduce the fluctuation in drug level.
- Improves the bioavailability of some drugs.
- Make use of special effects

DISADVANTAGES OF CONTROLLED RELEASE DRUG DELIVERY SYSTEMS

- Decreased systemic availability in comparison to immediate release conventional dosage forms, which may be due to incomplete release, increased first-pass metabolism, increased instability, insufficient residence time for complete release, site specific absorption, pH dependent stability etc.
- Poor In vitro – In vivo correlation.
- Retrieval of drug is difficult in case of toxicity, poisoning (or) hypersentivity reactions. Reduced potential for dose adjustment of drugs normally administered in varying strength[12-13].

ORAL DRUG DELIVERY SYSTEM

Oral route is one of the most extensively used routes of drug administration because of its obvious advantages of ease of administration, improved patient compliance and convenience. By definition oral controlled release products refer to those formulations in which a “controlling technology or component” is incorporated that is critical to modulate the drug release pattern in a predictable fashion or that controls the timing and subsequently the location of drug release within GIT[14]. All the pharmaceutical products formulated for systemic delivery via oral route of administration, irrespective of the mode of delivery – (immediate, sustained or controlled release) and the design of dosage forms (either solid, liquid or dispersion) must be developed within the intrinsic characters of GI physiology. Therefore a fundamental understanding of various disciplines including GI physiology, pharmacokinetics, pharmacodynamics and formulation design, is essential to achieve a systemic approach to the successful development of an oral pharmaceutical dosage form[15].

1.Physiochemical, pharmacokinetic and pharmacodynamic characteristic of the drug.
2. The anatomic and physiologic characters of GIT (surface area, length and transit time).
3. Physiochemical characteristics and drug delivery mode of dosage form design. Oral controlled release drug delivery is a drug delivery system that provides the continuous oral delivery of drugs at predictable and reproducible kinetics for a predetermined period throughout the course of GI transit[16].

Areas of potential [17]

- Development of a drug delivery¬ system.
- Modulation of GI transit time.¬
- Minimization of hepatic first pass¬ elimination.

Factor Influencing The Design And Performance of Controlled Drug Delivery System

Biopharmaceutic characteristic of the drug [18]

a) Molecular weight of the drug
b) Aqueous solubility of the drug
c) Apparent partition coefficient
d) Drug pKa and ionization physiological PH
e) Drug stability
f) Mechanism and site of absorption
g) Route of administration.

Pharmacokinetic characteristic of the drug [19]

a) Absorption rate
b) Elimination half life
c) Rate of metabolism
d) Dosage form index

Pharmacodynamic characteristic of the drug [20]

a) Therapeutic range
b) Therapeutic index
c) Plasma–concentration–response relationship

Molecular weight of the drug

Lower the molecular weight, faster and more complete the absorption. About 95% of the drugs are absorbed by passive diffusion. Diffusivity is defined as the ability of a drug to diffuse through the membrane is inversely related to the molecular size. Thus drugs with large molecular weight are poor candidates for oral controlled release systems[21].

Aqueous solubility of the drug

A drug with good aqueous solubility, especially if pH independent, serves as a good candidate for oral controlled release dosage form. Solubility of drug can limit the choice of mechanism to be employed for CRDDS, for example the diffusion systems are not suitable for poorly soluble drugs. Absorption of poorly soluble drugs is dissolution rate-limited hence control release device does not control the absorption process, so they are poor candidates[22].

Apparent partition coefficient

Greater the apparent partition coefficient of a drug, greater its lipophilicity and thus greater is its rate and extend of absorption. These types of drugs even cross the highly selective blood brain barrier. This parameter is also important in deciding the release rate of a drug from lipophilic matrix or device[23].

Drug pKa and ionization at physiological pH

For optimum passive absorption, the drugs should be nonionised at that site for an extend of 0.1-5%. Drugs that are exixting largely in ionosed forms are poor candidates for controlled delivery systems eg: hexamethonium[24].

Drug stability

Drugs that are unstable in the GI environment are not suitable candidates for controlled release systems. Drugs that are unstable in gastric pH can be designed to release in intestine with limited or no release in stomach and vice versa.

Mechanism and site of absorption

Drugs that are absorbed by carrier mediated transport process or through a window are poor candidates for controlled release systems,eg: Vitamin B.

Route of administration

For controlled release oral and parenteral routes are the most preffered which is the followed by transdermal[25].

Oral route

- the drug should have following properties to be a succesfull candidate
- It must get absorbed through the¬ entire length of GIT.
- Main limitation is transit time (mean of¬ 14 hours), which can be extended for 12-24 hours.
- Dose as high as 1000mg can be given¬ through this route.
- Intramuscular/subcutaneous route:¬
- This route is preferred because The action is to be prolonged for 24¬ hours to 12 months.
- Small amount of drug is administered¬ (2ml/2gm).
- Factors important are solubility of drug¬ in surrounding tissue, molecular weight, partition coefficient and pKa of drug[26].

Transdermal route

This route is selected for drugs which show extensive first pass metabolism upon oral administration or drugs with low dose. Important factors to be considered are partition coefficient of drugs, contact area, skin condition, skin permeability of drug, skin perfusion rate, etc[27].

PHARMACOKINETIC CHARACTERISTIC OF A DRUG

Absorption rate

A drug which is fabricated into a controlled release system its absorption must be efficient since the desired rate limiting step is rate of drug release. A drug with slow absorption is is a poor candidate for such dosage forms, as continuous release will result in a pool of unabsorbed drug. If a drug is absorbed by active transport, or transport is limited to a specific region of intestine, sustained-release preparations may be disadvantageous to absorption[28].

Metabolism

Drug selected for controlled release system should be completely metabolized but the rate of metabolism should not be too rapid. A drug which induces and inhibits metabolism is a poor candidate because steady states are difficult to achieve [29].

Drug-Protein Binding

The drug can bind to components like blood cells and plasma proteins and also to tissue proteins and macromolecules. Drug protein binding is a reversible process. As the free drug concentration in the blood decreases, the drug-protein complex dissociates to liberate the free drug and maintain equilibrium. A protein bound drug due to its high molecular size is unable to enter into hepatocytes, resulting in reduced metabolism. The bound drug is not available as a substrate for liver enzymes there by further reducing the rate of metabolism. The glomerular capillaries do not permit the passage of plasma-protein and drug protein complexes. Hence only unbound drug is eliminated. The elimination half-life of drugs generally increases when the percent of bound drug to plasma increases. Such drugs need not be formulated into sustained/controlled release formulations[30].

PHARMACODYNAMIC CHARACTERISTICS OF THE DRUG

Therapeutic range

A candidate drug for controlled release drug delivery system should have a therapeutic range wide enough such that variations in the release rate do not result in concenteration beyond this level[31].

Therapeutic index

It is most widely used to measure the margin of safety of a drug. TI = TD50 /ED50 .The longer the value of T.I the safer is the drug. Drugs with very small value of Therapeutic index are poor candidates for formulation into sustained release products. A drug is considered to be safe if its T.I value is greater than 10 [32].

CONCLUSION

Oral Sustained release (SR) products provide an advantage over conventional dosage forms by optimizing bio-pharmaceutic, pharmacokinetic and pharmacodynamic properties of drugs in such a way that it reduces dosing frequency to an extent that once daily dose is sufficient for therapeutic management through uniform plasma concentration providing maximum utility of drug with reduction in local and systemic side effects and cure or control condition in shortest possible time by smallest quantity of drug to assure greater patient compliance.

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RESEARCH ARTICLE

Taxonomic Diversity of the Weed Medicinal Flora used by Aboriginal People of Purulia District (W.B) India

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ABSTRACT

Most of the weeds are obnoxious and nuisance especially in the manmade desired productive ecosystem. But, the number of their plants as the pioneers of the secondary succession has been used by the people for the sake of the health and hygienic practices. The recent investigations suggest the impoundable importance of such entity due to its treasure of therapeutic value as repository of the secondary metabolites. The traditional knowledge system of the medicine reared by the tribal people has been acknowledged the weeds as a means of their usual health and hygienic practices. Purulia, the western most district of W.B, India is the treasure of the biodiversity along with the rich heritage of the aboriginal people habituated to use the plants in general and weeds in particular for the health and hygienic practices. The present paper mainly deals with the 60 species (Magnoliopsida and Liliopsida) of different genera belonging to different families having different phylogenetic distribution as per conventional point of view. This paper also tries to explore the weed diversity as per the APG IV classification along with their taxonomic attributes.

Keywords: APG IV, ITK, Medicinal Plants, Taxonomy, Therapeutics, Weeds.

INTRODUCTION

The tribal communities rear a treasure of indigenous traditional knowledge as far as their cultural aspects including food, health and hygienic practices. It is the outcome of the immemorial association of the bonding with the aboriginal people and the flora and fauna. The verbal and the non-documents precious knowledge is being transmitted since the time immemorial as a part of the socialization within the cultural and household contexts. This knowledge domain remains unexplored for a long period of time due to a fear of loss of traditional knowledge by the hands of the highly cultured mainstream refined civil society. But at the onset of the 21st century, the mindset of the aboriginal people has been subjected to change and the real stakeholders of this knowledge have been open due to change of the different socio-economic parameters in this regard. The documentation of the traditional knowledge is the call of the time before it is being lost to posterity and also to preserve the knowledge for the best of the future endeavour. The execution of the unplanned anthropogenic activities to meet up the sky skipping greed of the modern people has expedited the degradation and destruction of the ecosystem which has resulted the serious ecological imbalance and the degradation of the biodiversity. Not only that, the weed flora is also important for ensuring the productivity as it mitigates the reproductive biology of the desired standing crop plants. Therefore, for the sake of the productivity of the standing crop along with to keep up the ecological balance and the conservation of the biodiversity, the ethno botanical documentation can become a good avenue to conserve the traditional knowledge and to mitigate the needs of the different bioactive compounds required right now particularly at the age of the antimicrobial resistance of the different microbes against the traditional use of the antibiotics.

MATERIALS AND METHODS

Survey Area

The present paper deals with the enumeration of the diverse type of weeds used by the tribal people in the traditional health care system as a part of their traditional indigenous knowledge system. Purulia, one of the grand rocks of the world lies in the western most of the West Bengal and it shares the interstate boundary of Bokaro, West Singhbhum, East Singhbhum, Ranchi, Dhanbad district of neighbouring Jharkhand and the intra state boundary of the district of West Midnapore, Jhargram, Bankura and West Burdwan. It lays in between 23°42´ north 22°43´ south as far as longitudes and 86°54´ east to 85°49´ west as far as latitude is concerned. The total population is 29, 27, and 965 having the density of 468/ per sq km and the rural population is 87.3%. The S.T population is 5, 40,652 in which is about 18.45% of the total population and them, among the male is 2, 71,803 and female is 2, 68,849. The soil is mostly residual soil formed by the weathering of the bed rocks and the high temperature prevails during the summer having the relative humidity is 25-35% and the cool winter. The rainfall (Average) is 1100-1500 mm with 75-85% relative humidity. The economy is mainly catalysed by the agriculture, tourism and some industrial outcomes.

The tribal population mainly derived from Proto Australoid group and they mostly speak in Santhali, Gonda, and Kheria language. Out of the existing 20 tribal groups, Santhal are the major group contributing 60% of the total tribal population followed by Bhumij (19%), Sabar (7%), Munda (6%) and Birhors (1%). They used to enjoy different ecology, occupation as well as life style. After having an extensive literature survey, on the basis of ethno medicinal and floristic during the field trips, first a number of informants were selected and a questionnaire was distributed among the informants for the sake of the data collection. The methodology was mainly adopted on the basis of The Manual of Ethnobotany by S.k.Jain. The data collected from the informants were recorded in a data sheet for the further interpretation. The collected plant species as per the information available from the dependable sources were identified with the help of the different floras and standard literature. Very often the different apps from the web browser were also consulted to make it more scientific. The herbariums of the collected specimens have kept for further uses and the preservation has been done on the basis of conventional techniques (Jain & Rao, 1977).

Study Area Image I

Data Collection

The ethnobotanical study was carried out in the different seasons during 2017-19. The weeds collected mainly from the barren and non-cultivated land and brought to the laboratory. The field collection was done on the basis of the of the knowledge of the aboriginal people, aged rural folks, traditional medicinal men, the local herbal practitioners and the different informants as recruited from time to time to explore the data from the ground sources via the supply of the questionnaire maintaining a standard protocol as explored from the standard literature as referring the literature work by S. K. Jain on the Manual of Ethnobotany (2nd Revised Edition, 2010).The data has been collected using number of questionnaire in a printed fro supplied to the informant deployed to have the data regarding the use of the plants having therapeutic value in this regard. The questionnaire comprising of the following information-general information including the name of key informants, name of the person obtained the plant, local correspondence, ecological parameters, botanical investigation, pharmacological; investigation includes traditional values, time of collection of plant parts, storage, dosage forms used either monotypic or polytypic nature, method of use either external or internal, information on genuine therapeutic activity, any other information or observations, identification of the plants along with the person identifying the plant in this regard for authentication about the botanical knowhow..All sorts of the data were observed with degree of accuracy to explore the validation of the data as the plants use in this regard. Study area has been shown as image 1

WEEDS ENUMERATION: Table I

Ethnobotany is a field oriented study involving direct relationship of aboriginals with the surrounding plants. Field study in the tribal area gives first hand information where the ethno botanists apart from the collection of the plants, also discusses and records the uses of the plant with the help of the local informants. Great patience and the perseverance are indeed required to explore the true data in this regard. Familiarity with the local language, dialect is not only required to explore the data but also the sentiment is an important attributes in this endeavour. The different type of other issues like mythic stories and motifs are also addressed in this herculean tasks. The primitive or the ethnic populations have their own medical lore and some of the therapeutic practices have tremendous importance in the present context. In course of the investigation, it has been found that the same plant used by the different tribal groups in the different ways. The different weeds are basically attached with the different culture and rituals of the different ethnic groups but unfortunately, as part of the globalization and market economy, the continuous erosion of the rich and intangible culture are now at the verge of erosion. At the present context, this has become a nuisance to every nature lovers who are pondering over the culture and taboos of the aboriginal people. So, it is the high time for us to document the indigenous traditional knowledge as reared by the tribal group as a part of their social-economic attributes. It will also become helpful to make an inventory of the ITK of this district. Utmost care and deep respect to the verbal and non-documented knowledge were given paramount importance in course of the exploration for the conservation of the knowledge both in the physical forms and biological point of view.

TAXONOMIC DIVERSITY

The weeds observed and collected to use as medicinal plants having information directly and indirectly has been justified in the light of the modern taxonomic approaches by the APG IV system of Angiosperm phylogeny classification. The APG IV system of flowering plant classification as far as phylogeny is concerned mostly on the basis of molecular data and tit is an approach to find out whether the group of plants having therapeutic value offers any indication of relationship in this regard. The APG IV was published in 2016, seven years after its predecessor of the APG III system published in 2009 compared to APG III system, APG IV system recognises five new orders---Boraginalleles, Dilleniales, Icacinales, Mettuensiales, & Vahiales along with some new families making a total of 64 angiosperm orders and 416 families. This system is mainly based on monophyly discarding parphylya & synthesis of information from the different domain of knowledge of monophyly has been established. The total 60 weeds having ethnomedicinal values explored has been arranged as per the following to find out any homology as far as molecular data is concerned that offers a great degree of significance in this regard to give a concluding remarks in this regard.

As the APG IV classification of the flowering plants has been done on the basis of the molecular data, a number of families have been merged and the closely associated families enjoy a degree of relationship in this regard also reflect as far as the ethno medicinal properties is concerned. The plants on the basis of the phylogeny have been tabulated as per the following. The maximum number of the weeds having therapeutic values appeared from the family Fabaceae of 11.6% , Amaranthaceae of 10%, Asteraeae, Poaceae and Euphorbiaceae of 8.3% in each, Malvaceae 6.6% followed by Apocynaceae & Lamiaceae 3% in each followed by the rest of the members of the other families. Out of the total 60 plants species of different families, the species has been distributed in the following orders as per as the APG IV system is concerned. The highest in this regard is the members of the family Fabaceae followed by Amaranthaeae, Apocynaceae, Verbenaceae and the highest among them is the members of the family Asteraceae.

Chart I and II

Parts of the medicinal plants used

Aboriginal People of Purulia district harvest the different plant parts for the preparation of the indigenous traditional medicines (e.g. root, stem, leaf, flowers, fruits, seeds, rhizomes, whole plant etc) on the basis of the plant part value (PPV) index, leaf was found to be the dominant plant part for the preparation and the extraction of the active principles followed by whole plants, flowers, stem, seeds respectively. The leaf was used as the most dominant used part in this regard due to its easy availability, simplicity of the ready preparation followed by the quick and proper identification by the virtue of its biological property. Moreover, as the most of the secondary metabolites are the outcome of primary metabolic pathways like photosynthesis so, the leaf parts become dominant in this regard as the most used parts as far as the therapeutic value is concerned.

Pie chart I

Methods of remedy preparation

Active principles are the substances having phytomedicinal attributes and in order to facilitate the proper administration to have the potential use of the active principles, several methods are employed for the preparation of infusion, decoction, maceration, fumigation, and inhalation, cooking etc are employed to do the same. Out of the 60 medicinal weeds so far consideration is done in this regard, the majority of the remedies were prepared from the infusion followed by extraction and food supplements. The percentage of the other methods out of the three as stated below does not exceed a respectable limit. The infusion occupied the topmost order in this regard due to its property to collect the most active ingredients and to cancel the candidates out the toxic effect of the certain type of medication. As far as the literature is concerned in this regard, the infusion occupies the most outstanding result in this aspect.

Routes of administration

Herbal medicines acknowledge the routes of administration of the plant parts and it depends on the disease and the plant parts used. In general, most of the administration is done orally followed by message, rinsing or other traditional practices. The predominance of the administration is mostly orally and it is due to the high incidence of the internal ailments in the region. It is also thought that the most of the administration due to the non-toxic side of the bioactive compounds. The predominance of the oral administration of the different medicinal plants of the Purulia district is at par with the most carried out ethno botanical studies of the elsewhere region.

CONCLUSION

The ethnobotanical and Indigenous traditional knowledge in connection with the ethno pharmacological surveys reveal that the area of this study has a unique opposition as far as biodiversity is concerned due to presence of a variety of medicinal plants as far weeds are concerned. The weeds are mainly consulted which are exclusively found to occur in the barren land not on the crop field weeds. The rich flora indicates a high degree of the potential of

indigenous traditional knowledge to serve as a good substitute of the main stream healthcare practices. The distribution of the weeds found to occur from very ancient and primitive family like Araceae to highly developed family like Asteraceae. But a good number of plants species have been observed in the families like Fabaceae, Euphorbiaceae and Amaranthaceae. As far as PPIV index is concerned, the highest value appears from the leaf due to easy accessibility and smooth uses. On the basis of the result of the present studies, the recorded medicinal plant species would empower the future phytochemical screening to obtain the higher number of species in this regard. It also indicates the rich treasure of ITK and biodiversity knowledge of the aboriginal people in this locality. The wide diversity of uses of the plant species has been reflected in their other cultural practices. In this connection attention is desired to the loss of biodiversity due to different ongoing destruction of plant species for the sake of industrialization and the urban settlement. As a result, the genetic erosion can be minimised and the sustainable development can be ensured as a good substitute of modern practices of medicines to promote the green medicines to minimise the side toxic effect of costly medical protocol.

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Table 1: *Weeds Enumeration*

Sl. No.	Local Name	Botanical Name	Family	Parts Used	Ailments	Nature of Uses
1	Potari	*Abutilon indicum (L.) Sw*	Malvaceae	Leaf	Whole plant	Oral consumption as powder
2	Dadmari	*Ammannia baccifera L.*	Lythraceae	leaf	Burning	Paste of the leaf
3	Bethua	*Chenopodium album*	Amaaranthaceae	Leaves	Dysentery	Vegetables as food supplements
4	Bhabri	*Croton bonplandianumBaill.*	Euphorbiaceae	leaves	Blood clotting	Leaves extracts and latex
5.	Brahmi	*Bacopa monnieri (L.) Wettst.*	Scrophulariaceae/ Plantaginaceae	Whole plant	For rejuvenation and intelligence	Food supplement
6.	Dubbaghas	*Cynodon dactylon (L.) Pers.*	Poaceae	Leaf	Heart ailments and eye care	Food supplement
7.	Kesut	*Eclipta alba (L.) Hassk.*	Asteraceae	Leaves	Ski diseases	Fresh leaves with oil
8.	Kulata	*Astracanthus longifolia (L.) Nees.*	Acanthaceae	leaves	Blood purifier	Food supplements
9.	Kansira	*Commelina nudiflora Linn.*	Commeliniaceae	Whole plant	Skin disease	Food supplement
10.	Nuniasak	*Portulaca oleracea Linn.*	Portulaceae	Whole plant	Skin vitaliser	Food supplement
11.	Apang	*Achyranthes aspera Linn.*	Amaranthaceae	root	Urinary disorders	Oral admins.
12.	Ghetu	*Clerodendrum viscosum Linn.*	Verbenaceae	root	Blood dysentery	Decoxation orally
13	zhunzhuni	*Crotalaria prostrate Roxb.*	Fabaceae	Root	Swelling of body parts	Decoxation orally
14.	Soya lata	*Ichnocarpus frutescens (L.) R.Br.*	Apocynaceae	Root bark	Urinary problem	Decoxation orally
15.	Bheron	*Ricinus communis L.*	Euphorbiaceae	Petiole	Aerotitis and barotitis	Fumigants
16.	Sannalata	*Cuscuta reflexa Roxb.*	Cuscutaceae	Plant	Jaundice, Diabetes & cough	Decoxation
17.	Kundru	*Coccinia grandis (L.)*	Cucurbitaceae	fruit	Menstrual disorder	Food supplement
18.	Chiknishak	*Polygonum plabeium R. Br.*	Polygonaceae	Whole plant	Digestive stimulant	Food supplement

19.	Nuniashak	*Portulaca oleracea L.*	Portulacaceae	Whole plant	Dysentery and stomach disorders	Food supplement
20	Halkusa	*Leucas aspera (Wild.) Link.*	Lamiaceae	Leaves	Cough and cold	Decoxation as tonic orally
21.	Salantisak	*Alternanthera sessilis (L.)R.Br.ex.Dc*	Amaranthaceae	leaves	Asthma, Bronchitis	Decoxation orally
22.	Khudi note	*Amaranthus viridis L.*	Amaranthaceae	Leaves	Anti-microbial and anti-helminnthic	orally
23.	Dudhiya	*Euphorbia hirta Linn.*	Euphorbiaceae	Leaves	Asthma	Decoxation orally
24.	Kakmachi	*Solanum nigram Linn.*	Solanaceae	Fruits & leaves	Psoriasis &haemoridis	orally
25.	Dochanti	*Tridax procumbens Linn.*	Asteraceae	Leaf	Anti-fungal & insect repellent, Liver disorders	Leaf extracts orally
26.	Sahadevi	*Cyanthillium cinera (Linn.)H.Rob*	Asteraceae	Whole plant	Antibiotic and anti-helminthic	Leaf extracts
27.	Chanchi	*Gomphrena celosioides Mart.*	Amaranthaceae	Whole plant	Antimicrobial	Leaf extracts
28.	Sialkanta	*Argemone mexicana Linn.*	Papaveraceae	whole	Sin disorders and eye treatments	Decoxation juice
29.	Dochanti	*Ageretum conyzoides Linn.*	Asteraceae	leaves	antimicrobial	Decoxation
30.	Bon methi	*Melilotus indica Mill.*	Fabaceae	leaves	Antioxidant	juice
31.	Bon tepari	*Physalis minima Linn.*	Solanaceae	fruit	Analgesic	Leaf extract
32.	Bon labanga	*Ludwigia perennis Roxb.*	Onagraceae	fruit	Intestinal disorders	
33.	Kalkasunda	*Cassia tora Linn.*	Papillionaceae	leaves	Intestinal disorders	As juice mixed with lime
34.	Sialmutra	*Blumea lacera (Burm. F) DC.*	Asteraceae	Leaves	Wounds and blisters	Ointment
35.	Akanda	*Calotropis gigantean (L.)Dryand*	Apocynaceae	flowers	Elephantiasis	As ointment with admixture
36.	Begna	*Vitex negundo Linn.*	Lamiaceae	Leaves and flowers	Expectorant and tonic	Women's health and insecticide
37.	Bon nil	*Tephrosia purpurea (Linn.) Pers.*	Papillionaceae	Leaves and flowers	diarrhoea	Tonic and appetizers
38.	Jhatitulsi	*Leonotis nepetifolia (Linn.)*	Lamiaceae	Leaves	Stomach pain	Decoxation
39.	Bherenda	*Jatropha curcas (Linn.)R.Br.*	Euphorbiaceae	Leaves and flowers	Pain joints	As ointment
40.	Barela	*Sida cordifolia L.*	Malvaceae	Leaves and flowers	Healing of sores	Externally use

41.	Salpani	*Desmodium triflorum (Linn.) DC.*	Papillionaceae	Whole plant	Anti-inflammatory	extract
42	ChetraSak	*Dentella repens (Linn.) J.R.Forst& G.Forst*	Rubiaceae	leaf	sore	Extract
43.	Taramoni	*Lemna minor L.*	Araceae	whole	Animal fodder	leaf
41.	Bena/khaskhas	*Vetiveria zizainioides (Linn.) Nash.*	Poaceae	leaves	Antihelmintheic	Orally
42.	Durba	*Cynodon dactylon (Linn.)*	Poaceae	Leaves	Healing of wounds	Antimicrobial
43.	Boch	*Digitaria sanguinalis (L.)Scop.*	Poaceae	grains	Food supplements	Fruit
44.	Burashyma	*Echinochloa colona(L) link*	Poaceae	seeds	Food supplements	Seed
45.	Muthaghas	*Cyperus rotundus L.*	Cyperaceae	rhizome	fevers	Orally
46.	Bhabri	*Croton bonplandianum*	Euphorbiaceae	Latex	Blood clotting	Latex
47.	jhanti	*Barleria prionitis L.*	Acanthaceae	Leaves	Whooping cough	Leaf extracts
48.	Kamraj	*Byttneria harbacea Roxb.*	Malvaceae	Leaves	Food supplements	Leaf extracts
49.	kalimusli	*Curculigo orchioides Gaertn.*	Hypoxidaceae	Whole plant	Antidiabetic	Extracts
50.	Dadrumardan	*Senna alata (L.) Roxb.*	Fabaceae	leaves	antifungal	Extracts
51.	MorogJhuti	*Celosia argentea Linn.*	Amaranthaceae	leaves	Food supplement	food
52.	Ban-ochara	*Urena lobata Linn.*	Malvaceae	Leaves	Antifungal	Extracts
53.	Atasi	*Crotalaria pallida Aiton.*	Fabaceae	Leaves	Food adjuncts	Leaf
54.	Amrul	*Oxalis corniculata Linn.*	Oxalidaceae	Leaves	Food adjuncts	Leaf
55.	Chotonayantara	*Catharanthus roseus (L.) G.Don.*	Apocynaceae	leaves	Healing of wound	Extracts
56.	Lajjabati	*Mimosa pudica Linn.*	Fabaceae	Root	Leprosy and Venereal diseases	Root extracts
57.	Gimmasak	*Glinus oppositifolius*	Molluginaceae	Leaves	Anti-malarial	Extracts
58.	Hincha	*Enhydra fluctuans, Lour*	Asteraceae	Leaves	Appetite & digestion	Edible
59.	Kulekhara	*Hygrophila auriculata, Heine*	Acantahaceae	Fresh leaves	Anemia & Hair care	Edible
60.	Badnali	Lobelia alsinoides Lam.	Campanulaceae	Whole plant	Stomach disorders	Edible
Source: Field visit, Informants & other secondary sources						

Tale 2. Plants as per Family

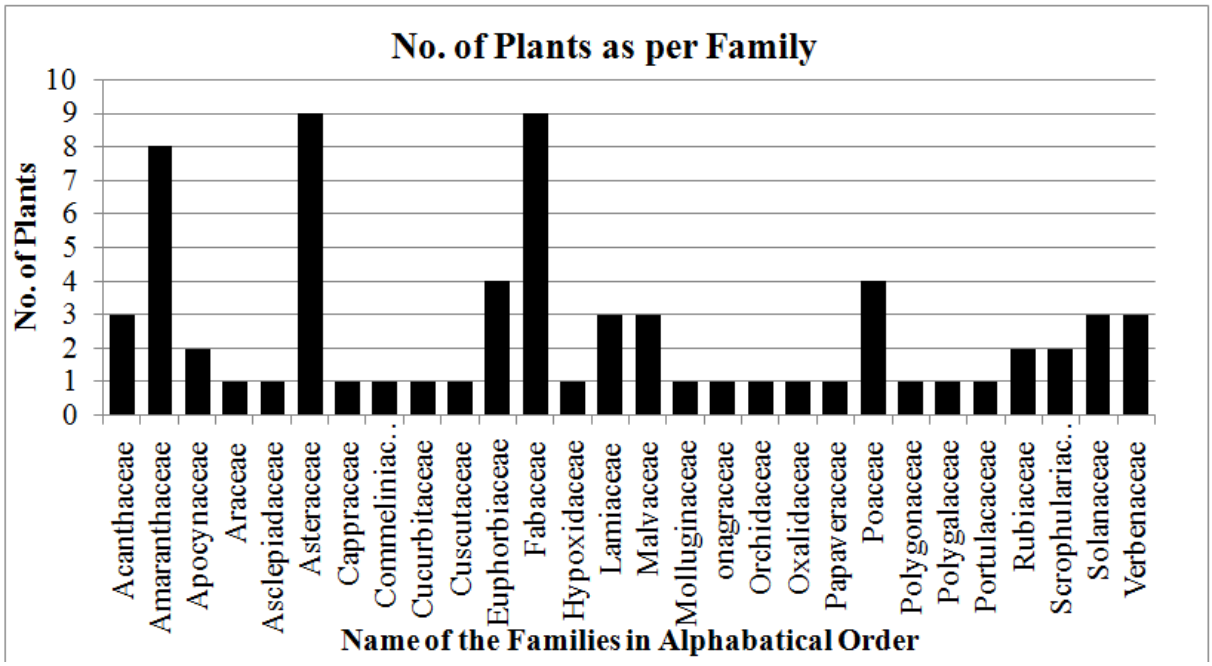

Figure 1: Study Area of Purulia District

Figure 2: *Cuscuta reflexa* Roxb. Convolvulaceae.

Figure 3: *Argemone mexicana* L. Papaveraceae

Figure 4: *Celosia argentea* L. Amaranthaceae

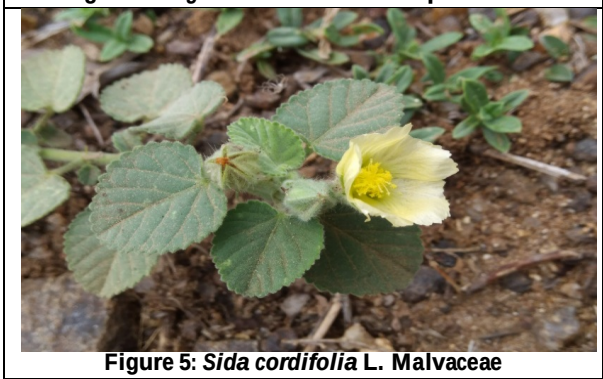

Figure 5: *Sida cordifolia* L. Malvaceae

Figure 6: *Ageretum conyzoides* L. Asteraceae

Figure 7: *Crotalaria pallida* Aiton. Fabaceae

Figure 8: *Leucas aspera* (Wild.) Link Lamiaceae

Figure 9: Author during collection

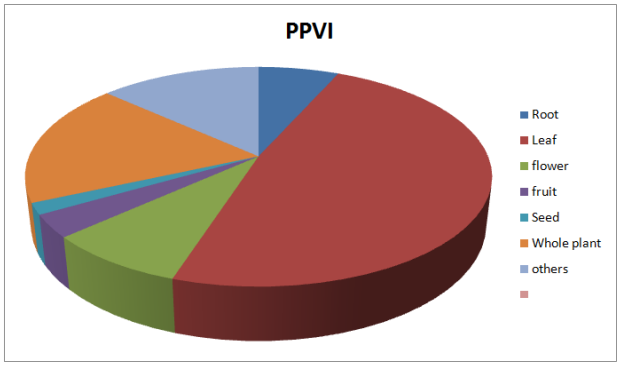

Figure 10. Plant Parts Value Index (PPVI)

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REVIEW ARTICLE

Review Article of Proteins, Peptides and Macromolecules Drug Delivery System

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ABSTRACT

The article provides a structure of proteins, and classification of proteins. And the various routes of administration for proteins and peptides drugs are Parenteral and Non parenteral systemic delivery system,(i.e. Oral route, Nasal route, Buccal route, Ocular route, Rectal route, Transdermal route, Pulmonary route), Development of delivery system for proteins and peptides based in pharmaceuticals, and used in Pharmaceutical approaches, application of drugs acting sites and the transport mechanism of macromolecules, Macromolecular conjugation and toxicity and safety of proteins therapeutics.

Keyword: Proteins, Routes of administration, Pharmaceutical approaches, Transport of Macromolecules

INTRODUCTION

Proteins and peptides are the building blocks of life and are now evolving as a very promising brand therapeutic entities. Proteins have increased dramatically in number and frequency of use the introduction of first recombinant protein therapeutic and human insulin were found before 25 years ago. Therapeutic proteins and peptides hold a chief role in all field of medicine, but this role is still only in its infancy. The foundation for the popularity of protein therapeutics was laid down with the regulatory approval of recombinant insulin by the US Food and Drug Administration (FDA) in 1982.A better understanding of molecular biology and biochemistry behind the macromolecular endogenous proteins, peptides and Peptidergic molecules, and their role in various body functions and pathological conditions has led to the realization of the huge therapeutic potential of proteins and peptides in the last few decades. Consequently, a variety of new therapeutic proteins have been developed showing therapeutic benefits in the treatment of ailments like diabetes cancer which offer several advantages over the conventional small-molecule drugs. Firstly, proteins often serve a highly specific and complex set of functions in the body that cannot be

mimicked by simple chemical compounds. Secondly, since the action of proteins is highly specific, there is often less potential for therapeutic protein to interfere with normal biological process and cause adverse effects. Thirdly, because the body naturally produces many of the proteins that are used for therapeutic purpose, these agents are often well-tolerated and are less likely to elicit immune responses. For the diseases in which a gene is mutated or deleted protein therapeutics can provide an effective replacement for the treatment without the need for gene therapy, which is not currently available for most genetic disorders. As a result of research efforts in both academic and industrial laboratories recombinant DNA, protein and peptide engineering and tissue culture techniques can now be used to obtain proteins and peptides for therapeutic use on a commercial scale which resemble an endogenous molecule and thus evoke fewer or minimal immunological responses. Though the initial problems related to obtaining non-immunogenic protein therapeutics in purer form at commercial scales have been overcome to quite some extent, their formulation and optimum delivery still remains the biggest challenge to pharmaceutical scientists[1].

Scientific advances in molecular and cell biology have resulted in the development of two new biotechnologies. The first utilizes Recombinant DNA to produce protein products, The second technology is Hybridoma Technology various proteins and peptides drugs are epidermal growth factor tissue plasminogen activator. Management of illness through medication is entering a new era in which a growing number of biotechnology produced peptide and protein drugs are available for the therapeutic use. Ailments that can be treated effectively by this new class of therapeutic agents includes cancers memory impairment mental disorders hypertension. Proteins and peptide drugs are therapeutically effective only by parental route repeated injections are required therapeutic applications of these drugs reply on successful development of viable delivery system to improve their stability bioavailability. It involves oral route transdermal route nasal pulmonary rectal vaginal. Challenges are large molecular size susceptibility to enzyme degradation short plasma half life ion permeability immunogenicity aggregation denaturation. Absorption of protein 90% of nutrient absorb in small intestine, Absorption is limited by acidic environment action of enzyme non absorptive nature of epithelial. Through Paracellular and trans-cellular mechanism they absorbed in blood and lymp[2].

Structure of proteins

The proteins are large molecules composite architecture. The peptide & protein are seldom linear & adapt a variety of specific folded three dimensional patterns & conformation. The Structure of a protein is straight related to its function, so that anything that severely distort the shape will also disrupt the function. There are classified into four types of protein structure.

1. Primary structure
2. Secondary structure
3. Tertiary structure
4. Quaternary structure

Classification of proteins

- functions
- location in living
- post translation modification

Classification by functions of proteins

- Enzymes : DNA and RNA polymerase
- Hormones : Endorphine and enkephalin.
- Transport proteins : Cytochrome C, Albumin, Haemoglobin.
- Antibodies : Interferon, Fibrin.
- Structural proteins : Collagen, Elastin.
- Motor proteins : Actin, Myosin.

- Recptors : Transmembrane proteins.
- Signalling proteins : GTPase.
- Storage proteins : Egg ovalbumin, milk casein.

Classification of proteins by location in the living cell

- Membrane proteins
- Internal proteins
- External proteins
- Virus proteins

Classification of proteins by posttranslational modification

- Native protein
- Glico protein
- Cleaved protein
- Protein with disulfide bonds
- Protein complexes
- Chemically modified proteins
- Prions

A protein drug has to face with a number of lipophilic and hydrophilic barriers to cross. The drug must first dissolve in the contents of the intestinal lumen if it is not already in solution; then there is a mucus layer and a water layer protecting the surface of the epithelial cells. The protein or peptide drug must have sufficient water - and lipid-solubility to pass through these layers. The epithelial tissue represents the next barrier. There are cases where a protein can be absorbed in to these cells by endocytosis, and then transported to the basement membrane on its way to the capillaries.

Various Routes of administration for protein or peptide drugs:

(1) Parenteral systemic delivery:
(2) Non-parenteral systemic delivery:
a. Oral route
b. Nasal route
c. Buccal route
d. Ocular route
e. Rectal route
f. Transdermal route
g. Pulmonary route

Parenteral systemic delivery

For the systemic delivery of therapeutic peptides and proteins, parenteral administration is currently believed to be the most efficient route and also the delivery method of choice to achieve therapeutic activity. Parenteral delivery consist of three major routes: intravenous (IV),intramuscular(IM), subcutaneous(SC). Among them intravenous administration is currently the method of choice for systemic delivery of proteins and peptides. Insulin, interferons, and gamma-globulins have been reportedly metabolized and/or bound to tissue at injection sites following IM administration, and as a result the systemic bioavailability of these protein drugs following IM administration is often less than that obtained by IV injection. The bioavailability of tissue plasminogen activator following IM administration was facilitated by the co-administration of hydroxylamine or by electrical stimulation of muscles, which results in prompt attainment of therapeutic blood levels and achievement of coronary thrombolysis. For SC administration, insulin is best example for the treatment of diabetes. The controlled delivery of peptide or protein

based pharmaceutical from subcutaneously implanted polymeric devices was reported by Davis. He used a gel formulation of cross linked polyacrylamide-polyvinyl pyrrolidone to achieve the prolonged release of immunoglobulin, luteinizing hormone, bovine serum albumin, insulin, and prostaglandin[4].

Non parenteral systemic delivery

Oral route

General consideration

The understanding of physicochemical properties of protein and peptide drugs is necessary to development of effective oral delivery system. These properties include molecular weight, hydrophobicity, ionization constants, and pH stability, as well as biological barriers that restrictprotein and peptide absorption from the gastro-intestinal(GI) tract, including pH variability, enzymatic degradation and membrane efflux. Potential problems associated with oral protein and peptide delivery like extreme pH condition, protease degradation, epithelial cell barrier, short plasma half-life, low bioavailability, tendency to undergo aggregation adsorption and denaturation. These problems create some challenge to pharmaceutical scientist45. Currently, only two protein drugs include Interferon alpha and human growth hormone are given orally in clinical development in the US46.The ease of administration and higher degree of patient compliance with oral dosage forms are the major reasons for preferring to deliver proteins and peptides by mouth.

Approaches

Chemical modification

Chemical modifications include some strategies like olefenic substitution, carboxyl reduction Dehydroaminoacid substitution, d-amino acid substitution, thiomethylene modification, PEGylation and retro inversion modification. Result gives more stable pro-drug with
increase plasma half life.

Amino acid substitution

This is demonstrated by vasopressin analogs 1-deamino-8-D-arginine vasopressin called desmopressin in which Larginine release by D-arginine. Desmopressin is twice as active at the 75th fraction of the dose, which is attributed to enhanced membrane permeation and enzymatic
stability.

Nobex Conjugated Technology

In this technology, an amphiphilic protein conjugate is prepared by attaching short chain PEG and alkyl group to the amino groups of the protein molecule, which splits off in the blood circulation to release the parent protein. Nobex's conjugated insulin consists of a short chain PEG linked to an alkyl group which, in turn, is linked to LYS-29 of the β chain. It is found to be more absorbed and effective

Nasal route

With greater interest in delivery of protein and peptide-based drugs to the lungs for topical and systemic activity, a range of new devices and formulations are being investigated. Pulmonary protein delivery offers both local targeting for the treatment of respiratory diseases and increasingly appears to be a viable option for the delivery of proteins systemically. The lung is easy to access, has decreased proteolytic activity compared with the gut, and allows rapid absorption and avoidance of first-pass metabolism for systemically delivered drugs. Hundreds of proteins and peptides are undergoing clinical investigation for a range of clinical conditions. These include growth factors, hormones, monoclonal antibodies, cytokines, and anti infective agents. For those being investigated for delivery via inhalation, the ultimate site of action may be the airway surface (e.g.DNase), the airway cells (e.g. cyclosporin), or the systemic circulation (e.g.insulin). Careful choice of carrier and device can facilitate delivery to a specific area of the lungs. Once delivered, a carrier can further influence the distribution and rate of clearance from the site of action..

The only protein for inhalation currently available on the market is DNase, but a growing number of proteins/peptides are in various phases of clinical trials. Systemic inhaled insulin is in late phase 3 trials. Other proteins/peptides in phase 3 trials include leuprolide and gamma-interferon. Bile salts have been used to enhance the nasal absorption of peptide based pharmaceuticals[5].

Buccal route

Oral mucosa, including the lining of the cheek (buccal mucosa), floor of mouth and underside of tongue (sublingual mucosa) and gingival mucosa, has received much attention in the last decade because it offers excellent accessibility and avoids degradation of proteins and peptides that occurs as a result of oral administration, gastrointestinal absorption and first-pass hepatic metabolism. Peptide absorption occurs across oral mucosa by passive diffusion and it is unlikely that there is a carrier-mediated transport mechanism. The penetration of macromolecules through oral epithelia has been studied by several investigators[6]. a developed a self adhesive buccal patch and reported that it is feasible to deliver peptide base pharmaceuticals such as protirelin and buserelin through buccal mucosa. Various types of polymers like sodium carboxymethyl cellulose, hydroxylpropylmethyl cellulose, polyvinyl pyrrolidone, acacia, calcium carbophil, gelatin, polyethylene glycol are used for delivery of proteins or peptides via buccal route. The anionic polyacrylate type hydrogel is the most commonly used polymer[7].

Various strategies employed for buccal delivery

1) Adhesive tablets: e.g. Adhesive tablet based on hydroxypropyl cellulose.
2) Adhesive gels: e.g. By using polyacrylic acid and polymethacrylate as gel forming polymers.
3) Adhesive patches: e.g. Protirelin in HEC patches and buserelin.
4) Adhesive promoters: e.g. Sodium lauryl sulphate, sodium myristate, bile acids, sodium glycocholate, citric acid.

Addition of absorption promoters/permeabilizers in bioadhesive dosage forms will be essential for a successful peptide/protein delivery system. Thyrotropin-releasing hormone, tripeptide, oxytocin, vasopressin,analogs, calcitonin, insulin have been easily applied through buccal route.

Advantages of buccal route

It is robust, much less sensitive to irreversible irritation even on long term treatment. Absence of enzymatic barrier. Well acceptable to the patients. Easy accessibility administration as dosage forms. It is attached or removed without any pain or discomfort[8].

Ocular route

The ocular route holds immense potential for peptides or proteins intended for pathological ophthalmologic conditions. The ocular route is the site of choice for the localized delivery of opthalmologically active peptides and proteins for the treatment of ocular disease that affect the anterior segment tissues of eye[9]. Christie and Hanzal reported the observation of a dose dependent reduction in blood glucose levels following the ocular administration of insulin to the rabbit. The use of nanoparticles liposomes, gels, ocular inserts, bioadhesives or surfactants are necessary to enhance ocular absorption of proteins or peptides. The polypeptide antibiotics like cyclosporine, tyrothricin, gramicidin, tyrocidine bacitracin and polymyxins have often been considered potential candidates for achieving local pharmacological actions in the eyes[10].

Rectal route [11]

The use of the rectum for the systemic delivery of organic and peptide based pharmaceuticals is a relatively recent ideas. The coadministration of an absorption promoting adjuvants such as sodium glycocholate, has been reported to enhance the rectal absorption of insulin[12]. Hyooglycemia in rats by administering insulin via rectal route, in a dosage form that contained polyethylene glycols and a surfactant. It was recently reported that a solid dispersion of insulin with sodium salicylate can produce a rapid release of insulin from the suppositories achieve a significant

reduction in plasma glucose levels in normal dogs, even at doses as low as 0.5IU/kg[13]. Bile salts, such as sodium salts of cholic,deoxycholic and glycocholic acids, have also been shown to enhance the rectal absorption of insulin in rats[14], and human volunteers, Vasopressin and its analogs[15], pentagastrin and gastrin[16], calcitonin analogs and human albumin[17]have been investigated for rectal delivery of protein or peptide based pharmaceuticals[18].

Advantages of rectal delivery

It is highly vascularized. It avoids first pass or presystemic metabolism. Drug can be targeted to the lymphic system. It is suitable for drugs that cause nausea/vomiting and irritate GI mucosa on oral administration. A large dose of drugs can be administered.

Transdermal route

Transdermal delivery has attracted considerable interest as a route for administering peptides and proteins. As early as 1996, Tregear investigated the feasibility of administering proteins and polymers through skin excised from human and animals[19]. More recently, studied the percutaneous absorption of elastin peptides through rat skin and its subsequent distribution in the body. The small peptides such as thyrotropin releasing hormone (TRH) ,vasopressin, have great difficulty in permeating the skin barrier[19,20].

Advantages of transdermal route

- Avoids the hepatic first-pass effect and gastrointestinal breakdown.
- Provides controlled and sustained administration, particularly suitable for the treatment of chronic disease.
- Reduces side-effects, often related to the peak concentrations of the circulating agent; Enables self-administration and improves patient compliance, due to its convenience and ease of use. Permits abrupt termination of drug effect by simply removing the delivery system from the skin surface.

Limitations of transdermal route

- A low rate of permeation for most of protein drugs due to their large molecular weight.
- High intra- and inter-patient variability.
- Because the skin has a relatively low proteolytic activity, the peptide
- drugs have poor skin permeability.

Pulmonary route

The respiratory tract offers an alternative site for systemic noninvasive delivery of peptides/proteins . Most of these drugs are readily absorbed through the lung, once they entered the deep lung tissues via transcytosis. They provide larger surface area (70 m2) as compared to the other mucosal sites including nasal, buccal, rectal and vagina.Three devices are currently available for the pulmonary delivery of the protein/peptide drugs : metered dose inhaler, nebulizer and powder inhaler (insufflator). Insulin can be delivered by this route by using devices such as aerosol, dry powder or as a administered with penetration enhancers like 1% azone, 1% fusidic acid or 1% glycerol. Calcitonin can be delivered as dry powder by this pulmonary route.

Advantages of pulmonary route

- Provide a direct route to the circulation.
- Reduction in dose requirement upto 50 fold and thus cost effective
- option for pharmaceutical industries
- Fast absorption
- Safe route for drug entry even in patient with lung diseases
- No triggering of immune function.
- Increase patient compliance with a minimum of discomfort and pain

Disadvantages of pulmonary route

- Most of the drug is delivered to the upper lung, an area with low
- systemic absorption.
- Only a small amount of drug can deliver[21].

Development of delivery system for proteins and peptide based pharmaceuticals

Formulation consideration

Preformulation studies of therapeutic peptides and proteins

Preformulation data must be generated to serve as the basis for the formulation development of dosage forms or for the design of delivery system to achieve optimum physicochemical stability and maximum systemic bioavailability.

Surface adsorption behaviour of peptide and protein molecules

Protein and peptide molecules have a tendency to be adsorbed to a variety of surfaces including glass and plastic.

Aggregation behavior of protein and peptide molecule

The potential problem is the self aggregation of peptide and protein molecules such as insulin This has been minimize by the incorporation of additives like urea, dicarboxylic amino acid such as aspartic acid and glutamic acid or other reagents such as glycerols EDTA, Lysine.

Pharmacokinetic considerations

Due to the very short half life of protein and peptides, it is more critical to get information about pharmacokinetic of therapeutic peptide and protein. Metabolic degradation by peptidases and proteinases can occur in the vascular endothelium, liver, kidney and non target tissues and even at a site of administration.

Analytical consideration

Bioassay method has been available for detection and potency determination of peptides and proteins, but it is very time consuming, labor intensive. Now a days spectroscopy, chromatography, electrophoretic methods and immune assays have been available for an analytical determination of protein or peptide. The most commonly used analytical methods include HPLC and RIA.

Regulatory consideration

Biotechnology products regulated under the authority of four federal agencies:

- The food and drug administration (FDA),
- The environmental protection agency (EPA),
- The occupational safety and health administration (OSHA).
- U.S.Department of agriculture (USDA)[22].

PolyXen®

PolyXen® is an qualify technology for protein drug delivery. It uses the natural polymer polysialic acid (PSA) to prolong the active life and improve the safety of therapeutic peptides and proteins. It can also be used for the small molecule drugs.PSA is a polymer of a sialic acid (a sugar). When used for protein and therapeutic peptide drug delivery, polysialic acid provides a protective microenvironment on conjugation. This increases the active life of the therapeutic protein in the circulation and prevents it from being recognized by the immune system. The use of PSA makes PolyXen® a particularly effective form of protein drug delivery. PSA is a naturally occurring polymer, and it is biodegradable, non-immunogenic and non-toxic. This is particularly important where a polymer is to be used to deliver therapeutics chronically or in large dosages[23].

Imu Xen®

ImuXen® is a group of liposomal technologies designed to improve the delivery and effectiveness of DNA, protein and polysaccharide vaccines.ImuXen® technology can help to generate strong protective immune responses, in some cases with a single injected dose[24].

The potential advantages of ImuXen® for DNA, protein and polysaccharide vaccines include

Vaccine protection against degradation. Efficient delivery of vaccines to the immune system Increased immune responses Protective immunity with a single injection Rapid simple and scaleable manufacture. The main benefits of ImuXen for DNA, protein and polysaccharide vaccines include and Multiple vaccines delivered with a single injection. Reduction in the number of doses required Reduction in side effects. Potential for oral administration of vaccines Humira is the best-selling new monoclonal antibody in the market. Pegasys is the best-selling newcomer to the therapeutic protein, and global pharma market. Epogen is the second best-selling therapeutic protein Aranesp Neulasta and Enbrel are the most successful therapeutic proteins in terms of growth Aranesp is the fastest-growing therapeutic protein

NEED OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. The protein and peptides are very important in biological cells and Organic Molecules[25].
2. In the Absence of proteins and peptides causes diseases like Diabetes mellitus. (Caused due to the lack of protein called INSULIN)[26].
3. Now a days R-DNA technology and hybridoma techniques also used in protein and peptide based pharmaceuticals[27].

PHARMACEUTICAL APPROACHES

The protein and Peptides are having Four Approaches they has Follows

1. CHEMICAL MODIFICATION
2. ENZYME INHIBITORS
3. PENETRATION ENHANCERS
4. FORMULATION VEHICLE
5. MUCOADHESIVE POLYMERIC SYSTEM

CHEMICAL MODIFICATION (PRODRUG APPROACH)

The Chemical Modification of Protein and Peptide Drug Delivery System of Drugs is Important to Improve the Enzymatic Stability as well as Membrane Permeations. It is Applicable for the reducing the Immunogenicity

The Chemical Modification is Includes in Two Types of Modifications as Follows

- Amino acid Modification
- Hydrophobization

Amino acid Modifications

The Modification of amino acid is one of the important approach in which the Substitution of the D- amino acid and L- amino acid is important to alter the Physiological Properties of Protein and Peptide Drug Delivery Systems.

Example: Desmopressin and Deaminovasopressin are the two important analogs of vasopressin, former involves deamination of first amino acid and replacement of last Larginine D-arginine to give Deaminovasopressin.

Application: The Amino acid modification is important to enhance the Membrane Permeability and Maintain the Enzymatic Stability.

Hydrophobization: It is having an important approach for the Lipophilic Moieties

Example: NOBEX INSULIN by the Palmitoylatios.

Description of Example: Conjugation of the Insulin Molecule to the 1,3-dipalmitoylglycerol containing a free amino acid groups of glycine, Phenylalanine and Lysine molecule to from mono and insulin is important to facillated the transfer the insulin across the mucosal membrane of the large intestines. It is important to improve the Stability against the enzymatic degradations[28-30].

ENZYME INHIBITORS (PROTEASE)

The enzyme (protease) inhibitors are the enzymatic approach of the Protein and Peptide drug delivery systems. GIT and Liver is play important role in Metabolization of the Protein and Peptides into smaller fragments of the two to ten amino acids with the help of the variety of Proteolytic Enzymes. This Protease inhibitors are CO- administered with Protein and Peptideto alter the Environment for the Enzyme stability to supress the Proteolytic activity. The enzyme proteases inhibiters are divided into four types they are Aspartic Proteases (Pepsin,Rennin), Cystinyl Proteases (Papain, Endopeptidase), Serinyl Proteases (Thrombin, Trypsin),and Metallo Proteases (Carboxypeptidase) [31].

PENETRATION ENHANCERS

Penetration enhancers are the one of the most important Component of Protein and Peptides formulation is responsible for the Disruption of the Mucosal Barriers and applicable to improve the Membrane Permeations of Large Macromolecular substances lie Proteins and Peptides. The Several classes of compounds are mainly used has a permeation enhancers are such as Surfactant (Polysorbate, SLS, Pluronic F-68), Chelating agent (EDTA), Fatty acids(Sodium Carprate), Mucoadhesive Polymeric systems (Thiomers, Cellulose derivatives),Phospholipids (PC). The basic Mechanism of Penetration enhancers are the, detergent and surfactant molecules are the increases the transcellular transport of the drug material is responsible to disrupting the structure of the lipid bilayer of lipid membrane are having more permeability. Another mechanism is the calcium chelates are the responsible for the Exert the action of complex formation of the calcium ions and they are passing through the tight junctions and they are facillated the Paracellular transport of the hydrophilic drugs materials. Fatty acids are the important for the improving the paracellular absorption by phospholipases C activations and up regulation of intracellular Calcium ions, is leading to the contraction of actine myosin filaments[32].

FORMULATION VEHICLES

The Protein and Peptide Drug Delivery system is important for the Oral Delivery of Protein and Peptides can be successfully achieved by using various carrier systems are like

1. Dry Emulsion
2. Microspheres
3. Liposomes
4. Nanoparticles

Dry Emulsion

It is important application in drug delivery system s to prevent the instabilities of the long term storage of multiple emulsions. The novel approach at which multiple emulsion is replaced by dry emulsions. Dry Emulsion is prepared by the Spraydrying, Lyophollization and evaporation Techniques. In dry emulsion preparation application of the PH responsive polymers like HPMCP, is important for the emulsions are the enteric coated and site specific achieved.

Microspheres

The uniform distribution of drug in oral drug delivery in Protein peptides drug are known as Microspheres. The PH responsive microspheres are the mainly used in oral delivery for the protection of the stomach from proteolytic degradations and Protection upper portion of small intestine from proteolytic degradations.

Liposomes

Liposomes are the small microscopic vesicles in which aqueous volume is entirely enclosed by the membrane composed lipid molecules. Liposomes in drug delivery system, the encapsulation of the insulin with sugar chain portion of mucin and PEG completely suppressed the degradation of the insulin molecules in intestinal fluid. The uncoated from of liposomes are suppressed it on partially surface coating of the liposomes molecules in PEG or mucin gained resistances against desgtion by salts and increased the stability of GI tract.

Nanoparticles

Nanoparticles are Nano sized colloidal structure having size is 10-1000nm. The particles in nanometric sized range of the particles are absorbed intact by the intestinal epithelium and they are the less prone towards the enzymatic degradations. The particle size surface charges are the influencing the uptake of nanoparticle system in GI tract[33].

APPLICATION

1. **CVS acing drugs Protein and Peptides** (Angiotensin 2 antagonist, Bradykinin, Captopril) is important for the Lowering blood pressure and improving peripheral circulation for Heart failure management[34].
2. **CNS active Protein and Peptides** (Cholecystokinin, B-endorphin) is important for the Suppressing appetite and Relieving pain[35].
3. **GI-active Protein and Peptides** (Gastrin antagonist, pancreatic enzymes) is important for the Reducing secretion of gastric acid and it is important for Digestive supplement[36].
4. **Immunomodulation of the Protein and Peptides** (Bursin, Cyclosporin, and Interferon) is important for Selective B-cell different iatingharmone Inhibits functions of T-lymphocyte Enhancing activity of killer cells[37].
5. **Metabolism modulating Protein and Peptides** (Insulin, Vasopressin) is important for treating diabetes mellitus and treating diabetes insipidus[38].

Transport mechanism of macromolecules

Large numbers of mechanisms are responsible for penetration such as simple diffusion (paracellular and transcellular), carrier-mediated transport, active transport and pinocytosis or endocytosis Proteins and peptides have very low logP (<0) value. Those drugs have lack of lipophilicity, no passive absorption can take place and are absorbed through paracellular pathways (restricted to small molecules, less than 100–200.The paracellular space lies between 10 and 30–50A° , therefore the paracellular route is not feasible for large macromolecules. But in the case of insulin, it is adsorbed on the apical membrane and is internalized by specific types of endocytosis processes. Few numbers of protein and peptides show practically active transport by binding to the cell surface receptor or binding sites in the epithelial lining of the small intestine (membrane bound vesicles). The most commonly used transport mechanism is passive diffusion withtwo ways of transport: first, paracellular (transport of drug through the intercellular space between the cells) and second, Transcellular (involves passage into or across the cells).

Transportation of drugs depends on overall molecular geometry, lipophilicity and charge of the transport pathway across the oral mucosa .A minimum level of lipophilicity is essential in drugs to partition into the epithelial membrane and absorbed through transcellular passive diffusion. Transport of therapeutic molecules from the gastrointestinal tract into the systemic circulation is through the mucosal layer then through the layer are olaror. Other two intestinal layers (areolaror submucosal) connect together the mucus and muscular layers Muscular and mucus layers are the strongest layers of the intestine which consists of the loose, filament ousareolar tissue containing lymphatics, nerves and blood vessels. Membrane perturbing in order to increase transcellular permeation, was shown on human Caco-2 epithelial cell mono layers when exposed at maximum concentration and demonstrated tolerance in vitro, but the best way is to attach any ligand on molecules that opens the tight junctions[39-42].

Acromolecular conjugation

Polypeptides can be conjugate to a macromolecular carrier, as a polymer or a protein. The advantage by using conjugation technology for improving peptide GI absorption that it will change only the molecular properties of the drug not the function of epithelial cells and might consequently avoid some of the side effects observed in using penetration enhancers. Amphiphilic polymers such as alkylated polyethylene glycol derivatives, have been developed by NOBEX[43] their insulin oral delivery system, co-developed with GlaxoSmithKlinein mid-Phase II clinical trials and preliminary reports are promising[44].

National and international status

The report points out that the protein engineering market in 2006 was worth almost $67 billion (10% of total pharma sales) and is forecast to rise to $118 billion (12% of pharma sales) in 2011. Even though their remarkable success protein drugs continue to suffer from drawback sespecially with respect to their delivery (subcutaneously or intravenously injected). The past three years have seen approvals of products for non-parenteral delivery alongside advances in parenteral protein and peptide drug delivery. The increased use of development and discovery of protein therapeutics will lead to increasing opportunities for drug delivery companies. The protein therapeutic market is largely immediate release, but there is a trend moving toward increased sustained release formulations.

CONCLUSION

Proteins are the every part of cell involves proteins in some way from the nucleus . Peptide and protein drugs will be produced on a large scale by biotechnology processes and will become commercially available for therapeutic use and the total amino acid of a food protein expressed in relation to some standard is a good indicitor of the potential nutritive valve. In the review, proteins, peptides and macromolecules plays a more role in the various efficient carriers for the delivery of proteins such as solid lipid, liposomes, nanoparticles etc Various efficient approaches were discussed for formulation development of therapeutic and pharmaceutical approaches of proteins and it can be implemented in large-scale production. and the product development costs remain major Due to their poor bioavailability the proteins are peptides drugs used in the parenteral therapies. The main need in the clinical and therapeutic regions has intensified the investigation for their convenient & effective delivery through noninvasive system.

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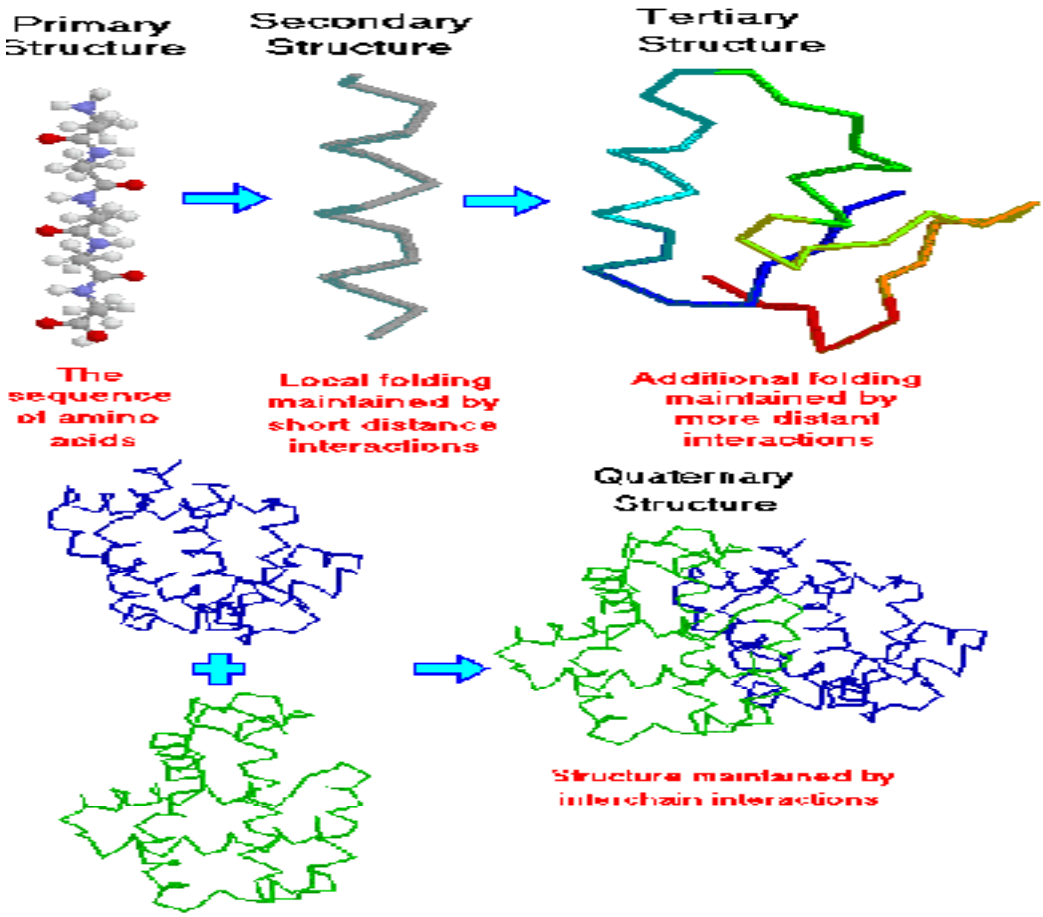

FIG 1: Structure of proteins[3]

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RESEARCH ARTICLE

Estimation of Antioxidant, Cytotoxic Activity of Attatic Curanam

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ABSTRACT

The study aims to investigate the phytochemical, antioxidant and cytotoxic activity of compounds from hydro-alcohol extract which will be pharmacologically effective. Phytochemical screening the presence of some compounds is found such as alkoids, flavonoids, phenols, steroidal compounds and saponins. This suggests it will be pharmacologically effective. Antioxidant properties will be analysed by DPPH test, Total reducing power test and total phenol content test. Cytotoxic activity was also found by Brine Shrimp Lethality test. The LD50 was measured for both the standard and the extract. The extract showed greater activity than the standard that means the hydro-alcohol extract of attatic curanam has potential cytotoxic effect. The result shows, that the drug attatic curanam low cytotoxic activity on the HepG2 cell lines.

Keywords: Antioxidant activity, Polyphenol, Flavonoid, Cytotoxicity, HPTLC Fingerprinting

INTRODUCTION

Siddha Medicine is an ancient Tamilakam system of traditional medicine in south india [1][2]. Agastyais considered the first siddha and the guru of all siddhars; the siddha system is believed to have been handed over to him by muragan son of shivaand paravti [3]. The term "antioxidant is used primarily for two different group of substances: Industrial chemicals added to oxidation-preventing products, and natural chemicals found in foods and body tissues that are said to have beneficial health effects [6] [7]. Siddhars have formulated different types of drug with antioxidant property one among such formulation is Attatic curanam made out of Cukku, Tippili, Cirakam, Karuncirakam, Intuppu, omam and Perunkayam. Attatic curanam is one of the siddha drug which is used to treat inflammatory diseases, loss of appetite and indigestion. Many enzymatic systems generate reactive oxygen species (ROS), such as radical superoxide anion, hydroxyl radical, and hydrogen peroxide, through oxygen consumption [8]. These ROS can be beneficial in small amounts as signal transducers and as growth regulators [9]. During oxidative

stress, however, large quantities of these ROS may favour certain conditions of human disease such as cancer, hepatic diseases, cardiovascular diseases, ageing and neurodegenerative diseases [10].

MATERIALS AND METHODS

Collection of the drug

The drug were procrured from the local markets, Chennai. Dr.K.N.Sunilkumar, Resaerch officer (Pharmacognosy), Siddha Central Research Institute, Chennai. The ingredients (*Zinger officinale, Piper longum, Piper nigrum, Cuminumcymium, Nigella sativa, Sodium chloride, Trachyspermumammi, Ferula assa-foetida*)for the test drug of each 10.7g were taken and powdered and kept in a earthern pot by adding ½ portion water and allowed to boil till becomes ½ the quantity and filtered.

Extraction procedure

For 48 hours, 75g of Attatic Curanam was soaked with 500mL of hydro-alcohol (1:1). The extract was filtered and concentrated under reduced pressure (100mbar) and reduced temperature (35c) using rotary evaporation. Using minimum quantity of ethanol, it was transferred to a porcelain dish and diluted over water bath to free ethanol.

Pre-Screening method (Determination of polyphenol content in the drug)

The amount of polyphenol in the drug is determined using the method folin-ciocalteau. Dissolved 300mg of the drug in 5ml of methanol: water: concentrated HCL (60:40:0.3). The contents were filtered through what filter paper man NO 1. 100 micro litres of 50% phenol reagent follins were added after 2 minutes.The tubes were incubated at room temperature for 30 minutes, and the absorption was read at 750nm using a UV Spectrophotometer. The standard use was gallic acid . The results have been expressed as a milligram of equivalent gallic acid.

Determination of flavonoid concentrations in the drug

The content of the flavonoid in the examined drug was determined using spectrophotometric method. The drug contained 2ml of methanol solution of the drug in the concentration of 1 mg/ml and 1ml of 2% Aluminium chloride solution was dissolved in methanol. At room temperature, the sample was incubates for an hour, and the absorbance was determined using 415nm spectrophotometer. The same procedure was constructed for the standard quercitin solution and the calibration line. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line, the content of flavonoids in drug was expressed in terms of quercitin equivalents

RESULTS

In this assay gallic acid was used as standard. The below table represents polyphenol content and absorbances of gallic acid and attatic curanam for their different concentrations (33.3 µg/ml – 166.7 µg/ml). The polyphenol content should be expressed in terms of GAE mg/g of drug. The average polyphenol content in the drug Attatic curanam is 337mg/g. In this assay gallic acid was used as standard. The below table represents polyphenol content and absorbances of gallic acid and attatic curanam for their different concentrations (10µg/ml – 50 µg/ml).

The flavonoid content should be expressed in terms of QE mg/g of drug. The average flavonoid content in the drug Attatic curanam is 242 mg/g. In this assay gallic acid was used as standard. The below table represents polyphenol content and absorbances of gallic acid and attatic curanam for their different concentrations (1.25µg/ml – 6.25 µg/ml). In this method, quercitin is used as standard. The below table represent the absorbance values and percentage of inhibition of quercitin at various concentrations (1.25 µg/ml- 6.25 µg/ml).The percentage of inhibition is calculated

with the control value of 0.339. In this assay quercitin is used as a standard. The below represents obsorbance valuesof quercitin and attatic curanam at various concentrations (0.28 µg/ml-1.14 µg). In this asay ascorbic acid was used as standard. The below table represents the total antioxidant content and absorbances of ascorbic acid and attatic curanm for their different concentrations (10 µg/ml – 50 µg/ml). The total antioxidant content should be expressed in terms of AE mg/g of drug. The average total antioxidant content in the drug Attatic curanam is 615 mg/g. The below table represents the absorbance values and percentage of inhibition of Attatic curanam at various concentration (1.25 µg/ml - 6.25 µg/ml). The percentage of inhibition is calculated with the control value of 0.210).
The IC_{50} values for ascorbic acid and attatic curanam is determined as (4.2µg/ml - 6.16 µg/ml)

CONCLUSION

The antioxidant potential of drug Attatic curanam was assessed and expressed in different ways by different methodologies such as Total antioxidant assay, DPPH, Alkaline DMSO and Pro-oxidant effect. The results were obtained as IC50= 8.88µg/ml and IC50=6.16µg/ml for DPPH and DMSO respectively, whereas 615mg/g (ascorbic acid equivalents) for Total antioxidant assay. For the absorbance value increases with respect to the concentration of drug. This indicates the reduction potential increases with the drug concentration. In prescreening methods, the polyphenol and flavonoid content were identified as 337mg/g (gallic acid equivalents) and 242 mg/g (quercitin equivalents) respectively. The cytotoxic activity of siddha drug attatic curanam On HepG2cell lines was assed by using MTT assay. The result shows, that the drug attatic curanam low cytotoxic activity on the HepG2 cell lines.

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TABLE 1 : PRE-SCREENING METHOD (ESTIMATION OF POLYPHENOL IN ATATTIC CURANAM)

Attatic curanam/ Gallic acid content µg	Concentartion µg/ml(Content/ total volume)	Gallic acid (O.D)	Attaic curanam (O.D)	Polyphenol content
100	33.3	0.210	0.203	321
200	66.7	0.221	0.221	295
300	100	0.412	0.254	387
400	133.3	0.454	0.270	331
500	166.7	0.496	0.299	352

TABLE 2 : DETERMINATION OF FLAVONOID CONCENTRATION IN ATTATIC CURANAM

Attatic curanam/quercitin content µg	Concentration µg /ml (content / total volume)	Quercitin (O.D)	Attatic curanam (O.D)	Flavanoid content (QE mg/g of drug)
40	10	0.206	0.120	260
80	20	0.331	0.150	252
120	30	0.481	0.172	228
160	40	0.558	0.200	227
200	50	0.707	0.240	247

TABLE 3 : DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

Quercitin content µg	Concentration µg/ml (content/ total volume)	Obsorbance 517nm	Inhibition%
5	1.25	0.313	7.67
10	2.5	0.308	9.14
15	3.75	0.270	20.4
20	5	0.220	35.0
25	6.25	0.218	35.7

TABLE 4 : DETERMINATION OF THE PRO-OXIDANT EFFECT OF THE DRUG

Attaticcuranam/ Quercitin content µg	Concentration µg/ml (Content/ total volume)	Attaticcuranam 0.D	Quercitin O.D
5	0.28	0.101	0.121
10	0.53	0.103	0.145
15	0.75	0.110	0.165
20	0.95	0.114	0.200
25	1.14	0.120	0.210

TABLE 5 : DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY OF THE DRUG

Attatic curanam/ Ascorbic acid content µg	Concentration µg/ml (content/ total volume)	Ascorbic acid O.D	Attatic curanam O.D	Total antioxidant content (QE mg/g of drug)
5	0.83	0.124	0.113	647
10	1.67	0.173	0.139	598
15	2.50	0.240	0.173	640
20	3.33	0.271	0.205	651
25	4.17	0.310	0.210	541

TABLE 6 : SCAVENGING OF SUPEROXIDE RADICAL BY ALKALINE DMSO METHOD

S. No	Concentration(µg)	Absorbance Value		% of Inhibition	
	Standard / Drug	Standard	Drug	Standard	Drug
1	1.25	0.152	0.198	27	5
2	2.5	0.131	0.183	37	12
3	3.75	0.112	0.149	46	29
4	5	0.093	0.120	55	43
5	6.25	0.068	0.109	67	48

TABLE 7 : CYTOTOXICITY OF THE DRUGS

Drug concentration (µg)/ml	DMSO Control %			5- Fluorouracil %			Attatic curanam%		
25	96.887	95.914	96.304	70.347	69.232	72.34	85.36	86.32	86.66
50	97.782	96.342	98.893	55.456	56.239	58.472	80.69	75.59	76.32
75	97.347	98.232	99.34	50.456	53.345	55.342	79.35	72.23	73.26
100	98.456	98.239	95.472	45.347	47.232	45.34	69.66	62.32	65.26
150	96.456	98.345	99.342	40.347	41.239	38.472	65.25	64.32	63.22
200	97.347	98.232	99.34	42.32	32.33	28.32	51.26	51.12	45.26
250	98.456	98.239	95.472	35.32	25.26	36.33	52.26	48.56	42.36

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RESEARCH ARTICLE

Lung Cancer and Its Types

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ABSTRACT

Non-enzymatic vitamin C (ascorbic acid) plays an important role in the medicinal field and acts as antioxidants use in fruits and vegetable such as lemon, orange, grapes, carrots, tomatoes, grapefruit, beans, broccoli, and mangos. It helps to prevent and stop of various diseases such as lung cancer, asthma, and wheezing and finding an antibronchospastic effect. Other factors such as diet have also been implicated in the development of lung cancer. Despite the extensive research conducted in this area, the relationship between diet and lung cancer is still not clear. Diets high in fat and low in vegetables and fruits may increase the risk of lung cancer and other fact eating of tobacco and smoking of cigarette. Lung tissue damage due to high levels of free radicals in cigarette smoke causes direct (tissue oxidation) and indirect (release of oxidizing agents and enzymes). Vitamin C is necessary for phagocytosis.

Keywords: Non-enzymatic antioxidant (vitamin C), Lung function, Lung diseases.

INTRODUCTION

Epidemiologic studies demonstrating a relationship between family history and an increased risk of lung malignancy gave the primary evidence of host susceptibility. Lung cancer vulnerability and risk also are enhanced in inherited cancer syndromes caused by uncommon germ-line mutations in p53 retinoblastoma and other genes and in addition a germ-line mutation in the epidermal growth factor receptor gene [1]. More recently, three vast genome wide societies' studies recognised a relationship between single-nucleotide polymorphism (SNP) variation at 15q-15q and vulnerability to lung disease. The locale of the SNP variation was recently connected to lung carcinogenesis and incorporates two genes encoding subunits of the nicotinic acetylcholine receptor alpha, which is managed by nicotine presentation [2] . Lung-disease vulnerability and risk increment with lessened DNA repair capacity that

results, for example, from germ-line alteration in nucleotide extraction repair genes such as ERCC1. Enhanced expression of DNA synthesis and repair genes, including RRM1 and ERCC1, in Non-small cell lung cancer relates with better prognosis generally, however no advantage from platinum-based chemotherapy presents gene abnormalities required in the development of various histologic types of lung cancer[3]. Lung cancer is the leading cause of cancer deaths worldwide. It caused 1.59 million deaths in 2012 i.e. about 20% of total cancer deaths , while American cancer society estimated that of all the cancer deaths in 2014 about 27% will occur due to lung cancer .Swamy and haul(2003) mentioned the antitumor activity of α-hederin from N.sativa against LL/2(Lewis lung carcinoma) in BDFI mice[4]. The diet supplemented with *N. sativa* and honey was shown to have protective effect against lung, colon and skin cancers . Kumara and Huat. TQ, extracted from the *N. sativa* seed, at concentration of 100 µM showed significant anti-cancer activity against the lung cancer cell line and demonstrated to inhibit cancer cell proliferation by about 90% [5].

Etiology

A combination of intrinsic factors and exposure to environmental carcinogens is involved in the pathogenesis of lung cancer. Preinvasive lesions such as adenocarcinoma in situ and minimally invasive adenocarcinoma are well described and show that there is likely a stepwise progression from dysplasia to malignancy. Familial and genetic variations can predispose a person to lung cancer, even nonsmokers. Many genetic mutations within tumors have been identified[6].

Pathology

Lung cancer is classified by its histologic appearance into small cell lung cancer (SCLC) or non–small cell lung cancer (NSCLC; eTable A). NSCLC is divided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma; these are further subclassified.16 NSCLC is sometimes poorly differentiated and only distinguishable by immunohistochemical stains and molecular testing. This is problematic when only a small amount of tissue is available for testing. The optimal choice of treatment relies on a complete phenotypic and genotypic characterization of the tumor[7].

Clinical presentation

These symptoms are a result of ectopic production of hormones from the tumor or the body's reaction to the tumor, and are not directly attributable to the tumor or metastasis. About 10% of patients with lung cancer present with a paraneoplastic syndrome, and this rate is higher in patients with SCLC.17 The best treatment for paraneoplastic syndromes is treatment of the underlying cancer.17 Digital clubbing is a common paraneoplastic syndrome finding that is poorly understood, and it is more common with NSCLC[8]. Most data about symptoms at presentation of lung cancer are from referral centers, making extrapolation to the primary care setting difficult.19 Two individual symptoms that significantly increase the likelihood of lung cancer are digital clubbing and hemoptysis[9]. Other independent predictors of lung cancer include loss of appetite, weight loss, fatigue, dyspnea, chest or rib pain, and an increasing number of visits to evaluate persistent cough[10]. Patients rarely present with only one symptom, and the positive predictive value is higher when two or more symptoms are reported. For example, the combination of weight loss and hemoptysis has a positive predictive value of 9.2%.19 Lung cancer should be highly suspected in any patient older than 40 years with risk factors and symptoms. However, physicians must remember that lung cancer can occur in younger persons and in individuals without known risk factors[11].

Small Cell Lung Cancer

Limited stage SCLC is treated with an intent to cure; treatment results in a five-year survival rate of up to 25%.34 For early limited stage SCLC, surgery may be indicated. For both limited stage and extensive stage SCLC, concurrent chemotherapy and radiation therapy with a platinum-based agent and at least one other chemotherapeutic agent should be pursued. The five-year survival rate is virtually zero for extensive stage SCLC. As with more extensive

NSCLC, a patient's comorbidities, the extent of disease, and patient preferences are integral to making treatment decisions, and palliative care should be initiated early[12].

Non–Small Cell Lung Cancer

The treatment of NSCLC is well detailed in the 2013 ACCP evidence-based practice guidelines.31-33 nuances of treatment are evolving, complex, and largely beyond the scope of this review, yet a few themes are significant. Morbidity and mortality outcomes may be improved for patients evaluated and treated by a surgical thoracic oncologist in conjunction with a multidisciplinary team at a lung cancer treatment center. Surgical resection is indicated in medically fit patients with resectable stage I or II NSCLC, preferably a minimally invasive approach such as video-assisted thoracic surgery[13]. The goal for stage III infiltrative NSCLC is eradicating known intrathoracic cancer while diminishing subsequent intrathoracic and systemic disease,32 usually through chemotherapy and radiation based on tumor histology and the patient's functional status. In stage IV tumors, multidisciplinary management options are also largely dictated by histology and patient status. Palliative care should be initiated early in patients with stage IV NSCLC, or at any stage if underlying morbidity or patient choice prevents intent-to-cure therapy. Early palliative care significantly improves quality of life, decreases the incidence of depression in patients with newly diagnosed NSCLC, and may prolong survival. Additional management decisions may be influenced by a patient's involvement in an approved clinical trial[14].

Prognosis

Prognosis is better if presenting symptoms are caused by the primary tumor rather than by metastatic disease or paraneoplastic syndromes. It is also better in earlier stages of cancer. Survival rates at five years can be greater than 50% for those with localized disease, but decrease to less than 5% in those with distant disease[15].

Screening

The U.S. Preventive Services Task Force (USPSTF) supports annual low-dose CT to screen for lung cancer in patients 55 to 80 years of age with at least a 30 pack-year history who currently smoke or have quit within the past 15 years.35 The USPSTF cites the National Lung Screening Trial, which found a number needed to screen of 312 to prevent one lung cancer death in five years with three screening examinations.36 The recommendation was also based on extensive modeling studies to refine estimates of benefit and harm.37 The American Academy of Family Physicians concludes that the evidence is insufficient to recommend for or against low-dose CT screening for lung cancer[16]. This conclusion is based on the fact that the National Lung Screening Trial was performed at major centers with strict protocols (not community hospitals) and 40% of patients required some type of follow-up study or intervention because of positive results, and that the long-term hazards from cumulative radiation exposure with this screening are unknown. In light of differing guidelines, an approach of shared decision making and educating patients on the potential benefits and risks in relation to their personal health and health care setting is essential[17].

Diagnostic Evaluation

The diagnostic evaluation includes three simultaneous steps: tissue diagnosis, staging, and functional evaluation.

Tissue Diagnosis

Although experienced physicians can often diagnose the type of lung cancer based on clinical presentation and radiographic appearance, an adequate tissue sample is imperative to optimize the diagnosis and plan treatment[18]. Molecular testing requires a significant amount of tissue. Targeted therapies can increase treatment options for patients with advanced disease or poor functional status. Molecular testing is also standard in never smokers with squamous cell tumors, making ample tissue all the more essential in such patients[19]. For small or peripherally located lung cancers, this can be challenging. A variety of diagnostic methods are available that yield cytology samples or small biopsies. The choice of procedure depends on the type, location, and size of the tumor; comorbidities; and accessibility of metastases[20]. In general, the least invasive method possible should be used.22 If

the procedure fails to obtain tissue, a more invasive method is needed. Conventional bronchoscopy works best for central lesions, whereas CT-guided transthoracic needle aspiration is typically the first-line method for peripheral lesions. Endobronchial ultrasound and electromagnetic navigation are some of the newer procedures that may increase the diagnostic yield of bronchoscopy for select patients with mediastinal or peripheral lesions[21].

Prevention

Never smoking is the best way to prevent lung cancer, and smoking cessation is helpful. The USPSTF recommends screening every patient for tobacco use and encouraging smoking cessation for smokers at every appointment[22]. Physician counseling techniques can be effective when tailored to a patient's willingness to change. A variety of pharmacologic modalities are available that work best when combined with social and behavioral support. Legislation such as smoking bans in public buildings, prohibiting marketing of tobacco products to minors, and taxation of tobacco products likely play a role in decreasing tobacco use[23].

CONCLUSIONS

Our findings have established that relative to the huge health, social, and economic burden associated with lung cancer, the level of world research output lags significantly behind that of research on other malignancies. Commitment to diagnostics, screening, and quality of life research is much lower than to basic science and medical research. The study findings are expected to provide the requisite knowledge to guide future cancer research programs in lung cancer.

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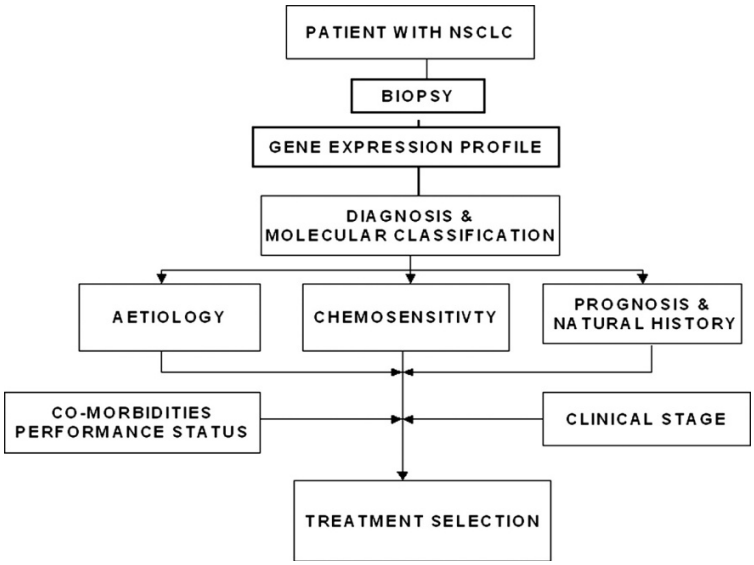

Fig.1 Flow chart

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RESEARCH ARTICLE

Studies on the Antioxidants Activities in *Gloriosa superba* L. among Five Different Accessions of Tamil Nadu State, South India

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ABSTRACT

Gloriosa Superba L is a medicinal plant and belongs to the family Colchicaceae. *Gloriosa superba* is the state flower of Tamil Nadu in India as well as national flower of Zimbabwe country. This is the most significant plant species having multiple medicinal values and used in Ayurvedic and Siddha medicinal system. A number of phytocompounds including alkaloids Gloriosine and Colchicine were present in the tubers and seeds. The antioxidant systems evaluated the extracts was assured by estimating various antioxidant enzymes such as DPPH activity, ABTS activity, Superoxide radicals, Peroxidase, Polyphenol oxidase and Phenylamine ammonialyase. The antioxidant potential assessed with the different accessions of Sirumalai (GA1) Mulanoor (GA2), Thuraiyur(GA3), Konganapuram (GA4) and Vedaranyan (GA5).The tuber contains biological active phytocompounds and a good source of crude drug can be utilized as a complementary and traditional source for modern medicine.

Keywords: Antioxidants, *Gloriosa superba*, Phytocompounds, Modern medicine, Tubers

INTRODUCTION

India's grown medicinal and aromatic plants are real emporium. Greater than 10,000 civilians have benefited from lot of medicinal plants resources in India. Around 8000 drugs, 4000 spices ingredients for cooking, 750 culturally significant economically for fibers, pesticides, diseases, insecticides, gum, adhesive and dye and 100 for incense and

perfume production .Based on plant species generally used for traditional medicine, local communities are estimated to be used by seven thousand plant species [1]. *Gloriosa superba* Linn. belongs to the family Colchicaceae. This species is endangered plants among the group of medicinal plants [2]. The plant is striking tuberous, climbing, wavy-edged, yellow and red coloured flowers. The flowering season from the months of every year November to March [3]. *Gloriosa superba* is a well known traditional medicinal plant used in the Ayurvedic system of medicine. The plant species is commonly used for gout, inflammations, gonorrhoea, rheumatoid arthritis and relieves the fever. Extracts of the *Gloriosa superba* tubers are reported to cure various diseases such as ulcer, skin diseases, leprosy, bleeding piles then it's have anti-dote property for snake bite.

The plant *Gloriosa superba* grown in sandy loam soil and red soil in deciduous forest ecosystem especially in sunny positions. Often, the plant species is tolerant in nutrient poor soils types. This plant species is found in the forest edges thickets and boundaries of cultivated regions in warm climatic countries [4]. In India, Tamil Nadu state cultivates the plant species for the production of glory lily seeds. The annual production of seeds more than 600 tons covering the area of about 6000 acres of land. It had been used to induce labor in the traditional Indian system because of its oxytocic and early it contains abortifacient activity due to the presence of alkaloid colchicines [5]. *Gloriosa superba* native is tropical Africa. The plan grown in many regions of tropical Asia as well as India, Burma, Malaysia and Srilanka [6, 7]. *Gloriosa superba* is an industrial crop of medicinal in South India. The alkaloid colchicine content also collected from wild in India [8, 9].The wild species is exploited by peoples and as well as in field cultivation face some consequences, it was extinction in condition. The plant tubers and seeds contain similar medicinal values [10]. Antioxidants are involved in chain reactions by removing free radical intermediates and inhibit other oxidative reactions by being oxidized themselves [11].

Antioxidant may be a molecules and it's having capacity of preventing or slowing the other molecules oxidation. Oxidation reactions are to produce free radicals. The free radicals are going to start chain reactions and damage cells. Oxidation may be a reaction that transfers electrons from a substance to an oxidant. The *Gloriosa superba* tuberous roots are useful in to cure various diseases such as bleeding piles, ulcers, leprosy, skin diseases, and snakebites [12, 13]. Different plant parts of *G. superba* have widely used in medicinal system of tradition. The plant root paste is an effective against major issues such as paralysis, rheumatism, snake and insect bites [14]. In recent reports says that, interest has considerably increased in finding naturally occurring antioxidants generally used in food or medicinal products to replace synthetic antioxidants [15, 16]. Natural antioxidants are being reduced the risk factors like carcinogenicity. However, there is know about the chemical compositions of the plant leaves and tubers [14].This is most significant major medicinal plant in India and cultivated for its seeds which are exported to developed countries for pharmaceutical applications.

Medicinal plants are commonly known as storehouses of phytochemicals and its constituents. Phytochemicals may play an important role in synthesis of new molecules and nanoparticles [17-19]. Now a day, phythotherapy, as the application of medicinal plant-based phytoproducts or herbal extracts, is a common approach worldwide. The chemical drugs are the results of various side effects and spent more money for mass production of synthetic drugs, the secondary compounds of medicinal plants can be suitable alternatives to synthetic drugs [20]. Plants have anti-oxidant molecules in form of phytochemicals that protect our cells from damage caused by free radicals [21]. *G. superba* contains a rich source of phytochemical constituents such as glycosides, alkaloids, flavonoids and saponins. These phytochemicals serve as anti-oxidant and it may reduce the risk of health related issues [22]. The natural antioxidants intake has been link with the reduced risk factors of cancer, cardiovascular disease, diabetes and other diseases associated with ageing [23]. Many studies evidenced that plant sources have antioxidant phytochemicals and its provides the disease protection [24].

Antioxidants are generally compounds and it's preventing oxidative or oxidative damage of free radicals, so they are potential carriers of free radicals or reactive oxygen species. The antioxidants are act as the process of reduction of free radical activity, free radicals cleaning, pro-oxidative metals potentially complex and quenching single oxygen.

Oxidative stress is an imbalance between ROS synthesis and penetrating ability to eliminate ROS [25]. Deoxy Ribo Nucleic acid, Ribo Nucleic Acid, fatty tissues, pigment carotenoids, vitamins and proteins are involved more damaging to all the bimolecular in microorganisms. Then oxidative stimulating free radicals result in cell membrane and membrane protein degeneration and mutation, which can continue to developed in many diseases like oxygen toxicity, lipo fuscinosis, atherosclerosis, aging and liver injury [26,27]. The present study is to find out the antioxidant activities in *Gloriosa superba* L. tubers collected from different accessions of Tamil Nadu state, South India.

MATERIALS AND METHODS

Plant tuber collection

The difference accessions of *Gloriosa superba* L. cultivated in various places such as Sirumalai (GA1), Mulanoor (GA2), Thuraiyur (GA3), Konganapuram (GA4) and Vedaranyam (GA5) belongs to five different districts such as Dindigul, Tiruppur, Thiruchirapalli, Salem and Nagapattinam. The plant specimen authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2018/TECH/2042).The voucher specimen was deposited in the Department of Botany, Arignar Anna Government Arts College, Namakkal, Tamil Nadu, India.

Geographical Location

Sirumalai (GA1) is a region of 60,000 Acres (200 Km^2) Situated 25Km away from Dindigul. The latitude is 10º 11' 39.28" N and longitude is 77º 59' 48" E. Elevation is 1092.63 meters (3584.75 Feet). Mulanoor (GA2) is located in Tiruppur district. Mulanoor is located at 10.77º N and 77.72ºE. It has an average elevation of 238 meters (780 feet). Mulanoor is a part of Glory Lily market. Thuraiyur (GA3) latitude is11º 8'29.2380" N and longitude is 78º35'40.100" E situated in Tiruchirappalli district. Konganapuram (GA4) is located at 11.58ºN, 77.92ºE. It has an average elevation of 300 meters (1000 feet) and situated in Salem district. Vedaranyam (GA5) belongs to Nagapattinam district. The latitude is 10º22'77 27.15" N and longitude is 75º 51' 27.66" E. The elevation is 2.36 meters (7.73 feet) (Figure 1).

Preparation of powder form the tuber

The tubers were collected from five different accessions of Tamil Nadu state, India from five different districts. The collected raw materials thoroughly washed with distilled water and dried for 3 to 4 weeks under shade dry then finally powdered by using electric blender. The dried powder material from the tubers of five difference accession were properly packed in polyethene bags and used for further extraction and laboratory purpose.

Preparation of extracts

The tubers of *Gloriosa superba* were involved the steps to shade dried and make a fine powder for the analysis purpose. The powders were extracted with constant agitation for 48 hours of duration. By using Whatman No 1 chromatographic filter paper and then extract were filtered. The chromatographic paper and then concentrated in vacuum at 40 °C using a Rotary evaporator and stored at 4°C. 10 gm of each of the powdered specimen samples of *G. superba* was selected for the analysis of antioxidant activity and extracted with 100 ml of methanol for three days. The extracts obtained from the plant materials were filtered separately and concentrated by vacuum evaporation.

DPPH Radical Scavenging Assay

Radical Scavenging activity of *Gloriosa superba* tuber against stable DPPH (2,2-diphenyl-2- picrylhydrazyl hydrate) was determined by spectrophotometer. When DPPH reacting with as an antioxidant compound, which is donate the hydrogen, it is reduced. The colour changes (deep violet to light yellow) were measured in UV visible light spectrophotometer at 515 nm. The solution of extract was prepared with the help of 10 ml of methanol by dissolving two gram of dried extract. The solution of DPPH in methanol (6x10 -5 M) was prepared freshly, before UV measurements. 3.9 ml of DPPH is added in different concentration of extracts to measure IC50 value in microcuvettes [21]. The powdered tuber samples were kept in the dark for 15 minutes at room temperature and then the decrease in

absorption was measured. The blank is used for absorption and the same amount of methanol and DPPH solution was prepared and used for the measurement in every experiments. Then radical scavenging activity was calculated with percentage of inhibition.

Antioxidant analysis

The methodology for the ability of scavenging activity in natural antioxidants of the crude extract towards the stable free radical DPPH was measured [28]. ABTS (2, 2- azino-bis-3-ethyl benzothiazoline-6-sulphonic acid) radical cation decolourisation. Superoxide scavenging ability of the crude extract was assessed by the method [29].

Screening for DPPH scavenging activity

The *Gloriosa suberba* tuber extract (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The reactive mixture was allowed for 30 minutes at room temperature to complete reaction. The blank is methanol and DPPH in methanol. Then without the extracts of tuber, acted as the positive control. Then thirty minutes of incubation time, the discolouration of the purple colour was measured in spectrophotometer at 518nm.

Screening for ABTS scavenging effects

The ABTS radical cations were produced by reacting solution of ABTS (7mM) with 2.45mM potassium persulphate. This mixture was generally allowed and stands in room temperature in dark condition for 12-16 hours before use. The aliquots (0.5ml) of the *Gloriosa superba* tuber extracts were added to 0.3ml of solution ABTS. The final volume was made up to 1ml with ethanol. The absorbance was taken with Spectrophotometer and at read at 745nm.

Screening for Superoxide radicals scavenging activity

Superoxide anions were generated in samples that contained in the series of 3.0ml, 0.02ml of the tuber extracts (20mg), 0.2ml of Ethylene Diamine Tetra Acetic acid, 0.1ml of Nitro Blue Tetrazolium, 0.05ml of riboflavin and 2.64ml of phosphate buffer solution. The control tubes were also prepared, where DMSO also added instead of *Gloriosa* tuber extracts. The test tubes are vortexes and the initial OD was measured in spectrophotometer at 560nm. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm.

Extraction method for Peroxidase, Polyphenol oxidase and Superoxide dismutase

The method of estimation of Peroxidase and polyphenol oxidase activities [30]. Superoxide dismutase (SOD) activity was proposed by Beauchamp and Fridovich [31]. One gram of tuber powder was homogenized with 20 ml of ice-cold extraction medium containing 2mM $MgCl_2$, 1mM EDTA. 10mM β-mercaptoethanol, 7 percent PVP and 10mM of sodium metabisulphate. The homogenate was strained through and two layers of cheese cloth was used for the filtration process and centrifuged at 10000 g for 15 minutes. With the addition of buffer solution the supernatant was made up to 20 ml and finally it's used as enzyme source.

Extraction method for Phenylalanine ammonialyase

According to Amrhein and Zenk Phenylalanine ammonialyase was extracted [32]. Phenylalanine ammonialyase preparations were obtained by homogenisation of *Gloriosa superba* Linn. tubers of five different accessions in fluid nitrogen and extracted with buffer.

Statistical analysis

The experiments were carried out with five replicate of experiments. All the data's are statistically analyzed. The data are expressed as Mean ± Standard Deviation. The one way analyses were used to assess the differences. P values are expressed <0.05 of were considered as statistically significant.

RESULTS AND DISCUSSION

The potential antioxidant is observed in tubers of *Gloriosa superba* from different accessions of Tamil Nadu State. The DPPH activity is showed in GA4 (57.62%) and GA5 (63.2%) accessions is high and lower level in GA2 (45.42%) accessions. These results suggested that in accordance with rich medicinal suitability of *Gloriosa superba* accessions.
The ABTS scavenging activity was analyzed in five different accession of *Gloriosa superba.* The highest percentage of inhibition was observed 72% in GA5 accession ad lowest 33% rewarded in GA3 accession. The second moderate level 63 percentage of inhibition was shown in GA4 accession. The superoxide anion radicals are formed from the photochemical reduction of riboflavin from the *Gloriosa superba* tuber extract of different accessions of Tamil Nadu state. The percentage of inhibitions was observed in 40% in GA3, 42% in GA1, 46% in GA2, 62% in GA4 and 65%in GA5. The highest level of GA5 and GA4 and lowest in GA3 accessions of *Gloriosa superba* respectively.

The peroxide activity was higher in 70% GA5 accession and followed by GA4 accession as 65% when compared to all other accessions. The lowest peroxidase activity was observed in GA1 Sirumalai accession. A significant increase and gradual synthesis of polyphenol oxidase activity was observed in different accessions of *Gloriosa superba* in Tamil Nadu State. The enzyme also responsible for the oxidation of phenolic substances when met the stress conditions in plants. The result shows that 49µ Purpurogallin is formed in GA5 and 40.3µl purpurogallin formed in GA4 accessions. Another accession recorded in lower level. Phenylalanine ammonialyase rewarded in higher level at GA5, followed by GA4, GA3, GA2 and GA1. PAL involves Phenylpropanoid pathway. PAL catalyzes the transformation is generally Phenylalanine converted into trans Cinnamic acid and levels to the formation of complex phenolic compounds such as flavonoids, Tannins and lignin's. Phenyllalanine ammonialyase enzyme and contribute to regulate flavonoid biosynthesis and transcription induced by photo stress [33]. Peroxidase is believed to use phenolic compounds as Co-substrate. The anionic peroxides are believed to utilize phenolic compounds and coniferyl alcohol and H2O2. Peroxidase activity is a significant antioxidant defense mechanism for scavenging H2O2.Polyphenol Oxidase and Peroxidase are two major key compounds [34]. PPO comprises a large group of enzymes, all of which are characterized by their ability to utilize molecular oxygen during oxidation of phenolic compounds. Polyphenol oxidase is divided into two types. i.e. Catechol oxidase and Laccase which catalyze the oxidoreduction of O-diphenol or P-diphenols. The superoxide dismutase enzyme is a metalloprotein and catalysis [35]. Super oxide dismutase catalyzed by the dismutation reaction O2- + O2- +2H+ H2O2 + O2. It plays an essential role in scavenging superoxide radicals, protecting cell against O2- or oxyradical reaction products [36]. SOD enzymes are generally metal co factor and their subcellular localization.

The results obtained in the present investigations showed that the concentration of flavonoids and phenolic compounds in both the plant *G. superba* and is very high. These flavonoids are an important phytocompounds and reported to have an anti-radical properties and antioxidant [37, 38]. The finding of the different phytochemical examines the plant to be rich in different biologically active phytocompounds [39]. These phytochemical compounds served as potential source of the crude drugs and used as traditional medicine in complementary system of medicine. However, DPPH anti-scavenging values of all the extracts are higher except chloroform. These results might suggest higher medicinal suitability of alcoholic extracts in various antioxidant applications [39, 40]. The research report in DPPH radical has broadly used to test an ability of phytocompounds are free-radical scavengers or hydrogen donors and contains antioxidative activity of different parts of plant extracts [41]. The scavenging properties between DPPH and ABTS analysis could be explained by the different mechanism of the reactions for the two radicals [42]. Naik *et al*. has been reported for other compounds that possessed ABTS scavenging activity, but did not exhibit DPPH scavenging activity [42,43]

Rios and Recio (2005) stated that the studies on the medicinal plants as a source of pharmacologically active phytocompounds have increased day by day in worldwide [44]. In many developing countries of the world, plants systems are the main medicinal sources used in treating various infectious diseases [45]. The biological role of

flavonoids apart from its antioxidant properties include protection against various categories allergies, inflammation, platelet aggregation, free radicals, microbes, ulcers, hepatoxins, viruses and tumors [46]. Flavonoids are reduced cancers by capable of produce the estrogen [46, 47]. The enzyme Superoxide anions are reactive species and to create with a transfer of single electron and involves to develop the reactive oxygen species as H2O2, hydroxyl radical or singlet oxygen in a living system [48]. Since, scavenging antioxidants activity have to reduce the power of effective managing the diseases such as stomach problems, cancer, ulcer, and AIDS. Antioxidant while reacts with nitric oxide forms peroxynitrite which can produce radicals in toxic condition such as hydroxyl radicals [49].

CONCLUSION

The results showed that *Gloriosa superba* tubers contain variety of Phytochemicals. The methanol extracts of *Gloriosa superba* reveals that free radical scavenging activity in all five different accessions. The maximum antioxidant enzymes such as DPPH ABTs activity, SOD, PO, PPO and PAL activities shows maximum in Vedaranyam (GA5) and Konganapuram (GA4) accessions.

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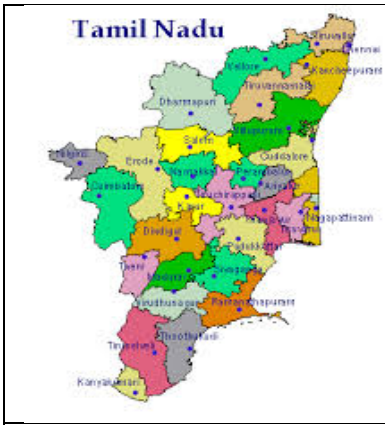

Tamil Nadu State

Dindigul district (GA1) Sirumalai Accession

Tiruppur district (GA2) Mulanoor Accession

Tiruchirappalli district (GA3) Thuraiyur Accession	Salem district (GA4) Konganapuram Accession	Nagapattinam district (GA5) Vedaranyam Accession

Figure. 1. Map shows different districts of Tamil Nadu for *Gloriosa superba* L cultivated area selected for accession studies.

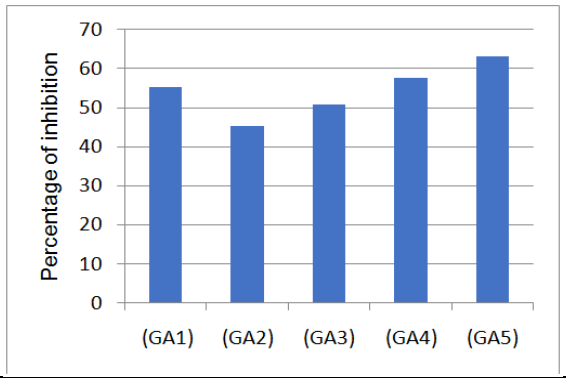

Fig. 2.Changes in the DPPH activity in the tubers of *G.superba grown in various accessions.* Values shown are mean ± S.E for five replicate experiments. Significant difference *p < 0.05.

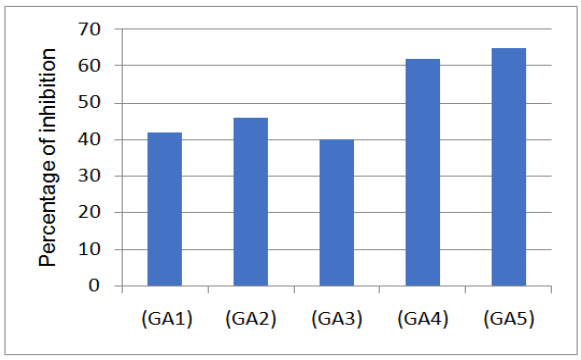

Fig. 3.Changes in the Superoxide radicals in the tubers of *G.superba grown in various accessions.* Values shown are mean ± S.E for five replicate experiments. Significant difference *p < 0.05.

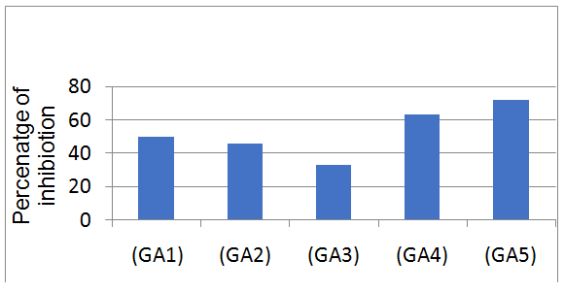

Fig. 4.Changes in the ABTS scavenging activity in the tubers of *G.superba grown in various accessions.* Values shown are mean ± S.E for five replicate experiments. Significant difference *p < 0.05

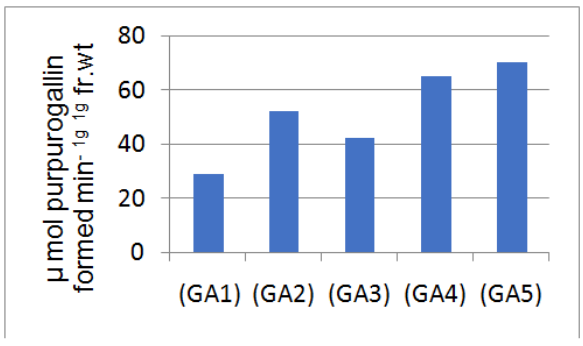

Fig. 5.Changes in the Peroxidase activity in the tubers of *G.superba grown in various accessions.* Values shown are mean ± S.E for five replicate experiments. Significant difference *p < 0.05.

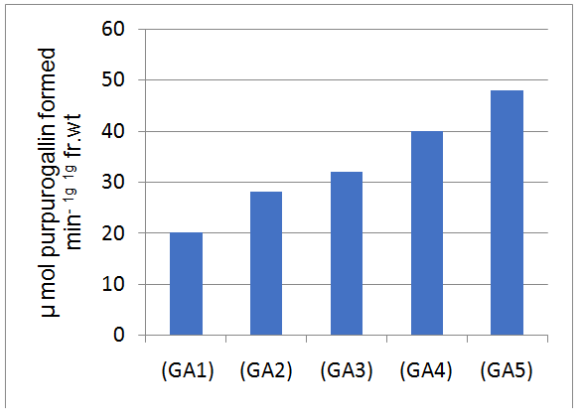

Fig. 6.Changes in the polyphenol oxidase activity in the tubers of *G.superba grown in various accessions.* Values shown are mean ± S.E for five replicate experiments. Significant difference *p < 0.05.

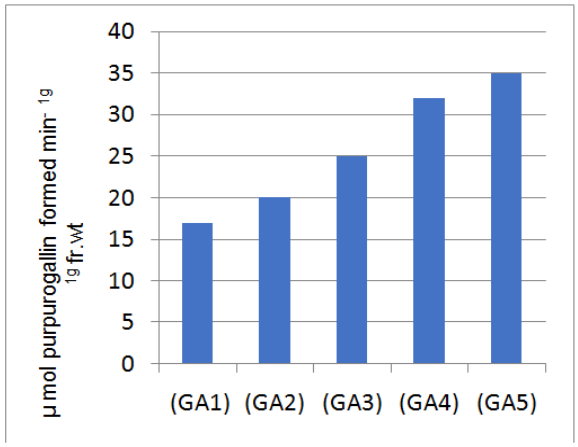

Fig.7.Changes in the Phenylalanine ammonialyase activity in the tubers of *G.superba grown in various accessions.* Values shown are mean ± S.E for five replicate experiments. Significant difference *p < 0.05

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REVIEW ARTICLE

Review of Hydrogels for Drug Delivery at Present

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ABSTRACT

Hydrogel products constitute three-dimensional networks which contains a group of polymeric materials, the hydrophilic structure of which renders them capable of holding large amounts of water. These biomaterials can integrate large quantum of biological fluids and swell. When swelled, they are soft & rubbery and resemble the living tissue, exhibiting excellent biocompatibility. Broad use of these products in a number of industrial and environmental areas of application is considered to be of prime importance. Today, drug delivery experience several challenges where hydrogel could be one potential answer to those. Due to the vast properties of hydrogel they are widely exposed to different biomedical fields. The primary objective of this article is to concerning classification of hydrogels on different bases, properties of hydrogels and its methods of preparations, specific therapeutic areas using hydrogels for drug delivery at present and its application.

Keywords: hydrogel, cross-linked, co-polymer, swelling, drug delivery

INTRODUCTION

Hydrogels are three-dimensional, crosslinked networks of water-soluble polymers. Hydrogels can be made from virtually any water-soluble polymer, encompassing a wide range of chemical compositions and bulk physical properties. Further- more, hydrogels can be formulated in a variety of physical forms, including slabs, microparticles, nanoparticles, coatings, and films. As a result, hydrogels are commonly used in clinical practice and experimental medicine for a wide range of applications, including tissue engineering and regenerative medicine, diagnostics, cellular immobilization, separation of biomolecules or cells and barrier materials to regulate biological adhesions. The unique physical properties of hydrogels have sparked particular interest in their use in drug delivery

applications. Their highly porous structure can easily be tuned by controlling the density of crosslinks in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity also permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule or macromolecule through the gel network. Indeed, the benefits of hydrogels for drug delivery may be largely pharmacokinetic especifically that a depot formulation is created from which drugs slowly elute, maintaining a high local concentration of drug in the surrounding tissues over an extended period, although they can also be used for systemic delivery.

Hydrogels are also generally highly biocompatible, as reflected in their successful use in the peritoneum and other sites in vivo. Biocompatibility is promoted by the high water content of hydrogels and the physiochemical similarity of hydrogels to the native extracellular matrix, both compositionally (particularly in the case of carbohydrate-based hydrogels) and mechanically. Biodegradability or dissolution may be designed into hydrogels via enzymatic, hydrolytic, or environmental (e.g. pH, temperature, or electric field) pathways; however, degradation is not always desirable depending on the time scale and location of the drug delivery device. Hydrogels are also relatively deformable and can conform to the shape of the surface to which they are applied. In the latter context, the muco or bio adhesive properties of some hydrogels can be advantageous in immobilizing them at the site of application or in applying them on surfaces that are not horizontal.

Despite these many advantageous properties, hydrogels also have several limitations. The low tensile strength of many hydrogels limits their use in loadbearing applications and can result in the premature dissolution or flow away of the hydrogel from a targeted local site. This limitation may not be important in many typical drug delivery applications (e.g. subcutaneous injection). More important, perhaps, are problems relating to the drug delivery properties of hydrogels. The quantity and homogeneity of drug loading into hydrogels may be limited, particularly in the case of hydrophobic drugs. The high water content and large pore sizes of most hydrogels often result in relatively rapid drug release, over a few hours to a few days.

Ease of application can also be problematic; although some hydrogels are sufficiently deformable to be injectable, many are not, necessitating surgical implantation. Each of these issues significantly restricts the practical use of hydrogel-based drug delivery therapies in the clinic. In this review, we focus on recent developments addressing three key clinically relevant issues regarding the use of hydrogels for drug delivery: facilitating the in vivo application of drug-eluting hydrogels, extending their duration of drug release, and broadening the range of drugs which they effectively deliver.

CLASSIFICATION OF HYDROGELS [7]

Hydrogels can be classified into two groups based on their natural or synthetic origins. Classification according to polymeric composition, the method of preparation leads to formations of some important classes of hydrogels:

Homopolymeric hydrogels: are referred to polymer networks derived from a single species of monomer, which is a basic structural unit comprising of any polymer network. Homopolymers may have cross-linked skeletal structure depending on the nature of the monomer and polymerization technique.

Copolymeric hydrogels: are comprised of two or more different monomer species with at least one hydrophilic component, arranged in a random, block or alternating configuration along the chain of the polymer network.

Multipolymer interpenetrating polymeric hydrogel (IPN): an important class of hydrogels, is made of two independent cross-linked synthetic and/or natural polymer component, contained in a network form. In semi-IPN hydrogel, one component is a cross-linked polymer and other component is a non-cross-linked polymer.

ADVANTAGES OF HYDROGEL [8, 9]

- Biocompatible
- Can be injected in vivo (in a whole, living organism) as a liquid that then gels at body temperature
- Protect cells
- Good transport properties (such as nutrients to cells or cell products from cells)
- Timed release of medicines or
- Easy to modify
- Can be biodegradable or bioabsorbable

DISADVANTAGES OF HYDROGEL: [10- 13]

- Low mechanical strength
- Can be hard to handle
- Difficult to load with drugs/nutrients
- May be difficult to sterilize
- Non-adherent

IMPORTANT PROPERTIES OF HYDROGEL: [14]

Swelling properties

All polymer chains in hydrogels are cross linked to each other either physically or chemically and thus, considered as one molecule regardless of its size. For this reason, there is no concept of molecular weight of hydrogels and therefore, sometimes called infinitely large molecules or super macromolecules. One of the variables that effects capacity of water absorption is the degree of cross linking and the type of cross linking agent used. A small change in environmental condition may trigger fast and reversible changes in hydrogel. The alteration in environmental parameters like ph, temperature, electric signal, presence of enzyme or other ionic species may lead to a change in physical texture of the hydrogel. These changes may occur at macroscopic level as precipitate formation, changes in size and water content of hydrogels. The amount of the aqueous medium incorporated in a hydrogel is determined gravimetrically and can be expressed by its swelling ratio.

$$\text{Swelling} = \frac{WS - WD}{WD}$$

Where, WS is the weight of hydrogel in swollen state and WD is the weight of hydrogel in dry state. The difference in concentration of mobile ions in the hydrogel interior relative to external solution (osmotic pressure), changes in solvent ph, drives the volume change. Hydrogels with acidic or basic functional groups respond to the fluctuations in the external environmental ph. degree of ionization of the functional groups dictates its swelling profile and hence the volume changes.

Mechanical properties

Mechanical properties of hydrogels are very important from the pharmaceutical and biomedical point of view. the evaluation of mechanical property is essential in various biomedical applications viz. ligament and tendon repair, wound dressing material, matrix for drug delivery, tissue engineering and as cartilage replacement material. The mechanical properties of hydrogels should be such that it can maintain its physical texture during the delivery of therapeutic moieties for the predetermined period of time. By changing the degree of crosslinking the desired mechanical property of the hydrogel can be achieved. Increase in the degree of crosslinking, a stronger hydrogel can be obtained through the higher degree of crosslinking decreases the % elongation of the hydrogels creates a more brittle structure.

Biocompatible properties

It is important for the hydrogels to be biocompatible and nontoxic in order to make it applicable in biomedical field. Most polymers used for this purpose must pass cytotoxicity and in-vivo toxicity tests. Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application. Biocompatibility studies consists of two parameters namely biosafety and bio functionality.

a) Biosafety i.e. appropriate host response not only systemic but also local (i.e. surrounding tissue), the absence of cytotoxicity, mutagenesis, and/or carcinogenesis and
b) Bio functionality i.e. the ability of material to perform the specific task for which it is intended.

This definition is particularly relevant in tissue engineering since the nature of tissue construct is to continuously interact with the body through the healing and cellular regeneration process as well as during scaffold degradation. Furthermore, initiators, organic solvents, stabilizers, emulsifiers, unreacted monomers and crosslinkers used in polymerization and hydrogel synthesis may be toxic to host cells if they seep out to tissues or encapsulated cells. To remove hazardous chemicals from preformed gels, various purification processes should be followed such as solvent washing or dialysis.

PREPARATION OF HYDROGELS:[15, 16, 17]

Hydrogels are polymeric networks. This implies that crosslinks have to be present in order to avoid dissolution of the hydrophilic polymer chain in aqueous solution. The various methods for crosslinking are as follows:

Crosslinking of polymers: In this method chemically crosslinked gels are formed by radical polymerization of low molecular weight monomers, or branched homopolymers, or copolymers in the presence of crosslinking agent. This reaction is mostly carried out in solution of biomedical applications.

Copolymerization/crosslinking reactions: Copolymerization reactions are used to produce polymer gels, many hydrogels are produced in this fashion, for example poly (hydroxyalkyl methylacrylates).

Crosslinking by high energy radiation: High energy radiation, such as gamma and electron beam radiation can be used to polymerize unsaturated compounds. Water soluble polymers derivatized with vinyl groups can be converted into hydrogels using high energy radiation.

Complex coacervation: Complex coacervate gels can be formed by mixing of a polyanion with a polycation. The underlying principle of this method is that polymers with opposite charges stick together and form soluble and insoluble complexes depending on the concentration and ph of the respective solutions (figure 2). One such example is coacervating polyanionic xanthan with polycationic chitosan. Proteins below its isoelectric point are positively charged and likely to associate with anionic hydrocolloids and form polyion complex hydrogel (complex coacervate)

Crosslinking using enzymes: Recently a new method was published using an enzyme to synthesize peg-based hydrogels. A tetrahydroxy peg was functionalized with addition of glutaminyl groups and networks were formed by addition of transglutaminase into solution of peg and poly (lysine-cophenylalanine). Several techniques have been reported for the synthesis of hydrogels. A chromia alumina hydrogel was prepared as in the preceding example except that ammonium nitrate was substituted as ammonium sulfate as Base Exchange solution. A portion of washed hydrogel was impregnated in 13 liters of an aqueous solution maintaining 775 g of copper acetate and 223 g of potassium acetate. The impregnated hydrogel was dried in 100% steam at 260-270o f and tempered 4 hours at 1100o f in a hydrogen atmosphere.

Polyvinyl alcohol–gelatin hydrogel was prepared, in short, 2.5 g of gelatin was dissolved in 100 ml of a 10% aqueous solution of pva. Concentrated hydrochloric acid (hcl, 0.05 ml) was added, and the resulting dispersion was stirred (using an overhead stirrer at 100 ± 5 rpm) at 70°c for a half-hour to carry out the esterification reaction between pva and gelatin. * Hydrogel sheets based on poly (vinyl alcohol) (pva) and poly (vinyl acetate) (pvac) have been prepared by the technique of acetalization of pva using formaldehyde and grafting of acrylic acid onto pvac by gamma irradiation. pva hydrogel (pvab) sheets have been prepared in geometrically stable shapes by compression moulding process.

semicrystalline crosslinked poly (vinyl alcohol) hydrogels in the form of films were prepared by electron beam irradiation and a subsequent slow dehydration process at 25 ± 1°, using various drying agents.1 as a result, hydrogels synthesized contain weakly acidic groups like carboxylic acids, or a weakly basic group like substituted amines, or a strong acidic and basic group like sulfonic acids, and quaternary ammonium compounds. The synthesis of hydrogel in industry is consist of solution and reversed suspension and reversed emulsion polymerizations.

SPECIFIC THERAPEUTIC AREAS USING HYDROGELS FOR DRUG DELIVERY AT PRESENT

Ophthalmic

Conventional eye drops have problems with sustained drug delivery and there is huge wastage of drug immediately following application through eye drainage. dextenza is a very recently FDA approved (3 december 2018) ocular therapeutic hydrogel formulation for human use. This is used for ocular pain following ophthalmic surgery and is the first intracanalicular implant developed for drug delivery and developed by the company ocular therapeutix (BEDFORD, MA, USA) [17]. Thermo-responsive polymer developed by mixing poly (acrylic acid-graft-nisopropylacrylamide) (PAAc-graft-PNIPAAm) with PAAc-co-PNIPAAm gel and incorporating epinephrine was used in the in vitro evaluation of ophthalmic drug release. The approach augmented the effect of intraocular pressure reduction from 8 h with the traditional drops to 36 h. the crosslinking density of the hydrogel affected the capillary network formation and offered a convenient controlled drug release method for ophthalmic drug delivery. [18].

Intra-ocular pressure (IOP) elevates during glaucoma and alleviating this pressure has been quite challenging. Hydrogels could be used to resolve this problem by using them to prepare soft contact lenses composed of polymers to form networks. The highly hydrated polymer networks of hydrogels cause the drug to elute out very rapidly and this is not favorable for glaucoma therapy, which mainly uses hydrophilic drugs. However, with suitable modifications, soft contact lenses have been developed using polymers of n,n-diethylacrylamide and methacrylic acid, which delivered the hydrophilic drug timolol for about 24 h, thereby opening up ways to allow sustained hydrophilic drug delivery using hydrogels. Storing the contact lenses in a hydrated state can leach out drug and to wear them all the time are the limitations though [19]. Inner layer-embedded contact lenses have been investigated for the sustained release of highly water-soluble drug betaxolol hydrochloride on the ocular surface. Cellulose acetate and eudragit s100 were selected as the inner layer of the contact lenses which showed a promising sustained drug release for over 240 h in tear fluid of rabbits in vivo to create a controlled-release carrier of the drug in ophthalmic drug delivery [19].

Controlled drug release behavior from hydrogels was also evaluated using nepafenac as the model drug. 3d cross-linked thermos and pH sensitive hydrogel was designed that was composed of carboxymethylchitosan (CMC) and poloxamer with glutaraldehyde as the cross-linking agent. The hydrogel was found to undergo reversible sol-gel transition at temperature and /or pH alteration at a very low concentration. Sustained release of the drug nepafenac was observed in the in vitro model and maximum release was observed at 35 °c and pH 7.4. Cytocompatibility of the hydrogel with human corneal epithelial cells was high [20].

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Oral, Intestinal

Gastroretentive drug dosage forms (GRDDFs) are particularly attractive for drugs that are absorbed in the proximal part of gastroinstestinal tract. Enhancing the retention time of the drugs in the GI tract is very important in order to improve their bioavailability and enhance their therapeutic effects. These dosage forms could be exploited for their muco-adhesion to the gastric mucosa, modified to float or sink in order to prevent leaving the stomach or increase their swelling behavior and make them as large to prevent passage through pylorus for prolonged periods. Based on these ideas, polyionic complex hydrogels of chitosan with ring-opened PVP have been developed for osteoporosis therapy. The formulation was used to release alendronate in the upper GI tract. Enhanced muco-adhesion, delayed clearance from swelling, minimal localized irritation, improved bioavailability and slower release of the active ingredients are the interesting aspects of the preparation. Also, in vivo experimentation showed that these hydrogels could provide optimized PK properties that maintained the drug in the therapeutic levels for a sustained period of time, minimizing fluctuations in therapeutic levels, hence also the possible side effects [22].

Inflammatory diseases such as irritable bowel syndrome have been recently treated using hydrogels. These provided safer alternatives to delivery methods that may cause systemic toxicity. zhang et al. developed negatively charged hydrogels that preferentially accumulated in the positively charged inflamed colon and acted as carriers of the corticosteroid drug dexamethasone (DEX). The hydrogel was prepared from ascorbyl palmitate which had labile bonds responsive to inflammatory conditions and was generally regarded as safe (GRAS) for administration. Enema administration to the colon of inflammation targeting (it) hydrogel microfibers not only reached the target site but also stayed there owing to charge interaction. The formulation was therapeutically very efficacious and revealed lesser systemic drug exposure than with free DEX in the IBS mice model in vivo [23]

complexation hydrogel prepared from poly (methacrylic acid-g-ethylene glycol) [P(MAA-g-EG) has been described. the targeting ligand used is the octarginine cell-penetrating peptide that causes specific delivery of insulin to the intestine. this method facilitated ideal targeting, absorption at target and allowed immediate release of insulin from absorption site. great hypoglycemic responses were achievable and increased insulin absorption was noted from diabetic rat models used for testing. 18% glucose reduction was observed immediately on administration of the hydrogel containing insulin[24]

Cardiac illness and cancer

Myocardial infarction is a leading cause of death and disability in the world. Intramyocardial administration of biomaterials such as hydrogels along the perimeter region of myocardial infarction has proven to be beneficial. chen et al proposed the use of a combination of curcumin (known for its anti-oxidant, antiinflammatory and anti-oxidation properties) and nitric oxide (known as an anti-angiogenesis agent) in a hydrogel to treat myocardial infarction. The mixed component hydrogel created with the combination drugs improved therapeutic efficacy synergistically. protective effects such as myocytic apoptotic death alleviation, reduced collagen deposition, increased vessel density (attributable to no in the combination) and upregulated silent information regulator 1 (SIRT-1), a histone deacetylase that confers resistance to the heart from ischemic injury were observed in diseased mice models in vivo. the hydrogel was prepared using peptide derivatives of curcumin and NO in a ratio of 4:1 and

showed sustained curcumin release at a low concentration of 2.5 μg per ml per 24 h. NO was released in presence of the enzyme β-galactosidase that could break glucosidic bonds to release no [25]

Growth factors and cytokines (paracrine factors) secreted by stem cells have been proven to be effective in repairing damaged myocardial tissue. The whole cocktail of the paracrine factors is referred to as a secretome and is isolated in vitro. The biomolecular composition of the secretome can be manipulated suitably by varying stem cell culture conditions. An injectable hydrogel to deliver to peri-infarct myocardium has been recently developed using secretome from human adipose derived stem cell secretome. Nano-composite hydrogel was formed from a combination of gelatin and laponite carryi ng the secretome and tested both in vitro and in vivo for their therapeutic effects via monitoring angiogenesis, scar formation and heart function. Significantly reduced scar area and improved cardiac function were observed in vivo in the secretome loaded hydrogel group in relation to the control [26]

The very recent development of a paintable hydrogel to serve as cardiac patch for treating myocardial infarction is worth mentioning in this context. The hydrogel eliminates the damage to tissue through suture or light triggered reactions as it is paintable. It has been constructed by a Fe3+ triggered polymerization reaction wherein the covalently linked pyrrole and dopamine undergo simultaneous polymerization with the trigger and the conductive polypyrrole produced also uniquely cross-links the network further. The functional patch is both adhesive and conductive and forms a suture-free alternative for reconstruction of cardiac function and revascularization. Bonding within 4 weeks to the beating heart boosts the transmission of electrophysiological signals with conductivity profiles equivalent to that of the normal myocardium [27]

Bio-material based immunotherapy platforms for targeted drug delivery to cancers are the latest trend observable in cancer therapy. based on this idea, novel STINGels have been developed by leach et al, that are peptide hydrogels to show controlled delivery of cyclic dinucleotides (CDns). Dramatic improvement in survival was observed in murine models of head and neck cancer in comparison to CDn alone or CDn delivered from a collagen hydrogel [28]. Thyroid cancer treatment using local drug delivery system formed of glycol chitosan (GC) hydrogel and doxorubicin hydrochloride (DOX·HCL) called gc10/dox has been recently developed (figure 7). Visible light regulated the storage and swelling aspects of the hydrogel and a controlled sustained release followed the initial burst release within 18hours. Potent antitumor effects were observed in vivo and in vitro in comparison to free DOX·HCL and this is a promising research direction for thyroid cancer therapy [29]

Injectable hydrogels responsive to reactive oxygen species that degrade in the presence of ROS and promote immunogenic tumor phenotype via local gemcitabine delivery is a recent discovery. The PVA cross-linked hydrogel with ROS-labile linkers enhance anti-tumor response with a localized release of immune checkpoint blocking antibody (anti-PD-L1 blocking antibody (aPDL-1) in in vitro and immunogenic in vivo mouse models. tumor recurrence prevention after primary resection is the therapeutic advantage of this chemo-immunotherapy. [30]

APPLICATIONS OF HYDROGELS IN DRUG DELIVERY [31- 33]

Hydrogels have attracted considerable attention as excellent candidates for bioadhesive devices, controlled release devices and targetable devices of therapeutic agents. Hydrogel-based delivery devices can be used for oral, rectal, ocular, epidermal and subcutaneous application. Various sites that is available for the application of hydrogels for drug delivery.

Drug delivery in the oral cavity

Drug is incorporated into hydrogels and delivers to oral cavity for local treatment of diseases of the mouth, such as stomatitis, fungal diseases, periodontal disease, viral infections, and oral cavity cancers.

Drug delivery in the GI tract

GI tract is the most popular route of drug delivery because of the facility of administration of drugs for compliant therapy, and its large surface area for systemic absorption. like buccal delivery, hydrogel-based devices can be designed to deliver drugs locally to the specific sites in the gi tract. for example, stomach-specific antibiotic drug delivery systems for the treatment of helicobacter pylori infection in peptic ulcer disease.

Wound healing

Hydrogels have the ability to hold water and drug in them due to their cross linked structure. Due to their water holding ability they can hold and retain wound exudates. Gelatin and sodium alginate based hydrogels when applied have the ability to cover and protect the wound from bacterial infection.

Hydrogels for brain

Blood brain barrier is also a challenge for drug delivery like other barriers in human body, concerning 98 % of the newly synthesized drugs fail to cross this barrier. Due to that reason a low number of drugs are present for drug delivery for cns. camptothecin having long termed sustained release drug is loaded with plga microspheres which was observed in rats. These microspheres increase the survival period in rats against malignant gliomas.

Rectal delivery

It is well known that drugs absorbed from the lower part of the rectum drain into the systemic circulation directly. Thus, the rectal route is a useful for the drug administration having first pass metabolism. Its primary applications have been for local treatment of diseases associated with the rectum, such as hemorrhoids.

Ocular drug delivery

Hydrogels are most widely used in ocular drug delivery system. Most of hard and soft contact lenses are formed of polymers in form of hydrogel films. In-situ forming hydrogels are attractive as an ocular drug delivery system because of their facility in dosing as a liquid, and their long-term retention property as a gel after dosing.

Subcutaneous Delivery

Hydrogels are biodegradable in nature by utilization of this property we can form biodegradable implantable hydrogels. Used in subcutaneous delivery of anticancer drugs is being prepared viz. cross linked PHEMA which is applied to cytarabine.

Transdermal Delivery

Various hydrogel based drug delivery device are formed to deliver drug through transdermal route. Swollen hydrogels can be used as controlled release devices in the field of wound dressing. Hydrogel based formulations are being explored for transdermal iontophoresis to obtain enhanced permeation of products viz. hormones and nicotine.

Topical drug delivery

Hydrogels have been used to deliver active component like desonide which is a synthetic corticosteroid usually used as an anti-inflammatory. The hydrogels have been formulated for better patient compliance having moisturizing properties therefore scaling and dryness is not expected with this drug delivery system.

CONCLUSION

Recently, many hydrogel based networks have been designed and personalized to meet the needs of different applications. When putted in contact with an aqueous solution these hydrogels is either ability to swell. The present review demonstrates about the classification of hydrogels on different bases, specific therapeutic areas using hydrogels for drug delivery at present, method of preparation and application. From the study we find that the hydrogels have fantastic properties that they will have abundant future applications as the next generation biomaterials. That's why hydrogels also called a smart or intelligent biomaterial. There are present various methods by which hydrogels can be prepared as mentioned above.

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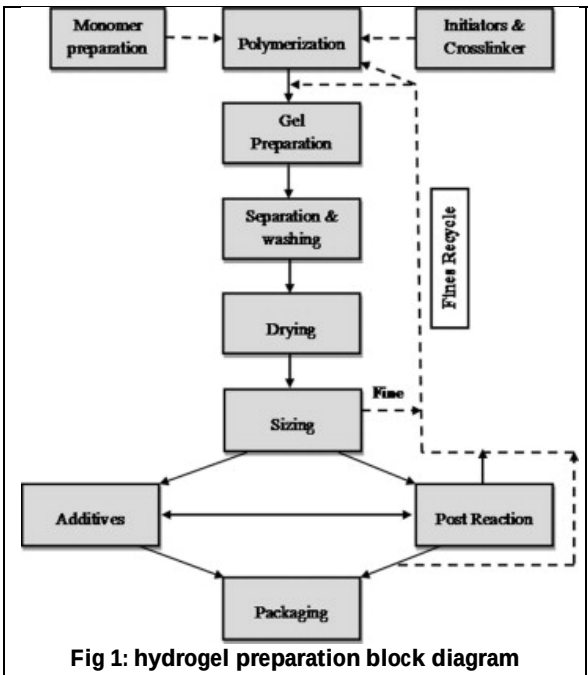

Fig 1: hydrogel preparation block diagram

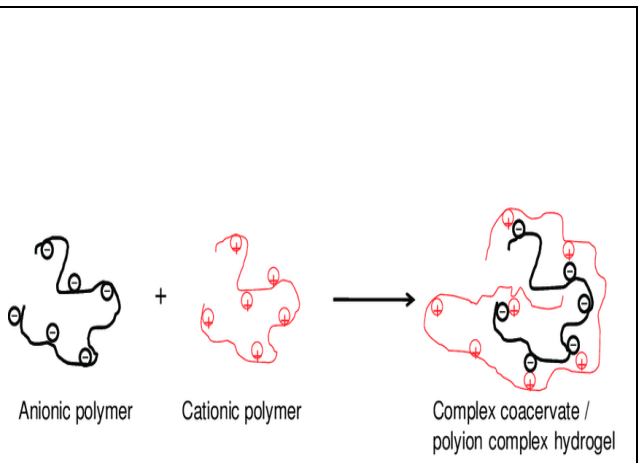

Fig 2: complex coacervation between a polyanion and a polycation.

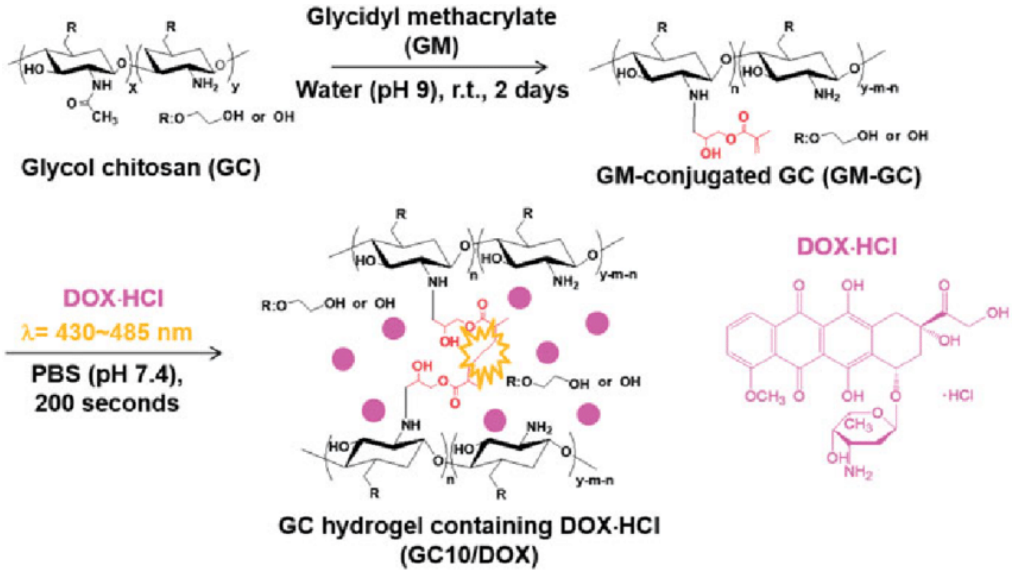

Figure 3: to glycol chitosan solution glycidyl methacrylate (GM) was added in water

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REVIEW ARTICLE

Electrospun Nanofibers- New Delivery System A Review

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ABSTRACT

Micro/ Nanofiber mats have been a subject of intensive research due to their high specific surface area and interconnected porous structure. These unique features, in addition to the intrinsic functionalities of polymers, impart nanofiber mats with many desirable properties for interesting applications in a multitude of fields. Biomedical applications of Nanofibers such as medical prostheses, drug release, wound dressing, tissue engineering. Methods have been applied for generating micro/nanofiber mats such as Electro spinning, Phase separation, Template synthesis, Melt-blown, Self-assembly, Three-dimensional (3D) printing. Electrospinning is a versatile and fascinating technology to produce ultra-fine fibers with diameter from several micrometers even to sub-nanometers. Electrospinning involves an electrohydrodynamic process and there is a unique approach using electrostatic forces to produce fine fibers.

Keywords: Nanofibers, Electospinning technique, Drug delivery system.

INTRODUCTION

Novel drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimization of drug related side effects due to controlled therapeutic blood levels instead of oscillating blood levels, improved patient compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered. [1, 2]

Liposomal and Targeted Drug Delivery System

Drug delivery systems can in principle provide enhanced efficacy and reduced toxicity for anticancer agents.[3] Liposomal have achieved highly efficient drug encapsulation, resulting in significant anticancer activity with reduced cardiotoxicity, and include versions with highly prolonged circulation such as liposomal daunorubicin and pegylated liposomal doxorubicin. Pegylated liposomal doxorubucin has shown substantial efficacy in breast cancer treatment both as monotherapy and in amalgamation with other chemotherapeutics. Additional liposome demolish are being developed for the delivery of other drugs. The next generation of delivery systems will include true molecular targeting immune liposomes and other ligand-directed demolish represent an combine the biological components capable of tumor recognition with delivery technologies. [4]

Lung-Specific Drug Delivery

Pulmonary drug delivery is a several advantages in the treatment of respiratory diseases over other routes of administration. The local pulmonary shipment and delivery of the administered drug facilitates a targeted treatment of respiratory diseases, such as pulmonary arterial hypertension (PAH), without the need for high dose subjection by other routes of administration. [5]

Intra-ventricular/ Intrathecal delivery

Here, using a plastic reservoir, which implanted subcutaneously in the scalp and connected to the ventricles within the brain by an outlet catheter. Drug injection into the CSF is a suitable strategy for sites close to the ventricles only. [6]

Intra-Nasal Drug Delivery

After nasal delivery drugs first reach the respiratory epithelium, the compounds can be absorbed into the systemic circulation by tran- cellular and para- cellular passive absorption, carrier mediated transport, and absorption through cytopempsis. When a nasal drug formulation is delivered deep and high enough into the nasal cavity, the olfactory mucosa may be reached and drug transport into the brain and CSF via the olfactory receptor neurons may occur. [7]

Transdermal Delivery

Bioadhesive liposomes relevance levonorgestrel as controlled drug delivery system has been studied.[8] The vesicles were mostly uni-lamellar and some were multi lamellar. Release was of zero order kinetics. Alcohol as compared to oils had greater effect on transdermal flux. In vivo studies showed that a significant lag phase was observed before the therapeutic levels were reached indicating the requirement for a loading dose. This pro liposomes system was found to be superior to PEG-based ointment system.[9] The drug delivery across human cadaver skin was very slow. In vivo studies affectation a longer duration of action in the case of liposomal formulation. [10]

Colon-Specific Drug Delivery

Delivery of drugs into systemic circulation through colonic absorption consider a novel mode of introducing peptide and protein drug molecules and drugs that are poorly absorbed from the upper gastrointestinal (GI) tract. [11] Oral colon-specific drug delivery systems offer clear advantages over parenteral administration. Colon targeting is naturally of value for the topical treatment of diseases of the colon such as Crohn's disease, ulcerative colitis and colorectal cancer. Sustained colonic release of drugs can be useful in the treatment of nocturnal asthma, angina and arthritis. Peptides, proteins, oligonucleotides, and vaccines are the potential candidates of interest for colon-specific drug delivery. [12]

Beaded Delivery Systems

Beaded delivery formulations are another method used to achieve long-acting drug levels associated with the convenience of once-a-day dosing. This system has been successfully linked to tolterodine tartrate and is available as Detrol LA. The drug delivery from this system is acid sensitive, in that drug levels are dependent on gastric acidity

for release. This process produces a pharmacokinetic pattern roughly similar to a zeroorder pattern, with C max obtained approximately 4 to 6 hours after ingestion and sustained levels observed for 24 hours after initial dosing. [13, 14]

Oral Drug Delivery

There is a great need in oral delivery of protein and peptide drugs, suitable devices for delivering the therapeutic agent incorporated microspheres selectively in the intestine. Gelatin capsules were coated with various concentrations of sodium alginate and cross linked with appropriate concentrations of calcium chloride and tested in vitro for resistance to gastric and intestinal medium. [15- 18]

Parenteral Drug Delivery

The advantage of this system is that the release kinetics of the drug from the system can be tailored by adjusting the plasticizer, homo polymer and cross linker composition. Chitosan microspheres of 45-300 μ were used for controlled delivery of progesterone.[19] Determination of in vivo bioavailability of the steroid from microsphere formulation by intramuscular injection in rabbits showed that a plasma concentration of 1-2 μg/ ml was maintained upto 5 months without a high burst effect. The data suggests that cross linked chitosan microspheres would be an interesting system for long term delivery of steroids. Cross linked dextran beads were developed as a carrier for development of a single contact vaccine delivery system. [20- 24]

RECENT DEVELOPMENTS IN NOVEL DRUG DELIVERY SYSTEM

Phytosome

Phytosomes are lipid compatible molecular complex which are composed of "phyto" which means plant and "some" meaning cell-like.[25] Complexing the polyphenolic phytoconstituents in the molar ratio with phosphatidyl choline results in a new herbal drug delivery system, known as "Phytosome". Phytosomes are advanced forms of herbal products that are better absorbed, utilized to produce better results than those produced by conventional herbal extracts. Phytosomes show better pharmacokinetic and therapeutic profiles than conventional herbal extracts. [26]

Nanoparticles

Nanotechnology is science of matter and material that deal with the particle size in nanometers. The word "Nano" is derived from Latinword, which means dwarf (1nm=10-9m). Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. [27] Nanoparticles offer some specific advantages such as they help to increase the stability of drugs/proteins and possess useful controlled release properties. It can be modified to achieve both active and passive targeting; drug loading is very high and can be administered by various routes such as parenteral, nasal, intra ocular and oral routes. [28]

Liposome

Liposome's are concentric bi-layered vesicles in which aqueous volume is entirely enclosed by a membranous lipid bi-layer mainly composed of natural or synthetic phospholipids. The liposome's are spherical particles that encapsulate the solvents which are freely floating in the interior. [29]

Emulsions

Emulsion is a biphasic system in which one phase is intimately disperse in the other phase in the form of minute droplets in ranging in diameter from 0.1μm to 100 μm. In emulsion, one phase is always water or aqueous phase, and the other phase is oily liquid, i.e. non aqueous. Among them, the micro emulsion is also called nanoemulsion, and the sub-micro-emulsion is called liquid emulsion. [30] Micro emulsion is a clear, thermodynamically stable, frequently in combination with a co-surfactant. [31]

Microsphere

Microsphere comprises of small spherical particles, with diameters in the micrometer range, typically 1 μm to 1000 μm (1 mm). Microspheres are sometimes referred to as micro-particles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres and ceramic microspheres are commercially available. Microspheres are classified as biodegradable or non-biodegradable. Biodegradable microspheres include albumin microspheres, modified starch microspheres, gelatin microspheres, polypropylene dextranmicrospheres, polylactic acid microspheres, etc. According to the current literature reports on non-biodegradable microspheres, polylactic acid is the only polymer approved to be used by people, and it is used as a controlled-release agent. Solid and hollow microspheres vary widely in density and therefore are used for different applications. [32]

Solid Lipid Nanoparticles (SLN)

It is a technique developed in the 1990s. It is a colloidal carrier used especially for the delivery of lipophilic compounds. The average mean size of solid lipid nanoparticles ranges from 50 nm to 1000 nm. Solid lipid nanoparticles are composed of lipid matrix, which becomes solid at room temperature and also at the body temperature. [33] The main features of solid lipid nanoparticles (SLNs) with regard to parenteral application are the excellent physical stability, protection of incorporated labile drugs from degradation. To cross blood brain barrier, it should be made for selection of lipids and surfactants. The SLNs are prepared by different methods such as homogenization and the warm micro-emulsion high-speed stirring ultrasonication and solvent-diffusion method. Lipids show compatibility with lipophilic drugs and increase the entrapment efficiency and drug-loading into the SLN. [34]

Niosomes

Niosomes are multilamellar vesicles formed from non-ionic surfactants of the alkyl or dialkyl polyglycerol ether class and cholesterol. Earlier studies, in association with L'Oreal have shown that, in general, niosomes have properties as potential drug carriers similar to liposomes. Niosomes are different from liposomes in that they offer certain advantages over liposomes. [35]

Proniosomes

Proniosomes gel system is step forward to niosome, which can be utilized for various applications in delivery of actives at desire site. Proniosomal gels are the formulations, which on in situ hydration with water from the skin are converted into niosomes. [36]

Ethosomes

The Ethosomes was developed and examined for their ability the topical absorption of Tetrandine through dermal delivery, and the relation of formulations to the pharmacological activity of Tetrandrine loaded in the formulation was also accessed. Result of the drug levels in rat plasma showed that when Tetrandrineloded Ethosomes were topically administered in rats the drug level was low to be detected in rat plasma. By providing fewer delivery of Tetrandrine into bloodstream, topical administration might offer favorable efficacy with reduced side effects, thus leading to improve patient's compliances. In conclusion, Ethosomes were demonstrated to be promising carrier for improving topical delivery of Tentrandrine via skin. [37]

TRANSDERMAL DRUG DELIVERY SYSTEM

Transdermal drug delivery system has been an increased interest in the drug administration via the skin for both local therapeutic effects on diseased skin (topical delivery) as well as for systemic delivery of drugs. But immense potential lies in transdermal drug as future smart drug delivery devices. [38]

Liquid Crystals

Liquid Crystals combine the properties of both liquid and solid states. They can be made to from different geometries, with alternative polar and non-polar layers (i.e., a lamellar phase) where aqueous drug solutions can be included. [39]

Hydrogels

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. They are used to regulate drug release in reservoir-based, controlled release systems or as carriers in swellable and swelling-controlled release devices. [40]

ELECTROSPINNING TECHNIQUES

Micro/nanofiber mats have been a subject of intensive research due to their high specific surface area and interconnected porous structure. [41] These unique features, in addition to the intrinsic functionalities of polymers, impart nanofiber mats with many desirable properties for interesting applications in a multitude of fields. [42- 44]
Several methods have already been applied for generating micro/nanofiber mats, such as:-
Phase separation. [45, 46]
Template synthesis. [47]
Melt-blown. [48, 49]
Self-assembly. [50, 51]
Three-dimensional (3D) printing. [52, 53]
Electro spinning. [54- 56]
Electrospinning is a versatile and fascinating technology to produce ultra-fine fibers with diameter from several micrometers even to sub-nanometers. So far, huge number of materials such as polymers, composite ceramics, metals, carbon nanotubes, even bacteria and virus can be fabricated/incorporated into micro/nano fibers by directly electrospinning or through post-spinning process. Many publications including reviews and research papers were focused on the process, the properties and the applications of electrospinning/electrospinning nanofibers. [57]
Electrospinning is a unique approach using electrostatic forces to produce fine fibers. Electrostatic precipitators and pesticide sprayers are some of the well known applications that work similarly to the electrospinning technique. Fiber production using electrostatic forces has invoked glare and attention due to its potential to form fine fibers. Electrospun fibers have small pore size and high surface area. There is also evidence of sizable static charges in electrospun fibers that could be effectively handled to produce three dimensional structures. [58]

Principle of Electrospinning: [59- 62]

Electrospinning involves an electrohydrodynamic process, during which a liquid droplet is electrified to generate a jet, followed by stretching and elongation to generate fiber(s). As illustrated in the basic setup for electrospinning is rather simple, making it accessible to almost every laboratory. The major components include a high-voltage power supply, a syringe pump, a spinneret (usually, a hypodermic needle with blunt tip), and a conductive collector. The power supply can be either direct current (DC) or alternating current (AC). During electrospinning, the liquid is extruded from the spinneret to produce a pendant droplet as a result of surface tension. Upon electrification, the electrostatic repulsion among the surface charges that feature the same sign deforms the droplet into a Taylor cone, from which a charged jet is ejected. The jet initially extends in a straight line and then undergoes vigorous whipping motions because of bending instabilities. As the jet is stretched into finer diameters, it solidifies quickly, leading to the deposition of solid fiber(s) on the grounded collector.

The electrospinning process can be divided into four consecutive steps:

- Charging of the liquid droplet and formation of Taylor cone or cone-shaped jet.
- Extension of the charged jet along a straight line.

- Thinning of the jet in the presence of an electric field and growth of electrical bending Instability (also known as whipping instability)
- Solidification and collection of the jet as solid fiber(s) on a grounded collector.

Advantages opf Electrospinning: [63- 69]

- A unique advantage of electrospinning is that complex hierarchical structures can be obtained via controlled calcinations
- Wet-chemistry methods such as polyol method, hydrothermal method and sol-gel synthesis have also been adopted to synthesize NWs.
- Hydro thermal method has been viewed as one of the efficient fabrication methods of inorganic nanomaterials. Specifically, TiO_2 NWs produced by this method have advantages of fine and controllable crystal form and good dispersibility.
- NWs prepared by sol-gel tend to display larger aggregated structures, which negatively affect their performance in an energy device.
- Considering the lengthy and complex procedures in the milling of NWs prepared by sol-gel , sol-gel is inferior to electrospinning. Generally speaking, electrospinning is a comprehensive, simple, and advantageous approach for fabrication of NWs or NFs.

Disadvantages of Electrospinning: [70]

- Electrospinning currently has several limitations. First, in the preparation of organic NFs, the variety of polymers used in electrospinning is limited and the structure and performance of NFs are not well researched.
- Second, the performance and range of application of electrospun inorganic NFs have been limited due to their friability after calcination, although inorganic NFs have a potential application in many fields such as energy devices, high temperature filtration, biological tissue engineering, and efficient catalysis.
- Third, electrospinning has been implemented at industrial level; however, in terms of producing fibers for the application of filters electrospinning is inferior to traditional methods due to its higher cost to produce fibers with large diameter.
- Furthermore, it remains a challenge to fabricate NFs with diameters less than 10 nm by electrospinning.

GENERAL APPLICATION OF NANOFIBERS

Lithium-Air Battery

Among many advanced electrochemical energy storage devices, rechargeable lithium-air batteries are of particular interest due to their considerable energy storing capacities and high power densities. As the battery is being used, lithium ions combine with oxygen from the air to form particles of lithium oxides, which attach to carbon fibers on the electrode. During recharging, the lithium oxides separate again into lithium and oxygen which is released back into the atmosphere. [71]

Optical Sensors

Polymer optical fibers have generated increasing interest in recent years. [72, 73] Because of low cost, ease of handling, long wavelength transparency, great flexibility, and biocompatibility, polymer optical fibers show great potential for short-distance networking, optical sensing and power delivery. [74, 75] developed a sensor that warns first responders when the carbon filters in their respirators have become saturated with toxic fume particles. [76]

Air Filtration

Electrospun nanofibers are useful for removing volatile organic compounds (VOC) from the atmosphere. Scholten et al. showed that adsorption and desorption of VOC by electrospun nanofibrous membrane were faster than the rates of conventional activated carbon. [77] Recent work with mining equipment manufacturers and the MSHA has shown

that nanofiber filter media can reduce cabin dust concentration to a greater extent compared to standard cellulose filter media. [78]

Oil-Water Separation

Nanofibers have the capabilities in oil–water separation, most particularly in sorption process when the material in use has the oleophilic and hydrophobic surfaces. These characteristic enable the nanofibers to be used as a tool to combat either oily waste- water from domestic household and industrial activities, or oily seawater due to the oil run down to the ocean from oil transportation activities and oil tank cleaning on a vessel. [79]

BIOMEDICAL APPLICATIONS

Most of human organs and tissues such as bone, collagen, dentin, and skin are present in a nanofibrous form. They are characterized by organized hierarchical fibrous structures that realign in nanometer scale, motivating and steering most of the nanofiber research towards biomedical and bioengineering applications. [80]

Medical Prostheses

Electrospun fibers exhibited high potential in prosthetic devices used in surgical operations. The soft texture and the nature of prepared fibers made them excellent candidates to be utilized as a coating for hard tissue prosthetic devices. This coating sheet with fibrous morphology inhibits the device failure by diminishing the stiffness mismatch at the tissue/device interphase by acting as interphase between the host tissues and the prosthetic system. It has also been reported that nanofibrous materials have been selected as unique candidates in a wide range of tissue prostheses including breast, vascular, blood vessel, etc. [81- 87]

Wound Dressing

Wound dressing represents a significant issue to be dealt with in the biomedical field. The warm, nutritious, and moist environment offered by wound beds offers a perfect condition for microbial growth.[88, 89] Excellent antimicrobial dressings should exhibit good broad-spectrum antimicrobial behavior, provision of a moist environment, gas permeation, and performance against antibiotic-resistant bacteria to improve healing processes. [90] Consequently, to avoid microbial infection, trans-epiderma water loss leading to an acceleration of wound regeneration and quick care of skin wounds are required. [91]

Drug Release

It is well known that the amount of drug actually delivered to the target site within the human body is much lower than the initial orally ingested drug dose, as it spreads to other healthy sites through the digestive organs. Hence, patients are required to take excessive amount of medication, causing unfavorable side effects. It has been found that the optimum drug amount is actually the minimum needed content of the drug to the target site and the minimum needed to be absorbed efficiently at the disease site. The rapidity of the uptake of the drug into the body is directly proportional to the size of the drug. Therefore, polymers including micelle and hydrogels were used to improve the drug carriers. [92, 93]

Tissue Engineering

The fabrication of promising matrices/scaffolds that mimic the biological function and structure of the natural extracellular matrix is one of the essential contests in the research of biomaterials and tissue engineering. [94] The smaller human cells can attach to the fibers with dimensions smaller than those of the human cells. Hence, nanofibers can offer a promising structure for cells to grow, migrate, and seed. The development of nanofibers for cell proliferation and adhesion is necessary for the organ and tissue regeneration. For this approach, it is essential to reproduce and create a three-dimensional biocompatible composite for cell growth for tissue replacement and repair

processes. Great attention has been given to design and develop scaffolds with biodegradable polymer and/or synthetic biopolymer nanofibers. [95- 97]

CONCULSION

The review presented in this article has focused on development of electospinning methods and electrospun nanofibers to suit or enable various application in many different fields such as biomedical, drug delivery, protective textiles, etc. Electrospinning method is a fascinating method to prepare members in micro or nano pore size. Electrospun nanofibers layers have higher porosity with uniform pore size distribution compared to conventional membranes.

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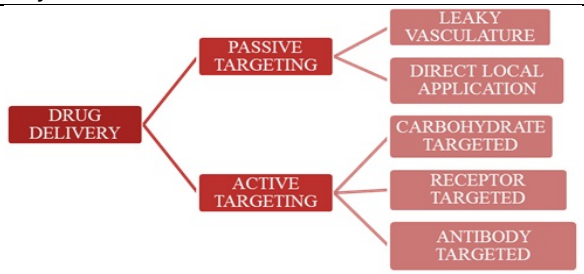

Fig.1: Various Drug Delivery Systems

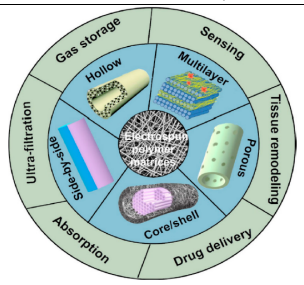

Fig. 2: Possible structures and applications of electrospun fibers.

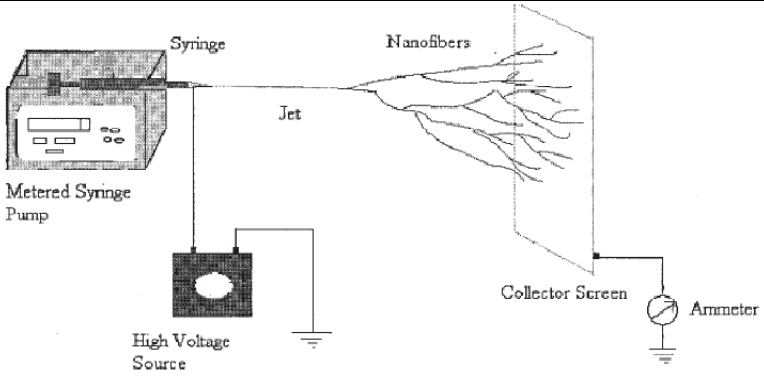

Fig.3: Schematic of electrospinning process.

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REVIEW ARTICLE

Biodegradable Nanoparticle Delivery System - An Overview

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ABSTRACT

The nanoparticles exhibit a unique physical, chemical and biological properties at nanoscale compared to their respective particles at higher scales. This phenomena is due to a relatively larger surface area to the volume, enhanced mechanical strength, increased reactivity or stability in a chemical process, etc. Biodegradable nanoparticles are colloidal particles (100 nm in diameter) with a gene of interest encapsulated inside a polymeric matrix. Biodegradable nanoparticles are used for site-specific delivery of drugs, vaccines and various other biomolecules. Biodegradable nanoparticles are prepared by using various materials such as proteins, polysaccharides and synthetic biodegradable polymers. Biodegradable polymeric nanoparticles are biocompatible, non-immunogenic, non-toxic and stable providing controlled/sustained release properties. Biodegradable nanoparticles provides a vast application in the areas of Drug delivery, Antigen detection, biocompatibility, Tracking and therapeutic agent.

Keywords: Nanoparticles, Biodegradable Nanoparticles, Vaccine/Gene Delivery, Cellular Trafficking

INTRODUCTION

Nanoparticles are particles size range between 1 and 100 nanometers (nm) with a surrounding interfacial layer, which is an integral part of nanoscale matter, fundamentally affecting all of its physico-chemical properties. The interfacial layer contains the ions, inorganic and organic molecules. Organic molecules are coating the inorganic nanoparticles they are known as stabilizers, capping and surface ligands, or passivating agents[1]. The nanoparticles has different shape, size and structure. It may spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc. Some of the nanoparticles are crystalline or amorphous with single or multi crystal solids it may be loose or

agglomerated. The nanoparticles exhibit a unique physical, chemical and biological properties at nanoscale compared to their respective particles at higher scales. This phenomena is due to a relatively larger surface area to the volume, enhanced mechanical strength, increased reactivity or stability in a chemical process, etc[2].

Advantages of nanoparticles [3-5]

- Nanoparticles are suitable for different route of administration.
- Carrying capacity of nanoparticles is high.
- Drug shelf-stability is increased.
- Ability to sustain and control drug release patterns.
- Suitable for combination therapy where two or more drug can be co-delivered.
- Both hydrophobic and hydrophilic drug can be incorporated.
- System increases the bioavailability of drugs.
- Imaging studies can be done by utilizing them.
- It is used for targeted drug delivery of drugs and development of new medicines which are safer.

Disadvantages of nanoparticles

- The manufacturing costs of nanoparticle are high which result in overall product cost, Solvents are toxic in nature which is used in the preparation process.
- Can start immune response and allergic reactions in body.
- Extensive use of poly (vinyl alcohol) as stabilizer may have toxicity issues.
- Nanoparticles are difficult to handle in physical form because particle-particle aggregation occurs due their small size and large surface area.[6,7,8]

Classification of Nanoparticles

Nanoparticles are broadly divided into various categories depending on their morphology, size and chemical properties. Based on physical and chemical characteristics, some of the well known classes of NPs are given as below (Fig.2)

Biodegradable Nanoparticles

Biodegradable nanoparticles are colloidal particles with a gene of interest encapsulated inside a polymeric matrix. They are typically 100 nm in diameter, and are formulated using FDA-approved, biodegradable, biocompatible polymers such as poly-D-L-lactide-co-glycolide (PLGA), polylactic scid (PLA), Poly-ε-caprolactone (PCL), Chitosan & Gelatin. Biodegradable nanoparticles have been used for site-specific delivery of drugs, vaccines and various other biomolecules.

Biodegradable Nanoparticles Preparation[9]

Biodegradable nanoparticles are prepared by using various materials such as proteins, polysaccharides and synthetic biodegradable polymers.

Selection of Polymers

The base polymer selection is based on various designs and end application criteria dependent on factors such as,

- Size of the desired nanoparticles.
- Properties of the drug (aqueous solubility, stability, etc.) to be encapsulated in the polymer.
- Surface characteristics and functionality.
- Degree of biodegradability and biocompatibility.
- Drug release profile of the final product.

Poly-D-L- lactide-co-glycolide (PLGA)

Poly-D-L- lactide-co-glycolide (PLGA) is one of the most successfully used biodegradable polymers in the preparation of nanoparticles. PLGA is synthesized by means of ring-opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. Polymers can be synthesized as either random or block co-polymers thereby imparting additional polymer properties[10]. PLGA undergoes hydrolysis in the body to produce the original monomers; lactic acid and glycolic acid. These monomers under normal physiological conditions are by products of various metabolic pathways in the body. Lactic acid is metabolized within the tricarboxylic acid cycle and eliminated via carbon dioxide and water. Glycolic acid is metabolized in the same way, and also excreted through the kidney[11]. PLGA NPs have been mostly prepared by the emulsification-diffusion, the solvent evaporation and the nano precipitation methods. PLGA nanoparticles have been used to develop the protein and peptide based nano-medicines, nano-vaccines, and genes containing nanoparticles for *in-vivo* delivery systems[12,13].

Polylactic acid (PLA)

PLA is a biocompatible and biodegradable polymer which is broken down to monomeric units of lactic acid in the body. Lactic acid is a natural intermediate product of anaerobic respiration, which is converted into glucose by the liver during the Cori cycle. Then the glucose is used as an energy source within the body. The use of polylactic acid nanoparticles is safe and devoid of any major toxicity. Mostly PLA nanoparticles are prepared by the solvent evaporation, solvent displacement, salting out and solvent diffusion methods[9,14]. The salting out procedure is based on the separation of a water- miscible solvent from aqueous solution by adding a salting out agent like magnesium chloride or calcium chloride. The main advantage of the salting out procedure is that it minimizes stress to protein encapsulants[12].

Poly-ε-caprolactone (PCL)

Poly-ε-caprolactone is degraded by hydrolysis of its ester linkages under the normal physiological conditions in the human body. PCL's slower rate of degradation compared to polylactides has make it better candidate for made long-term implantable devices. PCL nanoparticles have been prepared mostly by nano precipitation, solvent displacement and solvent evaporation[12,15,16]. Polycaprolactone (PCL) has been widely used in long-term implants and controlled drug release applications[17].

Chitosan

The chitosan salts are biocompatible and biodegradable making them useful as absorbable haemostats. The protonated chitosan is shattered by lysozyme in the body to glucosamine and therefore conjugate base of the acid (such as lactate or succinate), substances are naturally found in the body[18]. Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of crustacean derived natural biopolymer chitin. The preparation method for chitosan nanoparticles as ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex[19].

Gelatin

Gelatin is mostly used in food and medical products and is attractive for use in controlled release due to its non-toxic, biodegradable, bio-active and inexpensive properties. It is a polyampholyte having each cationic and anionic groups along with hydrophilic group. It is known that mechanical properties, swelling behavior and thermal properties depend significantly on the crosslinking degree of gelatin. Gelatin nanoparticles can be prepared by desolvation/coacervation or emulsion method[20].

Method of Preparation

Depending upon the selection of desired criteria for the preparation of nanoparticles, the methods can be classified as following,

- Dispersion of preformed polymers
- Polymerization of monomers
- Ionic gelation method for hydrophilic polymers

Specific Applications of Biodegradable NPs

Tumor Targeting

The rationale of using nanoparticles for tumour targeting is based on,

- NP's ability to deliver the requisite dose load of drug in the vicinity of the tumor due to the enhanced permeability and retention effect or active targeting by ligands on the surface of Nanoparticles.
- NP's ability to reduce the drug exposure to healthy tissues by limiting drug distribution to the target organ.

Active tumor targeting of Nanoparticles may be achieved with either direct targeting or the pretargeting method. In direct targeting method Nanoparticles are covalently coupled with the ligands. The ligand coupled Nanoparticles are received by the tumor cells expressing a homologous receptor on their surfaces. The specific ligand-receptor binding ensures that the NPs carrying drugs can get attached specifically to the tumour cells. This will facilitate delivery of drugs only to the tumour cells expressing receptor and not the normal healthy cells. In the pre-targeting approach, the therapeutic molecule is not coupled with the ligand and is administered after an appropriate delay time following the administration of the targeting ligand[21]. Expansive efforts have been devoted to achieving "active targeting" of nanoparticles in order to deliver drugs to the right targets. The molecular recognition processes such as ligand-receptor specificity or antigen antibody interaction plays important role in such targeting. Considering that folate receptors are over expressed on the surface of some human malignant cells and that cell adhesion molecules such as selectins and integrins are involved in metastatic events, nanoparticles bearing specific ligands such as folate may be used to target ovarian carcinoma while specific peptides or carbohydrates may be used to target integrins and selectins[22]. Demonstrated that the benefits of folate ligand coating were to facilitate internalization and retention of Gd-nanoparticles in the tumor cells/tissues[23].

Nanoparticles for Oral delivery

Oral delivery of drugs using nanoparticles has been shown to be far superior to the delivery of free drugs in terms of bioavailability, residence time, and bio-distribution[24]. Advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract. The drugs may also be exposed to gastrointestinal degradation by digestive enzymes. The advantage of using polymeric nanoparticles is to allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation[25]. The major interest lies in lymphatic uptake of the nanoparticles by the Peyer's patches within the GALT (gut associated lymphoid tissue). There have been many reports as to the optimum size for Peyer's patch uptake ranging from less than 1 μm to 5 μm[26,27]. However, it has also been shown that microparticles remain in the Peyer's Patches while nanoparticles are disseminated systemically[28,29]. Nanoparticles can be engineered not only for oral absorption, but can also be used to deliver a drug directly to the source for gastrointestinal uptake, thereby protecting the drug from low pH and enzymes in the stomach. The pH-sensitive nanoparticles made from a poly (methylacrylic acid and methacyrlate) copolymer can increase the oral bioavailability of drugs like cyclosporine- A by releasing their load at a specific pH within the gastrointestinal tract. The pH sensitivity allows this to happen as close as possible to the drug's absorption window through the Peyer's patches[30].

Nanoparticles for vaccine/gene delivery

Polynucleotide vaccines/ DNA vaccines/ plasmid vaccines work by delivering genes encoding relevant antigens to host cells wherever they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the

immune system[31]. The key ingredient of polynucleotide vaccines, DNA can be produced and has good storage and handling properties than the ingredients of the majority of protein-based vaccines. Hence, polynucleotide vaccines/DNA vaccines are set to supersede many conventional vaccines particularly for immunotherapy. However, there are many issues related to the delivery of polynucleotides which limit their application. These issues including the efficient delivery of polynucleotide to the target cell population, its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotides is maintained during delivery to the target site[32]. Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment[33].

Nanoparticles for drug delivery into the brain

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system[34]. The BBB is characterized by relatively impermeable endothelial cells with enzymatic activity, tight junctions and active efflux transport systems. It effectively prevents the passage of water soluble molecules from the blood circulation into the CNS, and consequently only permits selective transport of molecules that are essential for brain function[35]. Strategies for nanoparticle targeting to the brain rely on nanoparticle's interaction with the specific receptor mediated transport systems in the BBB. For example, transferrin receptor binding antibody (such as OX26), polysorbate 80/LDL, cell penetrating peptides, lactoferrin and melanotransferrin have been shown to be capable of delivery of a self non-transportable drug to the brain via the chimeric construct that can undergo receptor-mediated transcytosis [36-39]. It has been reported that poly(butylcyanoacrylate) nanoparticles were able to deliver hexapeptide dalargin, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB[38]. Despite some reported success with polysorbate 80 coated NPs, this system does have many shortcomings including desorption of polysorbate coating, rapid NP degradation and toxicity caused by presence of high concentration of polysorbate 80[40]. Nanoparticles were also functionalized with thiamine surface ligands. Average diameter of these particles are 67 nm, were able to associate with the blood brain barrier thiamine transporters and thereby increase the unidirectional transfer coefficient for the particles into the brain[41].

Applications of Biodegradable Nanoparticles other than drug delivery

Diagnosis and imaging are important applications of nanoparticles that are briefly described. The ability to encapsulate or conjugate fluorescent compounds into or onto biodegradable nanoparticles has been used extensively in imaging. Compounds that have been encapsulated into nanoparticles for imaging include gadolinium, fluorescein isothiocyanate (FITC)–dextrans, Bodipy, and the auto fluorescent anticancer drug doxorubicin. Nanoparticles encapsulating radioactive ligands, such as ^{99m}Tc-labeled colloids and ^{111}In, have been used in scintigraphic imaging[42]. In addition, fluorescent or radioactive moieties can be targeted by noncovalently or covalently tagging the nanoparticles through avidin – biotin conjugation and thiol formation[43]. In vitro imaging enables the dynamics of cellular internalization and localization of nanoparticles to be studied. For example, Bodipy loaded PLGA nanoparticles have been used to study their cellular disposition in-vitro[44] as well as the effect of storage temperature on their physical properties[45]. The bio-tinylated antibody, specific to the CD3 antigen on lymphocytes, was chemically conjugated to nanoparticles and their binding to leukemic and primary T lymphocytes investigated[42].

Advantages of biodegradable polymeric nanoparticles[46]

- Providing controlled/sustained release properties.
- Subcellular size and biocompatibility with tissue and cells
- Stability in blood and biodegradability
- Being non-toxic, non-thrombogenic, non-immunogenic, non-inflammatory
- Avoiding reticuloendothelial system.
- Suitable for the delivery of various molecules such as drugs, proteins, peptides or nucleic acids.

Disadvantages of Biodegradable Polymeric Nanoparticles[47]

- Small size and large surface area of nanoparticles based drug delivery systems can cause some physical stability problems like aggregation.
- Nanoparticle drug conjugates can be exposed to phagocytosis in the body.
- Low drug loading capacity and low loading efficiency are the other limitations of developing a nanoparticle drug delivery system.

Cellular trafficking of Biodegradable nanoparticles

Biodegradable nanoparticles are internalized by one or more of the following mechanisms: phagocytosis, macropinocytosis, clathrin and caveolin mediated endocytosis While phagocytosis by macrophages eliminates nanoparticles from the body, efficient cellular uptake occurs when high - affinity receptors capture the nanoparticles through receptor mediated endocytosis[48]. On the cell surface, nanoparticles activate caveolin, a dimeric protein, resulting in their internalization through caveolae. Clathrin - mediated endocytosis occurs when nanoparticles accumulate on the plasma membrane and clathrin coated pits are formed to transport the nanoparticles into the cell, resulting in the formation of endosomes. Macropinocytosis is restricted to larger particles, such as nanoparticles typically greater than 800 nm. The mechanism by which particles enter cells depends on the composition of the nanoparticles, type of the cell, and particle size. For example, the uptake of chitosan nanoparticles into lung epithelial and Caco - 2 cells was mediated, in part, by the clathrin mediated pathway[49,50].

However, PLGA nanoparticles of size 100 nm are internalized by the clathrin and caveolin independent pathway[51]. There are comprehensive reviews of the mechanisms responsible for the uptake of nanoparticles[52-54]. Nanoparticles that are internalized into cells by these mechanisms first enter the primary endosomes of the cell and are then transported into sorting endosomes. While some nanoparticles in the sorting endosomes are transported out of the cell by recycling endosomes, the remaining nanoparticles are transported into secondary endosomes that fuse with the lysosomes[55]. The surface charge of PLGA nanoparticles is reversed in the acidic lysosome, resulting in their escape into the cytoplasm[56]. A high external concentration of nanoparticles outside the cell prolongs their intracellular concentration within the cytoplasm[55]. Ligand mediated endocytosis targets specific cell surface receptors and these nanoparticles are internalized by a receptor mediated endocytic pathway. Examples of targeting transferrin, folate, lectins, and epidermal growth factor receptors are contained in the literature[57-59].

CONCLUSION

Biodegradable nanoparticles are used for site-specific delivery of drugs, vaccines and various other biomolecules. Biodegradable polymeric nanoparticles are biocompatible, non-immunogenic, non-toxic and stable providing controlled/sustained release properties. Biodegradable nanoparticles provides a vast application in the areas of Drug delivery, Antigen detection, biocompatibility, Tracking and therapeutic agent. The article focuses on an overview of Biodegradable Nanoparticles and their application in the field of Pharmaceutical/ Biological Drug delivery.

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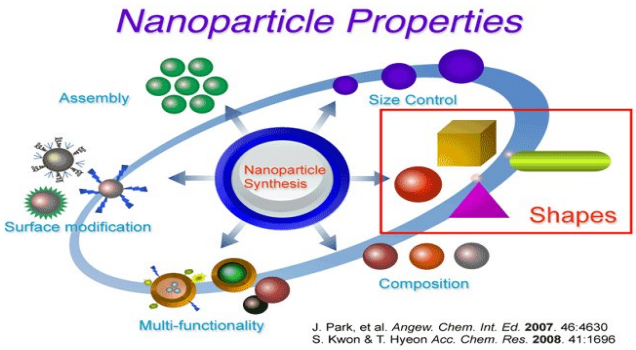

Fig. 1: Properties of Nanoparticles

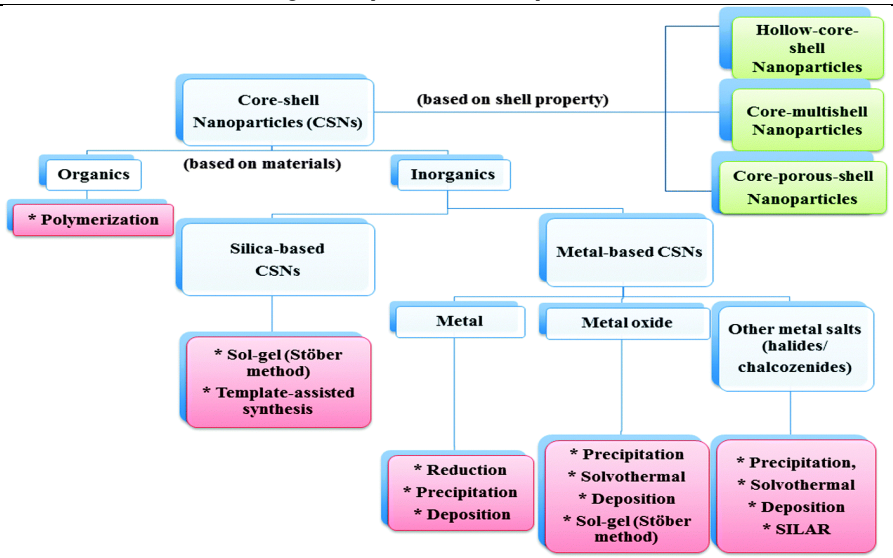

Fig. 2: Classification of Nanoparticles

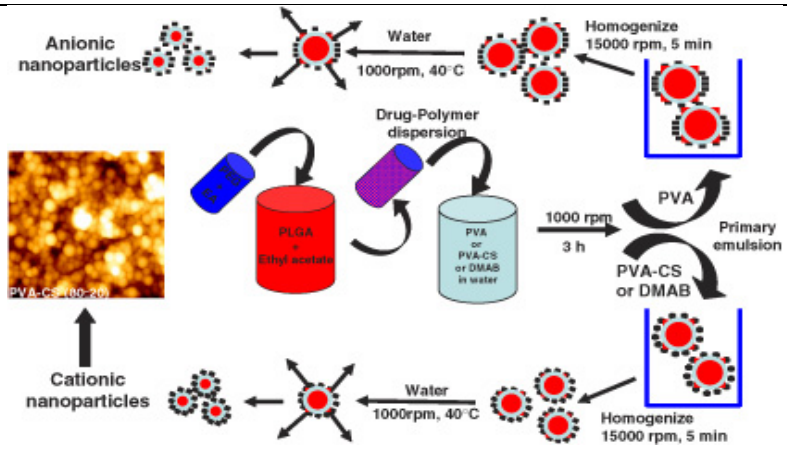

Fig. 3: Design of biodegradable Nanoparticles

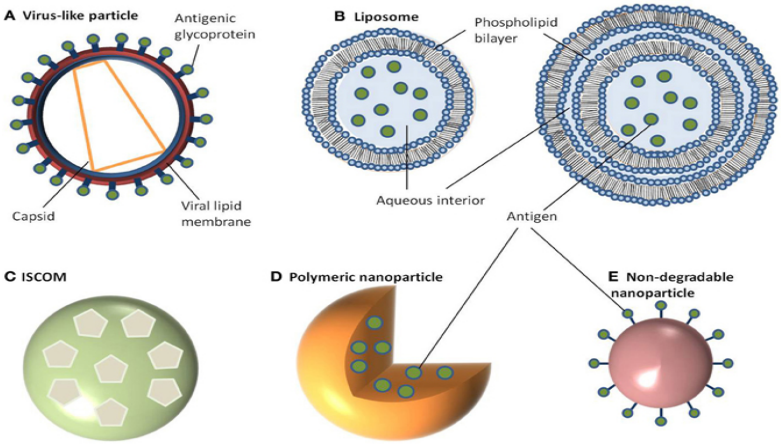

Fig. 4: Vaccine delivery using Nanoparticles

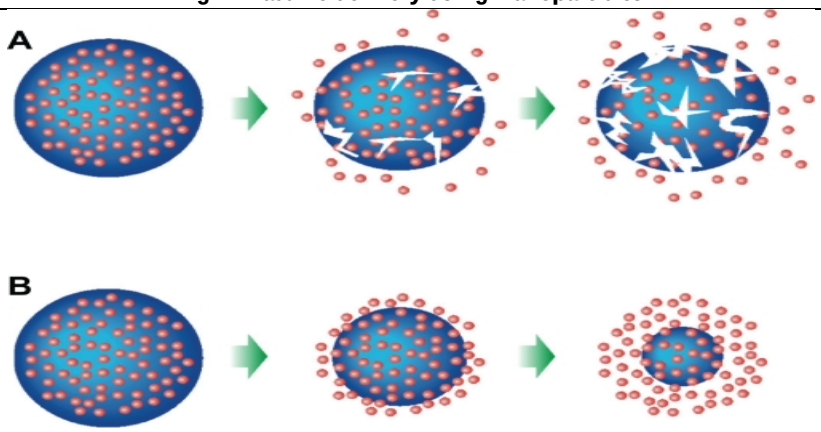

Fig. 5: Degradation mechanism of Biodegradable polymeric Nanoparticles

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RESEARCH ARTICLE

India's Globally Important *Sterculia urens* Roxb. in Crisis: Critical Insights into Conservation, Prospects and Challenges

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ABSTRACT

In the contemporary world of global climate change, conservation of plant resources is a priority area of research. India, being a mega-biodiversity centre, maintenance of phyto-diversity to avoid population and taxonomic extinctions requires substantial inputs. Usage of *Sterculia urens* Roxb. among tribal of India has prominently observed in recent literature. Besides, enormous phytochemicals, declining population, decreasing the production of gum, very little research and development are seen in favour of this species. Efforts are required in India to foster research and development for clinical trials and exploration of *S. urens* in the field of medicine. Moreover, there are numerous applications of *S. urens* in ethnomedicine documented by authors, which lacks validation and can be sourced as a lesser expensive medicine. The present review provides a comprehensive summary of the past research and immediate need for the conservation of *S. urens*, within the national perspective.

Keywords: Ethnomedicine, Karaya gum, Overexploitation, *Sterculia urens*, Tree conservation.

INTRODUCTION

India is a lavish plant biodiversity centre with over 15,000 species of plants, including around 120 plants, producing gum. India produces around 2,81,000 tons of gum yearly and about 5,000 tons of plant-based gum in particular [1,2]. Gum arabic (*Acacia nilotica*), gum ghatti (*Anogeissus latifolia*), and gum karaya (*S.urens*) are some commercially important gums produced in India. Traditionally, for 100 years India has been the largest producer and exporter of karaya gum. However, some peculiar physiochemical and inexpensive characteristics made it even more valuable than tragacanth[3].India has a world trade monopoly over some NWFPs (Non-Wood Forest Product) like Karaya gum, myrobalans, sandalwood chips and dust. India produces 20,000 tonnes of exudate gums in which gum karaya

alone contributes about 15,000 tonnes [4]. Its usage among tribal of India is prominently observed in recent pieces of literature. *S. urens* is found in Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu and Gujarat for its commercial potential [5]. Besides, India is the most significant contributor for the karaya gum production, high phytochemical values, declining population, decreasing the production of gum due to less availability of the tree resources, very little research and development are seen in favour of this species.

The exploitation of several species' crosses over sustainable levels due to a boost in human inhabitants, growing trade and mounting demand. One of the significant ever-present cause for the decrease of species richness are overexploitation and misuse of natural resources. At present, due to overexploitation, *S. urens* is one of the most threatened NWFP trees in India. It is nearly extinct in a few areas where it was common in the past. Earlier, to prevent the rapidly diminishing number of natural stands, several Indian states have prohibited trading this gum. However, in the process, they are depriving traditional gum collectors as a source of livelihood. The purpose of this review is to address the overexploitation of *S.urens*, ethnomedicinal significance, conservational challenges, and approaches with a view to national importance.

Sterculia urens Roxb.: A ghost tree with diverse significance

Sterculia urens Roxb. is from the Malvaceae family [6,7], commonly called "Karaya". It is recognized as 'gum karaya tree' for the globally significant gum, known as 'Indian Tragacanth, found chiefly in India [8,9,10].It is a medium-sized pale in colour tree native to India, Sri Lanka and was introduced to Burma lately [3,11,12]. The species of *Sterculia* is found inthe tropical Himalayas, central and western India, throughout eastern and Western Ghats of India and found on steep, rock-strewn slopes at altitudes between 400 and 800 metres (1,300 and 2,600 ft) [13,14,15].The main gum Karaya producing states are (1) Andhra Pradesh, (2) Maharashtra, (3) Gujarat, (4) Orissa, (5) Rajasthan, (6) Karnataka, (7) Bihar and is also available in Chhattisgarh and Madhya Pradesh. It is a common species and grows in deciduous forests, both wet and dry [16,17].

Phytochemical significance of *S. urens*

For phytochemical analysis, Nandagopalan [18] carried out GC- MS analysis for leaf powder extract in methanol solvent. The highest chemical constituent, phytol is one among the 27th biochemicals found from leaves of *S. urens;* it's a high quantity of phytol suggests its usefulness in the treatment of tuberculosis, as phytol shows anti-mycobacterial activity against Mycobacterium tuberculosis [19,20]. It is widely used in traditional medicine to fight and treat numerous illnesses due to its anti-inflammatory, anti-diabetic, antibacterial, anticancer, antioxidant, antispasmodic, analgesic, and diuretic properties of rich phytoconstituents. Hence, Gritto suggested it as a plant of pharmacological potential [21]. The seed of *S. urens* proved to have essential amino acid and fatty acid and can be used as a good source for dietary food. Moreover, Galla and Dubasi investigated seed phytochemistry and revealed that the cotyledons are lipids (39.2%) protein (30.88%), glutamic, arginine and aspartic acid-rich [15]. It can be majorly used in soap, detergent, and biofuel industry due to its high-quality lipid. Compared to the enormous phytopharmaceutical importance of leaf, a modest examination is done. Moreover, phytochemical screening, secondary metabolite production and antioxidant activity are yet to be explored.

Industrial and Pharmacological applications of *S. urens*

S. urens is majorly a commercially important tree due to multipurpose usage. It is overexploited for gum but ignored as a whole plant in terms of medicinal properties. Its application is seen in the industrial sector, while research in pharmacology is ignored.

Application of S. urens

S.urens gum is employed as a dye thickener for direct-colour printing and textile printing in the textile industry [22]. It is mainly used for cosmetics, lozenges, jellies, emulsions, lotions, sprays, pastes, laxatives, diarrhoea control in pharmaceutical and medicinal preparations. For the baking and dairy industry, it is used in binding,

dressing spread, for health care denture fixtures due to these welling and contracting properties in moisture presence. In the paper industry, long-fibred, lightweight papers produced using it [23]. In general, it is used for lactic products, dressing, whipped cream and frozen dessert, poorly used in Carnic industry, laxative elaboration, dental adhesive, release matrix of diltiazem, quetiapine fumarate and delivery of essential oil in patches [24,25,16,27,28,29,30,31]. It is commercially available from India. The European Union and EFTA permit to use E numbers are codes for food additives. Karaya gum has E number [E416][32]. *S. urens* is widely used for various commercial products due to its unique properties.

Ethnomedicinal importance

A review of ethnomedicinal significance reflects that local people using *S. urens* for a long have rich ethnomedicinal knowledge passed down from generation to generation only by oral communications. During the review study, it was also found that *S. urens* is an immensely useful ethnomedicinal tree, but pharmaceutical validations are hugely lacking in favour of it. A compilation of different tribes using different plant parts for different diseases is mentioned in (Table 1).

ASSOCIATED PROBLEMS AND SUSTAINABLE APPROACH FOR CONSERVATION

Key challenges during reforestation of S. urens

When in forest *S. urens* confronts multiple problems contributing to critical conservational challenges. Sunnichan in 2004 stated major hinderance for germination to be it's poor fruit set due to self-incompatibility [47]. Beside, pollination is caused by only *Apis indica* and its nectar unavailability causes only 56% pollination efficacy [47].The absence of wind pollination leaves seeds the only viable mode of propagation after. But major challenge of seed germination rate of stored seed is poor, freshly harvested (100%) > 10months, (70%) > 18 months, (60%)> 2 years and (42%)> 3 years [48]. Besides, Hard seed coat faces both physical and physiochemical dormancy [49]. Moreover, Lack of awareness regarding scientific and advanced gum tapping leads to fatal death. Over-seed collection for consumption and tree over exploitation for fibre extraction purposes by tribal [38]. Young trees reach gum-producing capability at 41.9 cm girth breast height experience high traditional (brutal) tapping and less gum production [50].

Sustainable conservational techniques: Approach for a better future

Germination: Freshly harvested seeds have 100% germination rate and can be extended up to 10 months as it reduces to 70%.The optimum germination ability can continue for a long period by retaining the standard moisture content required to germinate seeds. Besides, the storage of seeds requires optimum temperature (0-4 °C) in a polythene bag as it maintains moisture content essential for seed for germination [48, 49,51].

Dormancy: Apart from the germination challenge, this plant faces physical dormancy and chemical dormancy. Physical dormancy can be cut using acid and mechanical scarification, while for chemical treatment, phytohormone GA is proved to be supportive [49].

Scientific tapping: It is a sustainable technique to maximize the production and safeguard the existence of the tapped Karaya trees.

Nursery technique: Increased germination rate to 80%, seed vigour, and dry biomass of seedlings are achieved when 2 cm deep, the seed is sown in vertical alignment [52].

Gum tapping

Gum tapping is a brutal technique [53]; consequently, traditional tapping techniques, insubstantial distribution, illicit gum exploitation insignificant seed germination and survival rate, the tree in the Aravalli hills of Rajasthan is enlisted as an endangered plant species [54,55,56,18,57]. There is a pressing need for scientific gum tapping for minimum loss. Few tapping techniques using the physical and chemical tool has been discussed in (table 2).

***S.urens* restoration and conservation strategies: Future scope**

There is a pressing need for introducing new technologies to combat the low population of *S. urens*. Following are few approaches for promising future of this species:

(1) Artificial seed technology might be a promising approach to gain superior grade, healthy somatic embryos that can yield high frequency karaya plants in contrast to ordinary seeds.
(2) The micrografting technique might be an alternative way for propagation and development of high yielding trees.
(3) Embryo culture technique is a plant tissue culture technique that might be of immense help for the conservation of endangered species.
(4) Genetic diversity analysis using molecular markers and micropropagation of an elite tree is a significant choice for *S. urens* tree improvement programme.
(5) *S. urens* must be highly recommended by the ministry of forestry to tribal and farmers for agroforestry due to its potential for social forestry, .
(6) Moreover, the induction of rooting in stem cutting should be focused more as it remained unexplored.

***Sterculia urens* Roxb.: An asset for the sustenance of tribal**

Based on the 2001 Census report, 8.3 %, approximately 8.4 crores of the Indian population are tribes / Adivasis with NWFP as a significant source of their livelihood. Around 5 crore Indians depend on NWFP for livelihood and wage income. To sustain lives, tribal people have been sourcing this plant for diverse purposes (Table 1), which specifies its significance in their life. NWFP contributes about 50% of the total tribal income in Andhra Pradesh [63].

Moreover, the tribal residents are the most vulnerable and disadvantaged communes experiencing extreme economic dismissal. Due to the uncertain income, source of revenue, and lack of awareness, it was lethally used. Corruption is a chief struggle as they earn marginal income from diverse NWFPs mainly because of insufficient knowledge about the commercial value and utilization of these resources along with deficient value addition [17]. With the help of NGO and governmental bodies, gradual and steady change is seen in favour of tribal communities. The tribal community experienced gradual social uplift after increased *S.urens* population density such as(i) Livelihoods enhancement and community engagement (ii) Increased employability chance (iii) Reduced migration of tribes (iv) Introduction of social forestry caused upliftment financial gain.

CONCLUSION AND FUTURE SCOPE

In India, efforts are required to foster research and development for clinical trials and exploration of *S. urens* in the field of medicine. Moreover, there is a vast usage of *S. urens* in ethnomedicine, documented by authors, which lack validation and can be used as less expensive medicines. For conservation of this species, diverse approaches such as mass and clonal/ vegetative propagation of superior tree genotypes, genetic diversity analysis for in vitro propagation, micrografting, molecular breeding artificial seed technology and genetic transformation may be worked. Among these approaches, the in vitro culture technique is credited very efficiently for the germplasm conservation, chiefly in the case of rare or threatened species. Since karaya gum is crucial for the tribal economy, its commercial value is abundant, also with a status of an endangered tree, the population is leading to extinction; hence there is a pressing need to develop an intense activity for the conservation management of this species. If not conserved, it can be critically endangered.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Availability of data and material
Not applicable

Competing Interests
All the authors approve that they have no conflicting interests, which may seem to have affected the research documented in this paper.

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Authors Contribution
AS performed major contribution in writing the manuscript. NM was associated in supervising, advising positioning and structuring the manuscript. All authors read and approved the final manuscript.

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Study Involving Plants
As per the local and national guidelines and legislation and the required or appropriate permissions and/or licenses for the study.

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Table 1. Ethnomedicinal Importance of *Sterculia urens*

Sr. No.	Region	Part of Plant Used	Diseases	Literature Source
1	Dhinodar Hill, Kutch, Gujarat. (Local people)	Bark sap, Stem and Leaves	Bronchitis, Topological applicants	33
2	Bhiloda (West) forest range, Aravalli, Gujarat.	Gum	Removing thorns	34
3	Dang region, Rajasthan	Gum	Eating purpose	35
4	Visakhapatnam, Andhra Pradesh. (Local people)	Gum	Amoebic dysentery, Heel crack (bark)	36
5	Jharkhand (Birhore tribes)	Bark, Gum	To ease stomach-burn, urinary tract, Joints pain and muscular strain	37
6	Kanyakumari, Southern-Western Ghats. (Local people)	Gum,leaf	For bowel complaints, Throat problem, In cattle is used for pleuropneumonia	38
7	Wakal basin area, Udaipur, Rajasthan (Local people)	Seed	Eating purpose	39
8	Mannanur Forest, Mahaboobnagar, Andhra Pradesh.	Root	Body swelling	40
9	Western Ghats, Andhra Pradesh. (Adivasis)	Stem bark	Leucorrhoea	41
10	Sidhi district, Madhya Pradesh	Gum	Leucorrhoea	42
11	Adilabad district, Andhra Pradesh. (Gonds)	Stem bark	Oligospermia	43
12	Pachamalai hills, Tamilnadu	Leaf	Activates parturition	21

13	Kollihilla, Nammakkal, Tamil Nadu.	Leaf	Wound fractures and cracked skin leukemia	44
14	Similipal Biosphere Reserve and Mayurbhanj district.	Stem bark	Vomiting	45
15	The Eastern Ghats, Andhra Pradesh (Tribal communities)	Root bark	Bone fracture	41
16	The Eastern Ghats, Andhra Pradesh. (Jatapu Tribes)	Stem bark	Rheumatoid Arthritis	46

Table 2 Evolved Scientific tapping technique for sustainable karaya gum production

Sr. No.	Procedure and Precaution	Chemical/ physical tool	Gum Yield / Tree	Literature source
1	768mg of 2ml ethephon solution injected at 2.5m above ground and sealed with wax. Copious exudation from holes right above the treatment site yielded an immediate result	Chemical and physical	64.5g	58
2	300mg/ml ethephon injected using borehole tapping a 5cm hole of a tree at breast height of more than 40cm. Ethephon, bore technique proved better than sulphuric acid and V blaze technique, respectively.	Chemical and physical	135.20 g	59
3	3.9 % 4 ml ethephon+ 39% IAA injected into a battery drilled hole and sealed with moistened clay.	Chemical and physical	249.08 g	60
4	The girth of 90-135cm with two 45cm long & 25cm wide blazes using the sharp instrument at 4-5 tapping cycle.	Physical	1.5 to 3 kg	61
5	The girth of less than 90 with one, 47.5cm long & 12.5cm wide blaze, using sharp instrument above 30 cm from ground level.	Physical	2.5 to 5 kg	62

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RESEARCH ARTICLE

Fecundity of Catfish, *Sperata aor* (Hamilton, 1822) from Bhadra Reservoir, Karnataka

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ABSTRACT

Fecundity of catfish, namely *Sperata aor* was studied from Bhadra reservoir, Karnataka. The fecundity of, *S.aor* was 27,600 to 98,200. The rate of increase of fecundity is much higher with the increase in weight of the ovary, but lesser with the increase in length and weight of the fish. The regression equation correlation coefficient for fecundity and length of the fish is Log F = -1.742+2,764 log L (r = 0.914), for fecundity and body weight (Bw) Log F = -1.481+1.824 log (Bw) (r = 0.945), for fecundity and ovary weight (Ow) of the fish is Log F =-.912+1.789 log (Ow) (r = 0.970). The correlation between fecundity and length, ovary weight and body weight was significant.

Keywords: Bhadra reservoir, Fecundity, *Sperata aor*, Regression equation.

INTRODUCTION

Sperata aor, is a species of bagrid catfish found in Southern Asia. It grows to a total length of 180 centimeters (71 in) and is commercially used for human consumption. It is also a popular game fish (Froese et al.,2011). Knowledge on reproductive biology of a fish is of great importance in its rational exploitation through proper management of fishery resources, development of selective breeding, brood stock development, domestication and its genetics (Mollykurian and Inasu, 2003). A study on various aspects of reproduction is essential in the determination of population stock size, periodicity of the strength of broods, fecundity, spawning time and place, and sex composition of exploited stock. Fecundity, defined as the number of ripening eggs in the ovaries prior to spawning (Sindhe and Kulkarni, 2004) is estimated for several purposes: in connection with the estimation of population size, in studies on population dynamics and in racial investigations (Kaveri Borkotoki

and Dey, 2002). Egg production by a population of fishes is a function of the abundance of mature females, their fecundity and the proportion of females that release ova at spawning. The relationship between fecundity and the length or weight of fish can be utilized to estimate the fecundity of known length or weight. The main objective of this study was to add the information regarding the fecundity of the catfish, *Sperata aor* from Bhadra reservoir, Karnataka.

MATERIALS AND METHODS

Study Area

Bhadra reservoir is located near Lakkavalli village of Tarikere taluk in Chikmagalur district (Figure 1).This reservoir has been constructed at an elevation of 601 m above MSL The dam is located at latitude 13°42'00" N and longitude 75°38'20"E. The Bhadra basin gets the rainfall ranging from 117 to 573 cm and the temperature varies from 30.14 to 18.76 C. The reservoir is having 186 ft in depth. This is a multipurpose project for irrigation, drinking, fishery and hydroelectric power.

Fecundity Estimation

In the present study of fecundity the ovaries were preserved in Gilson's fluid (2 gm of mercuric chloride was dissolved in 88 ml of distilled water, to this 10 ml of 95% alcohol, 1,8 ml of concentrated nitric acid and 0.4 ml of glacial acetic acid were added), as described by Qasim and Qayyum (1961). Later the ovaries were agitated violently for the liberation of eggs. The adhering lumps of ovarian tissue were removed, eggs were separated and washed with water repeatedly and dried on the piece of filter paper kill they become translucent golden yellow. Fecundity was determined by the gravimetric method, which involves counting the number of mature eggs from a known weight of mature ovary. Fecundity was calculated by using the log transformation of power law $y= ax^b$ .i.e Log Y= log a + b log X where, Y= =fecundity, L= length, 'a' and 'b' constants to be calculated. The mathematical relationship between body length (L), body weight (W), ovary length (OL) and Ovary weight (OW) and fecundity has been calculated.

RESULTS AND DISCUSSION

The fecundity is the egg laying capacity of a fish or it is the number of eggs likely to be laid or laid by a fish in one spawning season. It is a measure of reproductive capacity of the fish. The success of any fishery is largely depends on the reproductive potentiality of the concerned species. The knowledge on fecundity is important for the successful management and exploitation of fishery. In the present study the observed fecundity of *Sperata aor* varied from 27,600 to 98,200 in fishes size ranging from 29.4 to 73.5 cm in length. The regression equations obtained with various variables are given in Table 1.The relationship of fecundity with length was parabolic (Fig. 2a). The relationship between fecundity (F) and body weight (w) and that of fecundity (F) and gonad weight (G) was linear (Table 1 and 2; Fig. 2b and 2c).

The fecundity of the freshwater fish *Sperata aor* was studied during May/June and September/October when the ovaries are in ripe stage. The mathematical relationship between fecundity with various parameters such as body weight (Bw), body length (Bl) and ovary weight (Ow) were calculated and presented (Table 1). The production models for fecundity based on the above mentioned variables were calculated by using the log transformation of power law and are mentioned below. Y = a X b i.e., log (F) = log a + b log x. The regression equation correlation coefficient for fecundity and length of the fish is Log F = -1.742+2,764 log L (r = 0.914), for fecundity and body weight (Bw) Log F = -1.481+1.824 log (Bw) (r = 0.945), for fecundity and ovary weight (Ow) of the fish is Log F =-.912+1.789 log (Ow) (r = 0.970). The correlation between fecundity and length, ovary weight and body weight was significant (Table 2).

The results obtained on the fecundity of *Sperata aor* of Bhadra reservoir revealed that there is a relationship between the length of the fish, weight of the fish and weight of the ovary with fecundity. The relationship between the lengths of the fish with fecundity was found to be curvilinear while the body weight (Bw) of the fish, ovary weight (Ow) with fecundity was found to be linear. Chacko (1955) reported the fecundity of *Sperata seenghala* that ranged between 200 and1,000; later Bhatt et al.(1977) and Saigal (1982) have documented the fecundity range of 20,064-46,443 and 1,31,820-4,28,376 respectively. Saigal(1982) has obtained high correlation between fecundity with ovary weight and total length in *Sperata seenghala*. Gupta (2015) has reviewed the biology of *Sperata seenghala,* a freshwater catfish of Indian subcontinent.

Simpson (1951) opined that fecundity is directly proportional to body weight. Begenal (1967) has pointed out that fecundity is more closely related with the weight of the fish than its length. Bhattacharya and Banik (2015) studied the fecundity of *Ompok pabo* in Tripura and it varied from 2500 to 19636.71 in the specimens measuring 133 mm to 192 mm. Studies on the fecundity of catfishes includes the works of Karmchandani et al. (1967) estimated the fecundity of *Rita pavimentata* from river Narmada and fecundity ranged from 2,970 to 30,000. Sharma (1978) made an observation on the fecundity of *Mystus cavasius* found to be 13,450 to 69,155. Kaliyamurthy (1981) estimated the fecundity of *M. gulio* (about 6,125 to 48,000) from Pulicat lake.

Rao et al. (1999) made a study on the fecundity in *M. vittatus* and the fecundity ranged from 3,500 to 18,000. Panigrahi (1987) made an observation on the fecundity of *Mystus vittatus* in relation to body length and weight. Krishna Rao (1990) studied the fecundity indices in *Ompok bimaculatus* and it was found to vary between 1,157 to 17, 269. Siva Reddy and Babu Rao (1991) made a study on the fecundity of *Heteropnestus fossilis* from Husain Sagar lake. Fecundity of the fish *Mystus seenghala* of river Godavari was estimated by Rao (1993), fecundity in their observation varied from 17600 to 73080 and they also concluded that fecundity was more closely related to length than to the weight of the fish. Inasu (1993) reported the fecundity of Mystus spp. from inland waters of Trichur. Rao et al. (1999) worked on the fecundity in Mystus spp. from Mehadrigedda stream of Vishakpatanam.

Das Gupta (2002) reported the fecundity of the *Mystus gulio* from salt water Bheries of West Bengal, found to be 425 to 18,199. The study revealed that the fecundity showed linear relationship with total length, total weight and also ovary weight. Mollykurian and Inasu (2003) worked on the fecundity aspects in *Horabagrus brachysoma* from inland waters of Kerala and found fecundity ranged between 1,500 to 21,184. Tapas Paria and Manna (2003) worked on the fecundity aspects of fish *Mystus tangra* (Bloch), the fecundity ranged from 1442 to 3721. They observed that the relationship between fecundity with body length, body weight and ovary weight was found to be linear. Kiran and Puttaiah (2003) studied the fecundity of the cyprinid fish, *Chela untrahi* (Day) from Bhadra reservoir and fecundity was found to range from 6729 to 26952 eggs, the average being 18130 eggs. Log linear relationships were noticed by them between fecundity and length-weight of fish as well as gonad weight. The ovary weight was found to be a better index of fecundity than total length or total weight of the fish.

CONCLUSION

To sum up, the obtained results of above studied parameter in *S.aor* from Bhadra reservoir, Karnataka had shown best for fecundity. It can be concluded that environment factors plays a role in the reproductive success of catfish. This study will provides an important information for brood stock management and conservation measures of *S. aor* in the Bhadra reservoir.

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Table 1: Showing statistical data of relationship between fecundity and various parameters in the fresh water fish *Sperata aor*

Sl. No.	Statistics	Total length (cm)	Body weight (gms)	Ovary weight (gms)
1	a	-1.742	-1.481	-0.912
2	b	2.764	1.824	1.789
3	r	0.914	0.945	0.970

Table 2. Showing statistical data of Correlation between fecundity and various parameters in the fresh water fish *Sperata aor*

Pearson Correlation	Fecundity
Length	0.952**
Body weight	0.964**
Ovary weight	0.981**

** Correlation is significant at the 0.01 level (2-tailed).

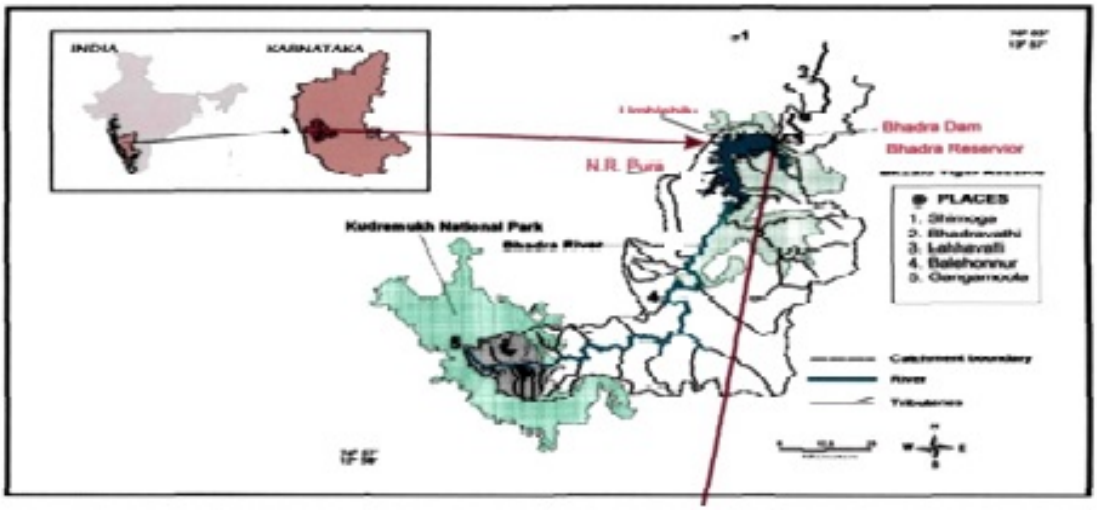

Figure 1: Location of the study area

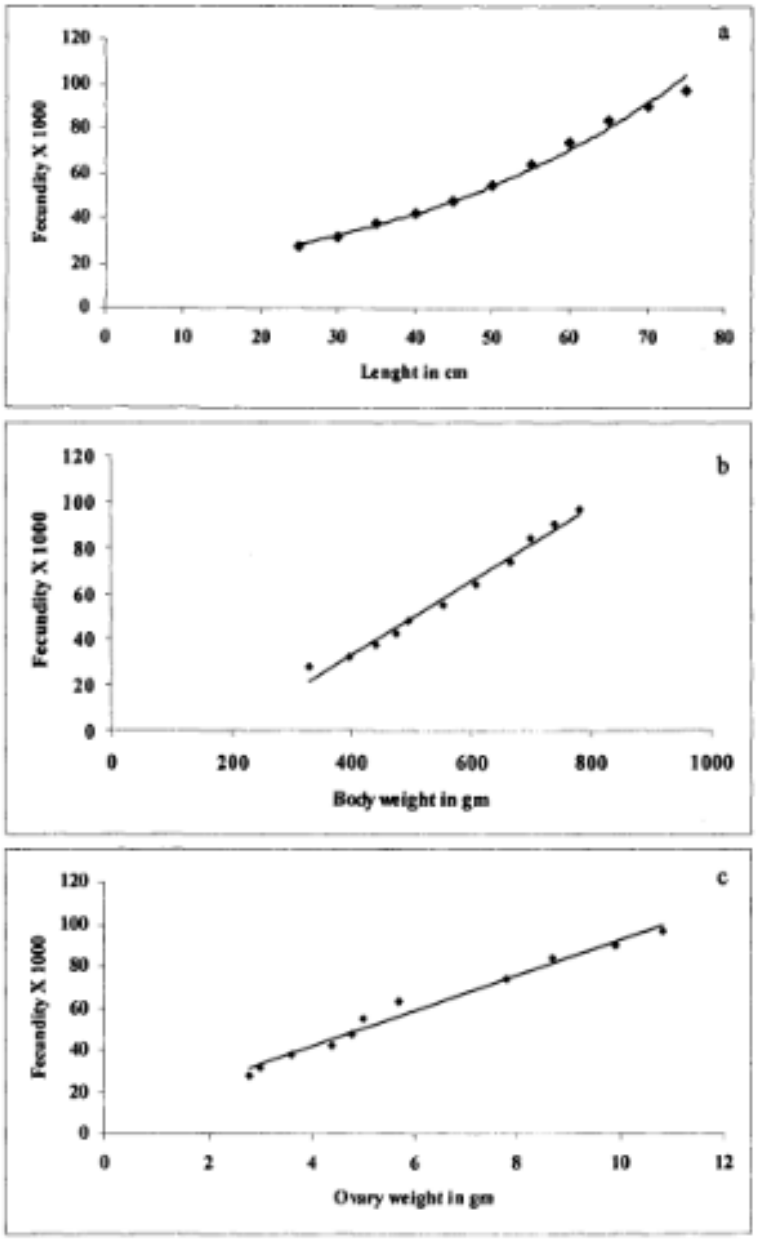

Fig.2: Scattered diagram showing relationship between fecundity and other parameters of the fresh water fish *Sperata aor*

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REVIEW ARTICLE

A Review on Anti-Hypertensive

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ABSTRACT

Hypertension, the leading risk factor for cardiovascular disease, originates from combined genetic, environmental, and social determinants. Environmental factors include overweight/obesity, unhealthy diet, excessive dietary sodium, inadequate dietary potassium, insucient physical activity, and consumption of alcohol. The increasing prevalence of hypertension and the continually increasing expense of its treatment influence the prescribing patterns among physicians and compliance to the treatment by the patients. It has been observed that type-2 diabetes has been promoted by not only hypertension but also by antihypertensive therapies. Several studies indicate and beta blockers and diuretics impair the glucose metabolism, hence not only the disease itself but also antihypertensive therapies may promote the development of new-onset diabetes. The antihypertensive medication prescribing pattern studies help in monitoring, evaluation and necessary modifications to the prescribing habits to achieve rational and cost-effective treatment. Prevention and control of hypertension can be achieved through targeted and/or population-based strategies. For control of hypertension, the targeted strategy involves interventions to increase awareness, treatment, and control in individuals. Corresponding population-based strategies involve interventions designed to achieve a small reduction in blood pressure (BP) in the entire population. As in this review we get information about Primary and secondary hypertension, Hypertension Pharmacotheraphy Guidelines for Prescription, Relation between Diabetes and Hypertension, Prevention and control of Hypertension, Diagnosis of Hypertension, Uses of antihypertensive drug therapy.

Keywords: Hypertension Pharmacotheraphy Guidelines for Prescription, Relation between Diabetes and Hypertension.

INTRODUCTION

Hypertension or high blood pressure is a worldwide problem that effects approximately 15-20% of all adults. Hypertension known as silent killer as it showed no symptom. Even though it is simple to diagnose and usually can be controlled by healthy diet, regular exercise, medication prescribed by doctors or a combination of these, untreated hypertension will cause serious condition. Hypertension is associated with cardiovascular disease, insulin resistance, obesity, carbohydrate tolerance, hyperuricacidemia, and atherosclerosis. Hypertension affects the structures and functions of small muscular arteries, arterioles and other blood vessels and can cause damage at variable rate to various target organs including kidney, brain and eye, related with the end stage of renal disease and to be the cause of stroke. It is associated with the alterations in the blood vessels wall that affecting the endothelium, the media and the adventitia, whereas alteration in the media leading to remodeling of the vessel wall. Patients with hypertension diepremature ly with the most common cause of death are heart disease, while strokes andrenal failure are frequently occurring, particularly in those with significant retinopathy.

Various antihypertensive drugs such as beta-blocking agents, hypotensive diuretics, calcium antagonist, angiotensin converting enzyme inhibitors (ACEI), angiotens in II receptor antagonists and alpha-receptor blocking agents were usually used to control hypertension and its alleviate symptoms clinically. Two or more antihypertensive drugs from different categories usually were combined to achieve optimal results as the efficacy of these drugs is only about 40-60%(1).

ESSENTIAL HYPERTENSION

95% of all hypertension cases were categorized as essential hypertension that also known as primary hypertension or idiopathic hypertension. It is a heterogeneous disorder as different patients have different factors that cause high blood pressure. The cause of essential hypertension is still unknown but it is considered as the sum of interaction between genetic and multiple environmental factors. Environmental factors including obesity, high alcohol intake, high salt intake, insulin resistance, low potassium intake, aging, sedentary lifestyles, stress, and low calcium intake contribute to the development of hypertension. Inherited blood pressure (Bp) known as blood pressure that are genetically determined, while hypertension genic factors are factors that cause high blood pressure such as obesity, high alcohol and salt intake.

Various of gene might involve in the development of hypertension can cause inherited blood pressure and the influences of these genes have been demonstrated by family studies that showed high blood pressure are associated among siblings and between parents and children. Obesity is known as important risk factor for type 2 diabetes and cardiovascular disease (CVD). It is associated with an incidence of arterial hypertension and known to be one of powerful risk factors for non-communicable diseases. Obesity also acknowledge as the main hypertension genic factor compared to high alcohol intake, high salt intake, stress, sedentary lifestyles, dyslipidemia, low potassium and low calcium intake. According to the study in Shanghai on Chinese adults age 40 years and above, subject with obesity are significantly has higher risk of hypertension and type 2 diabetes. Obesity can cause insulin resistance, adult-onset diabetes mellitus, left ventricular hypertrophy, hyperlipidemia and atherosclerotic disease. However, the mechanism of obesity raises blood pressure (Bp) is not fully understood(2).

SECONDARY HYPERTENSION

Secondary hypertension can be caused by medical conditions such as renal parenchymal disease, renal artery stenosis, hyperaldosteronism, or pheochromocytoma. Temporary high blood pressure also can cause by medications such as corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), cold medicines and birth control pills. Corticosteroids such as prednisone and prednisolone will lead to Cushing syndrome in long-term use. Usage of nonsteroidal anti-inflammatory drugs (NSAIDs) increase blood pressure as well as will interfere in anti-hypertensive treatment, and abolish its effect. NSAIDs interfere in some of the antihypertensive agents such as beta-blockers,

diuretics agents as well as angiotensin converting enzyme inhibitors (ACEI), except for calcium antagonist and central-acting drugs. NSAIDS such as indomethacin, naproxen and piroxicam were the greatest that involves in the increasing of blood pressure, while rofecoxib raise systolic blood pressure more than celecoxib. Cold medicines such as pseudoephedrine hydrochloride that used for upper respiratory decongestant may elevate blood pressure in hypertensive patients. Intake of birth control pills contributes in the increasing of blood pressure particularly in women above 35 years old that overweight and smokers(3).Treatment for primary and secondary hypertension were specifically different thus, it is important in terms of its diagnostic, therapeutic and prognostic to be determined before patient with such conditions were treated(4).

Hypertension is the most common modifiable risk factor for cardiovascular disease (CVD) and death; the increased risk associated with blood pressure (BP) elevation can be greatly reduced by treatment with antihypertensive drugs that lower both BP and related target organ damage. A total of 69 drugs in 15 different classes, many of which are also available in single pill combinations, have been approved for the treatment of hypertension in the United States. Hypertension if not diagnosed early and treated correctly may lead to myocardial infraction, renal failure, stroke, leading cause of mortality worldwide and even ultimate death as it is a hemodynamic disorder associated with increase in peripheral vascular resistance. Hypertension affects more than a third of adults aged 25 and above scoring about a billion people worldwide, this has been revealed in some of recent estimates of World Health Organization (WHO). Therapeutic options include diet and lifestyle changes (comprising weight loss, smoking cessation, and increased physical activity), antihypertensive medications, and in special situations surgery is required.

It is the second leading cause of chronic kidney disease (CKD). It is estimated that more than one billion adults are hypertensive worldwide and this figure is projected to increase to 1.56 billion by the year 2025, which is an increase of 60 % from 2000. Cardiovascular diseases and Hypertension are accounting for loss of 4 % gross domestic product for low and middle income countries annually which is amounting 500 billion USD [5]. Clinical evidence suggests that lowering blood pressure (BP) with antihypertensive drugs reduces the risk of myocardial infarction, stroke, heart failure, revascularization procedures and end-stage renal diseases in hypertensive patients [6]. The increasing prevalence of hypertension has been attributed to population growth, ageing and behavioural risk factors, such as unhealthy diet, excess use of alcohol, sedentary lifestyle, obesity, and exposure to persistent stress. A whopping approx10.4 million deaths occur worldwide every year because of hypertension [7], with it being responsible for about 50 % of mortality due to heart disease and stroke [8]. Epidemiological studies demonstrated that prevalence of hypertension is increasing rapidly in India, varying from 4 to 15 % in urban and 2-8 % in rural population [9,10].

The age-adjusted prevalence of systemic hypertension in the United States is 64% of older men and 78% of older women according to the American Heart Association (AHA) Statistics Committee and Stroke Statistics Committee(22) Patients with hypertension should be evaluated for other cardiovascular risk factors including smoking, dyslipidemia, diabetes mellitus, age older than 55 years for men and 65 years for women, body mass index ≥30 kg/m2, physical inactivity, microalbuminuria, an estimated glomerular filtration rate <60 ml/min/1.73 m2, and for a family history of premature cardiovascular disease (younger than 55 years in fathers or brothers and younger than 65 years in mothers or sisters) [**23]**. Patients with hypertension should also be evaluated for target organ damage and clinical cardiovascular disease including left ventricular hypertrophy, prior myocardial infarction, angina pectoris, prior coronary revascularization, congestive heart failure, stroke or transient ischemic attack, peripheral arterial disease, nephropathy, and retinopathy [**23]**.The higher the systolic or diastolic blood pressure, the higher the risk of cardiovascular morbidity and mortality [**24]**. Increased systolic blood pressure and pulse pressure are stronger risk factors for cardiovascular morbidity and mortality in older persons than is increased diastolic blood pressure [**25-26]**. An increased pulse pressure found in older persons with isolated systolic hypertension indicates decreased vascular compliance in the large arteries and is even a better marker of risk than is systolic or diastolic blood pressure [**25-26]**.

Hypertension Pharmacotherapy and Guidelines

Clinical practice guidelines suggest that antihypertensive classes are acceptable first-line agents for the management of uncomplicated hypertension. These classes include angiotens in receptor blockers thiazide diuretics, angiotensin-converting enzyme inhibitors, calcium channel blockers and beta-blockers.[11,12]. It is very difficult to achieve BP targets with monotherapy and most patients require a combination of two or three drugs to get to target [13].Since the need to improve the control of hypertension is well acknowledged, several guidelines on its classification and management have been developed. Some of the bodies which have developed guidelines are American Society of Hypertension/ International Society of hypertension (ASH/ISH), Joint National Committee (JNC) on Detection, Evaluation, and Treatment of High Blood Pressure, European Society of Hypertension (ESH)/European Society of Cardiology (ESC), National Institute for Health and Care Excellence (NICE) and Japanese Society of Hypertension. Guidelines regarding hypertensive drug therapy vary between countries, patient characteristics, and organizations [14].

Relation between Diabetes and Hypertension

Diabetes and high blood pressure are likely to occur together as they share certain physiological characteristics. High blood pressure is a dangerous condition that becomes even more troublesome with diabetes [16]. Hypertensive people are 2.5 times more likely to develop diabetes mellitus within five years. This may be due to the presence of an underlying metabolic syndrome and made worse by the type of antihypertensive drug used [17]. Accompaniment of insulin resistance generally causes hypertension. Increased hepatic glucose release and decreased insulin sensitivity occur by consequent elevations of angiotensin II and aldosterone and activation of the renin–angiotensin–aldosterone system (RAAS) [18].The coexistence of DM and hypertension is devastating to the cardiovascular system, and the results may increase the risk of stroke or even cardiovascular mortality [19]. Various analyses have indicated that some of the antihypertensive drugs promote the development of type-2-diabetes mellitus. Several studies indicate that, glucose metabolism is impaired by beta blockers and diuretics; hence not only the disease itself, but also antihypertensive therapies may promote the development of new-onset diabetes [20]. Furthermore, drugs for the treatment of hypertension exhibit a long record of potentially affecting also gluco-metabolic parameters, such as blood glucose, insulin sensitivity, and HbA1c. In particular, beta-blockers (also the so-called cardio-selective ones) and thiazide / thiazide-like diuretics have been known to increase blood glucose for many years, including the precipitation of hyperosmolar hyperglycemic coma at high dose by the latter. So, it seems to be an important issue whether antihypertensive drugs which increase blood glucose should be avoided, i.e. drugs which may worsen glycemic control in patients with diabetes or induce new onset diabetes in patients with hypertension [21].

Hypertension pharmacotherapy and guidelines for prescription

Antihypertensive drugs are prescribed mainly to reduce the morbidity and mortality caused by hypertension and its complications. Many a time, patients require more than one drug for effective control of hypertension. Various classes of antihypertensive drugs like diuretics, inhibitors of the renin-angiotensin system, calcium channel blockers (CCB) and beta blockers (BB) have been shown to reduce complications of hypertension and may be used for initial drugtherapy [**27]**. The JNC 8 guidelines published in 2014 are the most recent guidelines for the management of hypertension in different clinical settings. These guidelines were developed based on a systematic review of literature to help clinicians, especially the primary care physicians [**28]**. Despite these guidelines, and also evidence showing that hypertension is a major public health concern, many clinicians fail to assess BP routinely, and in those with a diagnosis of hypertension, do not start treatment or titrate the dosage of the drugs effectively [**29]**. The available guidelines recommend different goal BP levels and drug treatment options according to patients' individual clinical need (see Table 2).Studies have shown that the application of guidelines to clinical practice improve the treatment outcomes. According to a retrospective study by Jackson et al. on 19,258 patients, applying JNC-7 guidelines to practice helped in achieving better BP control. Blood pressure control in the before-JNC 7 cohort was 40.8 % vs. 49.3 % in the after-JNC 7 cohort ($p < 0.0001$) [**30]**. In another older study conducted to assess whether the publication of JNC 6 (1997) and WHO/ISH (1999) guidelines, and the development of new drugs improved BP control, follow-up of 150 patients from 1991 to 2001 showed that BP control increased from 31 % initially, to 43 % in

1996 and finally to 57 % in 2001. Both younger and older patients showed similar improvement during these 10 years. The authors concluded that improved BP control was because of increased use of ACEIs and CCBs, lifestyle modifications and improved awareness about the disease condition and the need for effective management [**31]**.

Prevention and Control of Hypertension

Genetic and epigenetic predisposition

Hypertension is a complex polygenic disorder in which many genes and/or combinations of genes infuence BP. Although several monogenic forms of hypertension, such as glucocorticoid-remediable aldosteronism, Liddle's and Gordon's syndromes, and others, have been identified in which single-gene mutations completely explain the pathophysiology of the hypertension, these disorders are rare. Common genetic variants influencing BP have been identified at over 300 independent genetic loci. However, these genetic variants typically have effects on the order of only 1.0 mm Hg SBP and 0.5 mm Hg DBP per BP-raising allele. Individually, these genetic variants each explain <0.1% of BP phenotype and collectively ≤3.5% of total BP variance. Because primary hypertension is a highly heritable condition, but genetic variants only explain a miniscule fraction of phenotypic variation and disease risk, the term *missing heritability* has been introduced. Missing heritability is the difference between estimated and observed phenotypic variance. Recent studies have suggested that missing heritability in hypertension may be due, in part, to pathological events during embryonic, fetal, and early postnatal life (e.g., nutritionaldeprivation of the fetus during pregnancy leading to low birth weight) having persistent effects on CVD homeostasis and thereby increasing CVD risk, including hypertension, with advancing age. These fetal programming events may be mediated by epigenetic mechanisms (i.e., alterations in gene expression in the absence of changes in DNA sequence, including posttranslational histone modification, DNA methylation, and noncoding microRNAs). During early life, epigenetic mechanisms seem to be strongly influenced by the environment, and environmentally induced epigenetic modification is heritable through multiple generations(40).

Environmental (lifestyle) factors

Although the genetic predisposition to hypertension is nonmodifiable and conveys lifelong CVD risk, the risk for hypertension is modifiable and largely preventable due to a strong influence by key environmental/lifestyle factors. The most important of these factors, which often are gradually introduced in childhood and early adult life, are weight gain leading to overweight/obesity, unhealthy diet, excessive dietary sodium and inadequate potassium intake, insufficient physical activity, and consumption of alcohol. The greatest impact can be achieved by targeting lifestyle areas of highest deficiency and combining more than 1 of these lifestyle modifications, as the individual BP reductions are often additive. Nevertheless, only a minority of adults change their lifestyle after a diagnosis of hypertension, and sustainability is difficult, posing a substantial challenge for successful implementation of lifestyle modification. The evidence underlying each of the environmental/lifestyle factors that promote elevation of BP and hypertension will be briefly reviewed(41).

Heart-healthy diet

Consuming a healthful diet lowers BP. The Dietary Approaches to Stop Hypertension (DASH) eating plan is especially effective for lowering BP. The DASH diet is rich in fruits, vegetables, whole grains, nuts, legumes, lean protein, and low-fat dairy products, and is markedly reduced in refined sugar, saturated fat, and cholesterol. The combination of low sodium intake and the DASH diet provides substantially greater BP reduction than sodium restriction or the DASH diet alone. Both the DASH diet and sodium reduction, therefore, are recommended in adults with elevated BP and hypertension (42).

Excess sodium intake

Sodium is an essential nutrient and dietary requirement for all humans. However, excessive sodium intake is an important determinant of hypertension. Sodium intake is positively correlated with BP in cross-sectional and prospective cohort studies and accounts substantially for the age-related rise in BP.

In the United States, most (≥70%) of the sodium intake results from addition during processing of foods, including breads, salted meats, canned goods, cereals, pastries, and food preparation (fast-food and sit-down restaurants). Modeling studies suggest that even a small reduction in population sodium intake would prevent thousands of deaths attributable to hypertension (e.g., heart disease and stroke) and save billions of dollars in health care costs annually(43).

Inadequate potassium intake

Increasing potassium intake lowers BP in hypertensive adults, especially among those who are black, older, or consuming a high intake of dietary sodium. Because of its BP-lowering effects, increased potassium intake would beexpected to prevent CVD events, and several studies have demonstrated an inverse relationship of potassium intake to stroke as well as other forms of CVD. Increased potassium intake can be achieved either by augmenting dietary potassium intake or by use of potassium supplements. The former approach is preferable, with the DASH diet providing the recommended daily consumption of 4,700 mg for a 2,000-calorie intake(44).

Inadequate physical activity

Observational studies have consistently demonstrated a protective effect of physical activity in preventing hypertension and controlling BP among those with hypertension. Even moderate levels of physical activity have been associated with a reduction in the risk of incident hypertension. Randomized trials suggest that the best form of physical activity conveying BP-lowering benefits is aerobic exercise (5– to 7–mm Hg reduction), but dynamic and isometric resistance exercise are also effective (4– to 5–mm Hg reduction). The mechanisms of physical activity in preventing hypertension are unclear, but may include decreased cardiac output, diminution of sympathetic nervous system and reninangiotensin system activity, decreased total peripheral vascular resistance and insulin resistance, and improved endothelial function (45).

Overweight and obesity

Studies in various populations demonstrate a direct, nearly linear association of body mass index (BMI) with BP. The risk of hypertension increases continuously with increasing anthropomorphic measurements (waist circumference, waistto- hip ratio, and waist-to-height ratio) in parallel with BMI. About 40% of adults with hypertension in the United States are obese (BMI ≥30 kg/m), and over one-third of the obese population has hypertension (SBP/DBP ≥140/90 mm Hg ortreatment with antihypertensive medication), compared with less than one-fifth of normal-weight individuals(46). Clinical studies have repeatedly demonstrated that weight loss reduces the risk for hypertension and BP in adults with hypertension. Several pathophysiological mechanisms seem to contribute to the development of hypertension in obesity, including insulin resistance, chronic low-grade inflammation, oxidative stress, adipokine abnormalities (e.g., high leptin, reduced adiponectin), increased sympathetic nervous system and renin-angiotensin-aldosterone system activity, endothelial dysfunction, intestinal microbiota, and increased renal sodium reabsorption with volume expansion(47).

Overall Strategies for Prevention and Control of Hypertension

Prevention and control of hypertension can be achieved by application of targeted and/or population-based strategies. The targeted approach is the traditional strategy used in health care practice and seeks to achieve a clinically important reduction in BP for individuals at the upper end of the BP distribution. The targeted approach is used in the management of patients with hypertension, but the same approach is well-proven as an effective strategy for prevention of hypertension in those at high risk of developing hypertension. The population-based strategy is derived from public health mass environmental control experience. It aims to achieve a smaller reduction in BP that is applied to the entire population, resulting in a small downward shift in the entire BP distribution(41). An appeal of the population-based approach is that modeling studies have consistently suggested that it provides greater potential to prevent CVD compared with the targeted strategy. This finding is based on the principle that a large number of people exposed to a small increased CVD risk may generate many more cases than a small number of

people exposed to a large increased risk. For example, a general population DBP-lowering of as little as 2 mm Hg would be expected to result in a 17% reduction in the incidence of hypertension, a 14% reduction in stroke risk, and a 6% reduction in the risk of coronary heart disease. Because they use the same interventions, the targeted and population-based strategies are complementary and mutually reinforcing.

Diagnosis of Hypertension: BP Measurement

Accurate BP measurement

The science underpinning prevention and treatment of high BP has progressively become stronger, but much remains to be done to ensure that this knowledge is translated to clinical practice. A fundamental need is to improve the quality of the BP measurements used for diagnosis and management of hypertension. Estimation of BP is highly prone to systematic and random error, but simple guideline-recommended approachesminimize these errors. Unfortunately, the quality of BP assessments in clinical practice is very poor. Improvements in the quality of office-based measurements through training of clinicians or designated staff members is essential to translation of clinical practice recommendations for detection and management of hypertension. An important complement or alternative is to train patients in accurate measurement of BP. Initiatives such as Million Heartsand Target BPare important steps in reaching this goal.

Hypertension awareness

Awareness of hypertension has increased markedly over the past one-half century in the United States. For example, according to the NHANES (National Health and Nutrition Examination Survey), the percentage of U.S. adults with hypertension (SBP ≥140 mm Hg, DBP ≥90 mm Hg)(48), or treatment with antihypertensive medication) who were aware of having this condition increased from 70% in 1999 to 2000 to 84% in 2011 to 2014(49). A number of programs have been implemented to increase awareness of hypertension. Many of these programs have been directed toward populations with a high prevalence of hypertension, including blacks and individuals with low socioeconomic status. These programs appear to have been effective in raising hypertension awareness and subsequent hypertension screening.

Hypertension treatment

Following the diagnosis of hypertension, initiation of treatment is the next step toward achieving control of BP and reducing the risk for CVD. The 2017 ACC/AHA BP guideline recommends nonpharmacological treatment for all adults with hypertension(50). According to NHANES 1999 to 2004, 84% of U.S. adults who are aware of their diagnosis of hypertension report having been advised by a health care provider to make lifestyle modification changes to lower their BP. Most U.S. adults with hypertension report being advised to reduce their dietary sodium intake (82%), increase their physical activity (79%), and lose weight (66%), whereas only 31% were advised to reduce their alcohol consumption. Of those with hypertension who were advised to undertake nonpharmacological approaches to lower their BP, 88% reported adhering to these recommendations (51).

Connected Health for Hypertension Control

Improving patients' hypertension management behaviors, and medication adherence, although critically important, can be complex and time-consuming. In a typical primary care setting, time limitations, competing demands, and the burden of comorbid illness, along with inadequate mechanisms for follow-up, constitute barriers to effective hypertension risk factor .Many aspects of hypertension risk reduction, however, do not require a physical examination, and BP can be measured at home; thus, much of the care for hypertension could be accomplished outside of the traditional confines of office-based clinical care (52). Advances in health information technology, including electronic health records and high-speed communications, provide new opportunities for improving the care of chronic conditions, including hypertension. Additionally, the Institute of Medicine's blueprint for meeting the *Crossing the Quality Chasm*goals for delivering of state-of-the-art health care suggests that patients should "receive

care when they need it and in many forms, have unfettered access to their ownmedical information and that there should be active collaboration and information exchange between clinicians" (53).

Telemedicine

Telemedicine refers to interactive communications, which can be as simple as telephone-based care or interactive video and digital technologies, enabling direct communication between patients and their care team from remote sites. Automated interactive voice response uses computer technology to telephone patients, collect data, and provide tailored interventions based on their responses (54). Telemedicine or remote monitoring in patients' homes has been offered as a plausible solution to improving ambulatory medical care. However current reimbursement models do not encourage these in-person and remote primary care interventions (55).

Telephone interventions

Medical office visits are usually focused on the management of symptoms with little time remaining for comprehensive risk factor management. An intervention that is delivered in patients' homes may be more successful in non–symptom-based approaches to health care. Telephone contact has been shown to be effective in changing multiple patient behaviors (56). In addition, telephone interventions allow increased numbers of patients to be contacted, and these interventions may be more convenient and acceptable than in-person medical ofiice interventions (57). Telephone interventions may increase cost-effectiveness because they are associated with lower intervention costs and visit rates. Clinicians thus can follow a much larger panel of patients over which to spread the intervention costs than is possible with medical office intervention. Thus, the use of telephones to implement the intervention allows individualized, personal interaction at minimal cost and without the time and transportation barriers that accompany in-person programs. This personal interaction allows the intervention to be adapted and tailored to participants' current concerns, health goals, and specific barriers to achieving these goals (58).

Mobile health interventions, such as smartphone applications (apps), have been advocated as promising strategies to assist in the self-management of hypertension. These tools have the potential to address non adherence by providing reminders for medication taking and refilling, tracking biometric results, offering education, and facilitating social interactions that provide support and motivation (59). From 2012 to 2015, there was a 515% increase in adherence apps available for download, and an estimated 107 unique apps have been evaluated for hypertension (41). However, rigorous evaluation of these technologies is still warranted, as indicated by a recent study among those with poorly controlled hypertension, in which patients randomized to the use of a standalone smartphone app had a small improvement in self-reported medication adherence but no change in SBP at 12 weeks of follow-up compared with control subjects (60).

Lifestyle counseling/monitoring

Clinicians would benefit from help in other areas as well, especially in counseling and monitoring recommendations for lifestyle change. This can be achieved by designation of a practice-based champion for lifestyle change who is sufficiently knowledgeable in behavior change techniques to be effective in patient counselling (41). Policy changes resulting in financial support for such endeavors would greatly enhance the opportunity for patients to improve their lifestyles and reduce the burden for busy clinicians.

Effect of antihypertensive therapy in reducing cardiovascular events

Numerous prospective, double-blind, randomized, placebo-controlled studies have shown that antihypertensive drug therapy reduces the development of new coronary events, stroke, and CHF Older patients with hypertension if treated appropriately will have a greater absolute reduction in cardiovascular events such as major coronary events, stroke, CHF, and renal insufficiency and a greater reduction in dementia than in younger patients (61). Therapy with antihypertensive drugs reduces the incidence of all strokes 38% in women, by 34% in men, by 36% in older persons, and by 34% in persons older than 80 years (62). The overall data suggest that the decrease of stroke in older persons

with hypertension is related more to a reduction in blood pressure than to the type of antihypertensive drugs used. In the Perindopril Protection Against Recurrent Stroke Study, perindopril plus indapamide reduced stroke-related dementia by 34% and cognitive decline by 45%. In the Systolic Hypertension in Europe trial, nitrendipine reduced dementia by 55% at 3.9-year follow-up. In 1900 older African-Americans, antihypertensive drug treatment reduced cognitive impairment by 38% . In the Rotterdam Study, antihypertensive drugs decreased vascular dementia by 70%. At 1.8-year follow-up of 3, 845 patients aged 80years and older (mean age 83.6 years) in the Hypertension in the Very Elderly Trial (HYVET) antihypertensive drug treatment reduced the incidence of the primary end point (fatal or nonfatal stroke) by 30% (p = 0.06) [38]. Antihypertensive drug treatment reduced fatal stroke by 39% (p = 0.05), all-cause mortality by 21% (p= 0.02), death from cardiovascular causes by 23% (p = 0.06), and heart failure by 64% (p<0.001) (63).

Although the optimal blood pressure treatment goal has not been determined, a therapeutic target of less than 140/90 mm Hg in patients younger than 80 years and a systolic blood pressure of 140-145 mm Hg if tolerated in patients aged 80 years and older is reasonable. We should also be careful to avoid intensive lowering of the blood pressure, especially in patients with diabetes mellitus and coronary artery disease, as this might be poorly tolerated and might increase cardiovascular events (the Curve phenomenon) (64). Until additional data from randomized controlled trials (including the Systolic Blood Pressure Intervention Trial-SPRINT) comparing various blood pressure targets in the elderly and younger become available, existing epidemiologic and clinical trial data suggest a diagnostic and therapeutic threshold for hypertension of 140/90 mm Hg remains reasonable in adults younger than 80 years and of 150 mm Hg of systolic blood pressure in adults 80 years of age and older (65).

Use of antihypertensive drug therapy

A meta-analysis of 147 randomized trials including 464,000 patients with hypertension showed that except for the extra protective effect of beta blockers given after myocardial infarction and a minor additional effect of calcium channel blockers in preventing stroke, use of beta blockers, angiotens in-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), diuretics, and calcium channel blockers cause a similar reduction in coronary events and stroke for a given decrease in blood pressure. The proportionate decrease in cardiovascular events was the same or similar regardless of pre-treatment blood pressure and the presence or absence of cardiovascular events (66).

Diuretics, ACE inhibitors, ARBs, calcium channel blockers, or beta blockers may be used as initial therapy in the treatment of primary hypertension in older and in younger patients. Atenolol should not be used. Beta blockers such as carvedilol, nebivolol, and bisoprolol are preferred (66). Centrally acting agents, such asclonidine, reserpine, and guanethidine, should not be used as monotherapy because they have been associated with a high incidence of significant side effects, including sedation, depression, and constipation (67). Most patients with hypertension will need 2 or more antihypertensive drugs to control their blood pressure. If the blood pressure is more than 20/10 mm Hg above the goal blood pressure, drug therapy should be initiated with 2 antihypertensive drugs (68).

The initial antihypertensive drug should be given to older patients at the lowest dose and gradually increased to the maximum dose (69). If the anti-hypertensive response to the initial drug is inadequate after reaching the full dose of drug, a second drug from another class should be given if the person is tolerating the initial drug (70). If there is no therapeutic response or if there are significant adverse effects, a drug from another class should be substituted. If the antihypertensive response is inadequate after reaching the full dose of two classes of drugs(71), a third drug from another class should be added.

Before adding new antihypertensive drugs, the physician should consider possible reasons for inadequate response to antihypertensive drug therapy, including nonadherance to therapy, volume overload, drug interactions (use of nonsteroidal Anti-inflammatory drugs, caffeine, antidepressants, nasal decongestants, sympathomimetics, etc.), and

associated conditions such as increasing obesity, smoking, excessive ethanol intake, and insulin resistance. Causes of secondary hypertension should be identified and treated in accordance with current guidelines (72).

Use of antihypertensive drugs with associated medical conditions

Patients with prior myocardial infarction should be treated with beta blockers and ACE inhibitor. In an observational prospective study of 1,212 older men and women with prior myocardial infarction and hypertension treated with beta blockers, ACE inhibitors, diuretics, calcium channel blockers, or alpha blockers, at 40-month follow-up, the incidence of new coronary events in patients treated with 1 antihypertensive rug was lowest in those treated with beta blockers or ACE inhibitor. In patients treated with 2 antihypertensive drugs, the incidence of new coronary events was lowest in hose treated with beta blockers plus ACE inhibitors. Beta blockers should be used to treat patients with complex ventricular arrhythmias with abnormal or normal left ventricular ejection fraction and with CHF with abnormal or normal left ventricular ejection fraction. Beta blockers should also be used to treat patients with hypertension who have angina pectoris, myocardial ischemia, supraventricular tachyarrhythmias such as atrial fibrillation with a rapid ventricular rate, hyperthyroidism, preoperative hypertension, migraine, or essential tremor. In addition to beta blockers, patients with CHF should be treated with diuretics and ACE inhibitors and with aldosterone antagonists if needed. ACE inhibitors or ARBs should be administered to patients with diabetes mellitus, chronic renal disease, or proteinuria.

Diuretics and ACE inhibitors are recommended to prevent recurrent stroke in patients with hypertension. Thiazide diuretics should be used to treat patients with osteoporosis. It is also very important to treat other cardio vascular risk factors in patients with hypertension to reduce cardiovascular events, and mortality. Smoking must be stopped. Dyslipidemia must be treated. Diabetes mellitus must be controlled (73).

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Table 1: Preferred treatment options for general population by guideline [15]

Guidelines	Initial treatment	Two-drug combinations	Three-drug combinations
JNC 8	Thiazide diuretic, CCB, ACEI or ARB (Alone or in combination)	Add a drug from another class: thiazide diuretic, CCB, ACEI or ARB	CCB + thiazide + ACEI or ARB
ASH/ISH	Stage 1: ACEI or ARB Stage 2: 2 drugs	CCB or thiazide + ACEI or ARB	CCB + thiazide + ACEI or ARB
AHA/ACC / CDC	Stage 1: Thiazide for most patients or ACEI, ARB, CCB Stage 2: Thiazide + ACEI, ARB or CCB OR ACEI + CCB	Add a drug from another class, either, thiazide diuretic, CCB, ACEI or ARB	Not specified
CHEP	Thiazide, β-blocker, ACEI, ARB, CCB (Consider combination if SBP C ≥20 mmHg or DBP ≥10 mmHg above target)	Add a drug from another class, either, thiazide, b-blocker, CCB, ACEI or ARB	Not specified
ESH/ESC	Stage 1: Diuretics, ACEI, ARB, CCB or β-blocker Stage 2: 2 drugs	Preferred combinations: Thiazide + ARB or ACEI Thiazide + CCB, CCB + ARB or ACEI	Add a drug from another class: thiazide diuretic, CCB, ACEI, ARB or β-blocker
France	Initiating treatment with ARB or ACEI (better persistence versus diuretic or β blocker), CCB (for adherence) is preferred versus diuretic or β-blocker		

NICE	<55 years, ACEI or ARB >55 years CCB	CCB + ACEI or ARB	CCB + thiazide + ACEI or ARB
Taiwan	Stage 1: Thiazide diuretic, CCB, ARB or ACEI Stage 2: 2 drugs	ACEI or ARB + CCB or thiazide	CCB + thiazide + ACEI or ARB
China	Stage 1: Thiazide diuretic, CCB, ARB, ACEI or β -blocker Stage 2: SPCs of 2 drugs	CCB + ACEI or ARB OR ACEI or ARB + thiazide OR CCB + thiazide. OR CCB + β-blocker	CCB + ACEI or ARB + thiazide OR CCB + ACEI or ARB + β-blocker OR ACEI or ARB + thiazide + α- blocker

Abbreviations: AHA/ACC/CDC- American Hypertension Association/American College of Cardiology/Centers for Disease Control and Prevention, ASH/ISH- American Society of Hypertension/International Society of Hypertension, CHEP- Canadian hypertension education program,
ESH/ESC- European Society of Hypertension/European Society of Cardiology, JNC8 - Eighth Joint National Committee,
NICE- National Institute for Clinical Excellence (UK), ACEI - angiotensin converting enzyme inhibitor, ARB- angiotensin receptor blocker,
CCB - calcium channel blocker, SPC- single pill combination

Table 2 Guideline comparisons of goal BP and initial drug therapy for adults with hypertension

Guideline	Population	Goal BP, mmHg	Initial drug treatment options
JNC 8: 2014 Hypertension Guideline [**33]**	General ≥60 y General <60 y Diabetes CKD	<150/90 <140/90 <140/90 <140/90	Nonblack: thiazide-type diuretic, ACEI, ARB, or CCB; black: thiazide-type diuretic or CCB ACEI or ARB
ESH/ESC 2013 [**33]**	General nonelderly General elderly <80 y General ≥80 y Diabetes CKD no proteinuria CKD + proteinuria	<140/90 <150/90 <150/90 <140/85 <140/90 <130/90	 ACEI or ARB
Canadian Hypertension Education Program (CHEP) 2014 [**34]**	General <80 y General ≥80 y Diabetes CKD	<140/90 <150/90 <130/80 <140/90	Thiazide, BB (age <60y), ACEI (nonblack), or ARB ACEI or ARB with additional CVD risk ACEI, ARB, thiazide, or dihydropyridine CCB without additional CVD risk. ACEI or ARB
American Diabetes Association (ADA) 2013 [**35]**	Diabetes	<140/80	ACEI or ARB
Kidney Disease: Improving Global Outcome (KDIGO) 2012 [**36]**	CKD, no proteinuria CKD + proteinuria	≤140/90 ≤130/80	ACEI or ARB

NICE 2011 **37**	General <80 y General ≥80 y	<140/90 <150/90	<55 y: ACEI or ARB ≥55 y or black: CCB
International Society for Hypertension in Blacks (ISHIB) 2010 [**38]**	Black, lower risk Target organ damage or CVD risk	<135/85 <130/80	Diuretic or CCB
Korean Society of Hypertension Guidelines for the Management of Hypertension 2013 [**39]**	Elderly (>65 years) Diabetes Stroke,	<140/90 <140/85 140/90	ACEIs, CCBs and diuretics; BBs should be limited to special scenarios CAD and CKD Combination therapy of ARBs, CCBs and diuretics

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RESEARCH ARTICLE

Measurement of Acoustics in Kolli Hills-An Ecotourism Place of Eastern Ghats of Tamil Nadu State, South India

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ABSTRACT

Kolli hills are an important ecotourism place in an Eastern Ghats of Tamil Nadu situated in Namakkal district. There are fourteen different panchayts in Kolli hills. The transport system is bus, care, bike, lorry and minidoor. The kolli hills contain variety of medicinal plants. The acoustics is recorded in every five kilometer distance by using decibel app. The results are discussed with the available literature.

Keywords: Kolli hills, Eastern Ghats, Tamil Nadu, Ecotourism

INTRODUCTION

The vegetation of the forest is most important to develop the forest ecosystem. Number of studies reveals that the advantages and disadvantages of the vegetation of the forest. Forests are providing energy sources to the ecosystem. Human beings are utilized the forest and forest products in various ways. Forest are provides lot of utilities to the human societies. Forests are maintaining the greening of the nature and have an advantage not only an active contribution to the society, environment and ecosystem. The greening of the forest ecosystem is most and an important due its significance and provides shelter to all living things in the forest ecosystem including flora and fauna. Forests are also maintain the local climatic conditions and also produce more amount of oxygen and maintain pure environment including air, water and its surroundings. Here, these green forms are essential for dust control, for humidification and the cold air generation and hence to the promotion of human Health. They have therefore, like a green roof, the function of a local and natural air conditioning [1]. Forest is also providing the sustainable environment for those who are living in its surroundings. Forests are grate and provide nutrient sources to soil and water and also maintain healthy environment, when its without human disturbances.

Few studies shows that, the study of forest landscapes especially sounds capes have gained to increase the special attention within the several areas of research field, including landscape design and planning. The sounds cape approach has contributed new insights into how, not only acousticians, but also landscape architects can benefit from considering sound [2-7]. Based, on the landscape model, soundscape and its design is useful for the consideration of different aspects such as materials, masking, screening, functions, creation of biotopes for birds, animals, human beings the introduction of water features. Western Ghats are continuous range of forests and the Eastern Ghats are discontinuous range of mountains. Both the mountains are having scattered strong connectivity of flora and fauna in their natural habitats. The most of the research reports reveals that organisms are recognize the landmarks. The landmarks are generally used as the identification factors such as auditory, visual, chemosensory, magnetic, olfactory, and tactile and also demonstrating the heterogeneous as well as landscape structure. Forest systems are always involves the better settlement of living things.

But in addition to the landmarks, most other organisms also have an inborn "positional awareness." The inborn position identity, and also creates territorial attachments [8].This attachment configure based on the behavioral phenomena of flora and fauna. Due to the developments needs, the word is using the limited resources and scarce available on the earth. But, now a days, the drsastic changes is happen due to the disturbance of natural systems by human beings. Even, establishment of rural to urban development, most of the villages turn back to urbanization process and also deplete the natural ecosystems including forest environments. Pollution is the major threats to the forest, but now a day's increasing the vehicles transport via forest to reach human settlement in the forest environment, so we disturbed the natural environment. The green architecture of the forest maintenance is very difficult now a day.

Most of the energy sources obtained from the forest for the benefits of living systems in and around the environment. The green energy system decrease the urban heat, reduce the carbon and mitigation of pollution reduce the runoff water and soil improvement of soil. Then improve a storm-water quality, reduction in interior noise levels, noise absorption [9]. Depending on the types of plants and soils, a GLS can provide a natural habitat for animals, insects, and plants and can increase the biodiversity of an urban area reported by Durhman *et al*. [10]. Ultimately, the acoustical characteristics of a system are governed by the properties of the multiple layers of fluids (density), solids (mass and stiffness) and poroelastic (porosity) materials and the fluid-structure interaction [11]. Mostly the vegetated roofs are implemented to reduce the heat in most of places. In a natural system of environment conserve the plant resources in terms of in situ ecological succession of the different plant species. The vegetated green roofs are acted as a root barrier, water reservoir, drainage layer, substrates, filter fabric and plants. Then the forest vegetation is also increase the aerial biomass and also maintains the microclimate in forest.

The mountain hazards have to be limited and to develop a harmonious coexistence pattern between humans and nature [12]. This is to realize the sustainable development of forest ecosystem in a particular region. Residential is the place where human community live, work and play. It is the best place to educate young people to be aware and responsible in taking care of the Mother Nature and understanding the environment that we live in. The present study deals with the measurement of acoustics in different heights especially hair pin bends in Kolli hills, an ecotourism spot in Eastern Ghats of Tamil Nadu state.

MATERIALS AND METHODS

Profile of the study area

The Garden of Namakkal District is Kolli hills. It is a part of Eastern Ghats and is located in the extreme Eastern part of Namakkal District. It is bounded on the North side by Namagiripettai Block and on the other sides by Uppliyapuram Block. It divides two taluks of Namakkal District, comprising Namakkal and Rasipuaram Taluk. Kolli

Hills Block comes under Rasipuaram Taluk but from Kolli hills block got taluk status. It includes two revenue firkas, namely Valavanthi Nadu and Thiruppuli Nadu, and is locally governed by Kolli Hills Panchayat Union called Nadu, having 16 revenue villages and 14 village panchayats. The Head Quarters of the Block, located at Valavanthi Nadu named as Semmedu is the main bus junction of the Block. The profile of Kolli Hills Block includes the sociological, economic, climate and demographic information of the study area. The block is filled with naturally diverse eco-system such as hills, plains, forests, evergreen fields, drought prone areas, tanks, etc. Due to the presence of the mountain pass, major parts of the block are benefitted by the south-west monsoon. The pass which is commonly known as Palghat Gap has an enduring influence on the trade and commerce that are centered in and around Kolli Hills and Namakkal District.

Location of Geographical Area

Kollimalai is lsituated in Namakkal district of central Tamil Nadu. Its covers some part of eastern portion of the hill lies in the Perambalur district. Kolli Hills coverage area is around 282.92 sq.km. It stretches 29 kms from north to south and 19 km from east to west. Kollimalai is also called as Sathuragiri or square hill. But the hill contains high rising peaks and ravines. The highest point in Kollimalai is 4663 feet above sea level, but the general level of the upper surface of the hill is not more than 3500 feet (1000 m). Its eastern and northeastern flanks drain either into Thuraiyur valley or the valley of the Periyar. Kollimalai region covers a total of 37,961 ha of geographical area. The MSL is 1200 meter. Forest system occupies nearly 44 percentage of the entire geographical area and related to agricultural activities covers 51.6 percentage. The all other activities occupy less than five percent of the total area. As per 2011, population report, 39,716 peoples living in the Kolli hills region and density of 150 persons per square kilometer in the region [13].

The tribal peoples are Malayalis are inhabitant of Kolli hills, one of the tribal peoples in India. The majority of the peoples are economically very poor and also they are doing agriculture in small and marginal scale. They are cultivating rice and millet crops especially traditional varieties. Then fruit crops such as jackfruit, hill banana, well known for flavor and taste. Some other crops such as coffee, pineapple, and spices such as black pepper. Kolli hills spices are very famous in Tamil Nadu. More recently, tapioca (cassava) has become common, often replacing small millets in upland farming areas. This increase of tapioca production, particularly as an industrial crop, is posing a threat to the genetic diversity of traditional crops such as small millets [14].

Demographic Characteristics

The change in land use pattern created serious concerns among the agricultural planners to evolve suitable development strategies. Land is one of the factors of production; it is virtually fixed in quantity although the supply of useful land may be increased by the use of fertilizers, irrigation and machinery and it can be contracted rapidly on large scale by neglecting the principles of soil conservation. The detail of land use pattern block shows increasing trend of fallow lands (both current and other fallows) due to drought situation causing reduction in cropping intensity.

Classification of forest

The Kolli hills forest classified as forest, barren and uncultivable land, land out to non-agricultural uses, cultivable waste, permanent pastures and other grazing land, land under miscellaneous tree crops and craves not included in Net area sown, current fallow, other fallow land, net area sown, geographical area according to village papers, total cropped area, area sown more than once.

Crops Cultivated in Kolli Hills

The following crops are cultivated in Kolli hills such as paddy, ragi, maize, pulses, samai, thinai, sugarcane, coffee, pepper, pineapple, banana, jackfruit, tapioca and mango.

Noise pollution

Noise pollution is generally harmful impact of propagation and impact of harmful on the activity of human beings or animal life. Worldwide, the sound pollution is generally created through transport and system of propagation systems. Noise pollution have an adverse effect on animals especially birds and other insects and flies. The noise may disturb in the natural ecosystem and affect their communication and reproduction and navigation aspects. These effects then may alter more interactions within a community through indirect effects. Environmental noise is the major issues in various ecosystems. The noise pollution is raised from various transport systems, industrial and recreational activities. The effects of noise pollution to effects in human of exposure to an environmental noise and its varies from the emotional, physiological and psychological changes. Government also creates awareness about the noise pollution and its effects on an ecosystem. The measurement of noise is very difficult in the different types of ecosystems. The noise is measured by decibel app and recorded the value. The measurements are typically taken over a period of weeks, in all-weather condition.

Decibel meter

A microphone is very useful for the measurement of sound in the forest ecosystem. Meantime the decibel app installed in the mobile and measured the sound in every hairpin pends in Kolli hills. This is to measure the sound with the help of mobile sensitivity. The instrument is able to measure the noise pollution very accurately and convert the electrical signal back due to sound pressure and measure the decibel units finally.

RESULTS

The noise pollution was measured 5km distance from the Karavalli. It is located in the foot hills of Kolli hills. The noise pollution was measured by using decibel app. The noise pollution create by vehicles such as bike, car, bus, lorry, minidoor, across be Kolli hills road. The maximum sound was recorded in lorry (70.7db) and maximum was recorded in bus (59.4db) (Fig.4). The second noise pollution reading was taken 10 km from Karavalli, foot hills of kolli hills. In this location there is no report in lorry during survey. The maximum 69db was recorded in bus and minimum 63.5db was recorded in car. (Fig.5). The third noise pollution reading measurement was taken bike, bus, car, lorry, and minidoor. The maximum value 69.2 db was recorded in bus. Minimum 63.2 db was recorded in bike. The above reading was taken at the height of 15 km away from Karavalli (Fig.6). The forth reading was taken 20 km away from the Karavalli region. The following vehicles were crossed such as bike, car, bus and lorry. The maximum sound was recorded in 68.9db in lorry and 58.9db in car (Fig.7). In 1000m height, the sound level was recorded in deep forest. The bike sound shows that 55.5db at maximum level. Car sound was recorded around 42.3db. The fifth point in 25 km away from Karavalli. The following vehicles such as bike, car, bike, bus and lorry was crossed. The maximum 69db was recorded in bus 59.7db car was lowest recorded (Fig.8).

In Kolli hills, Nachiamman temple is located at 1120m MSL. When pilgrims enter in to the temple, they are pulling down the bell. The bell sounds was recorded by using decibel app. The maximum sound is recorded 74.3db and maximum 49.3db was recorded (Fig.9). One of the falls is Agaya Ganga. It is located in the top of Kolli hills. Most of the tourists listed the place for the entrainment. The falls receive higher level of water dropping monsoon and low level of dropping summer. The maximum falls found was recorded 49db and minimum 42.3 db. (Fig.10).

DISCUSSION

The mostly bamboos are reducing the noise pollution in the forest ecosystem with the three meters height and both the road sides. This is because the noise sources in this configuration are closer to the screen, causing the detour of the noise over the screen to be bigger.

Green plants have been studies in last three decades show very effective and reduce the heat energy in urban and forest systems. Then green plants are also involves to improve the quality and protect the natural system and reduces electromagnetic waves. So many researchers evaluated the acoustic performance. On the contrary green walls have often been seen as pure decorations, with the unique functional application represented by green road noise barriers. The species selection is based on three fundamental parameters which were identified .These are: (i) ability of a plant to grow in indoor climate conditions, (ii) ability of a plant to survive without direct sunlight, (iii) ability of a plant to branch in horizontal conditions. The maintenance of humidity also important in natural systems.. Fur-thermoses, it can be possible to find habitats in which plants species grow naturally in horizontal position, as in waterfall sides or river blanks. According to the World Health Organization the noise is a major source of environmental pollution in urban centers, affecting directly their quality of life. Many research reports shows that, noise also bring disturbances and lower in performance activity and even changes in blood pressure, increasing the risk of cardiovascular diseases [15-16].

The vegetation of foliar plants to act as barrier for noise pollution in the natural systems. Meantime, through reflection and sound also scattering in the foliage and branches are also involved to make possible to reduce the sound significantly. Few incident of noise is absorbed by the thermo viscous layer of air that surrounds the leaves, and by the sound energy into mechanical vibrations, so dissipated as heat [17]. The impacts on carbon in a natural forest ecosystem and as well as tourist area of the forest have demonstrated the forestry carbon and its effects. Then the rural people to be involved in such interventions and also examine the interactions between responses of the carbon reduction [18-20].

CONCLUSION

A Kolli hill is an important plant for biodiversity, agriculture, cultivation of spices and condiments, and ecotourism, most of the medicinal plants occupies the biodiversity. Kolli hills contributor now a day a good place ecotourism. Mostly can, bike, jeep passed for transportation of people. Lorry and minidoor is used for transportation of goods. Karavalli is located in the foot hills of Kolli hills. The around noise pollution (acoustics) is measured from 5,10,15,20 and 25km away from Karavalli. The sound level was recorded car, bike, bus, lorry and minidoor by using decibel app. The results show that different height different decibel. The natural ecosystem of Kolli hills container birds, butterflies, cattle's, poultry, wild animals etc., sound effect disturb the natural ecosystem. Finally, reduce the sounds of transportation and usage of air horn prohibited except hairpin bend is highly appreciable and this is the recommendation for the society.

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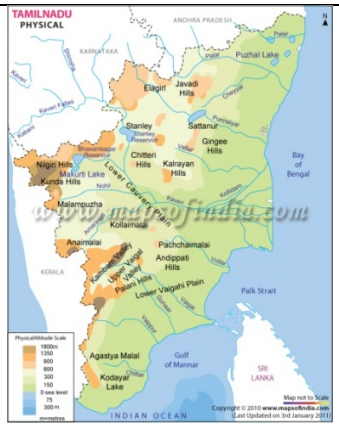
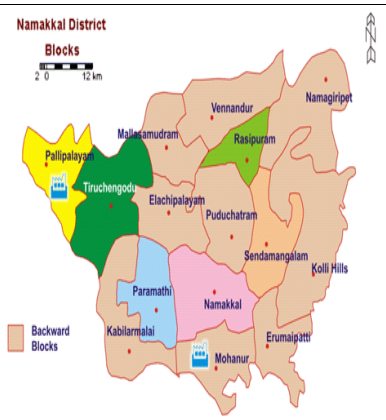
Table.1. Different Panchayat Villages in Kolli hills, Easter Ghats of Tamil Nadu.

S.No	Panchayat village	S.No	Panchayat village
1.	Peraikkarinadu	2.	Gundurnadu
3.	Bailnadu	4.	Valappurnadu
5.	Chithurnadu	6.	Ariyurnadu
7.	Edappulinadu	8.	Valavanthinadu
9.	Thiruppulinadu	10.	Thinnanurnadu
11.	Alathurnadu	12.	Devanurnadu
13.	Gundaninadu	14.	Selurnadu



Tamil Nadu Map



Namakkal District Map

Figure.1. Location of Kolli Hills, Namakkal district of Tamil Nadu State, India

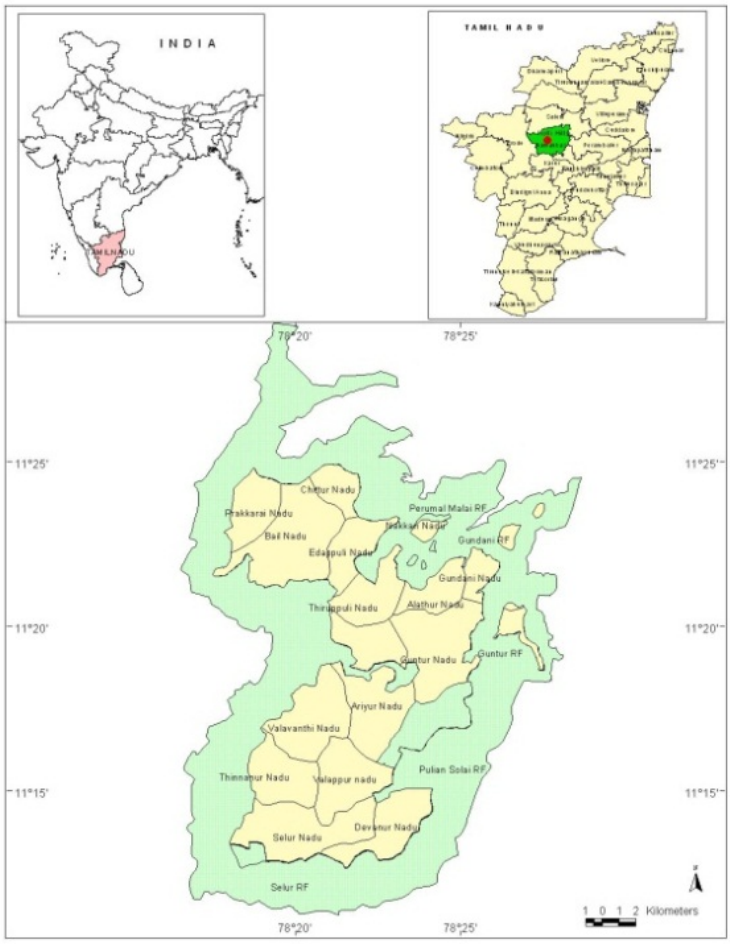


Figure.2. Location of showing different panchayats Kolli Hills, Eastern Ghats of Tamil Nadu State, India

Figure.3. Different locations of hair pin bends in Kolli hills, Eastern Ghats of Tamil Nadu for the measurement of acoustics through decibel app

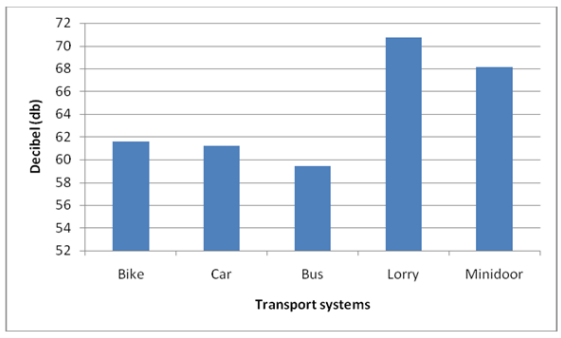


Fig.4.Sound recorded around 5km distance from Karavalli

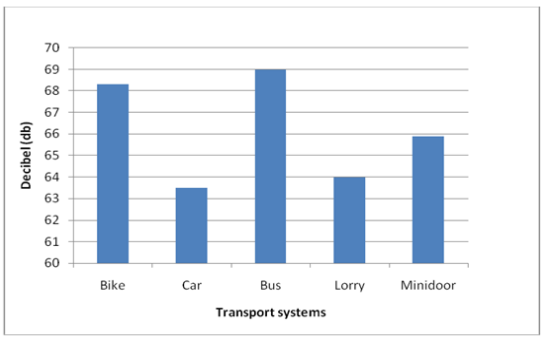


Fig.5.Sound recorded around 10 km distance from Karavalli

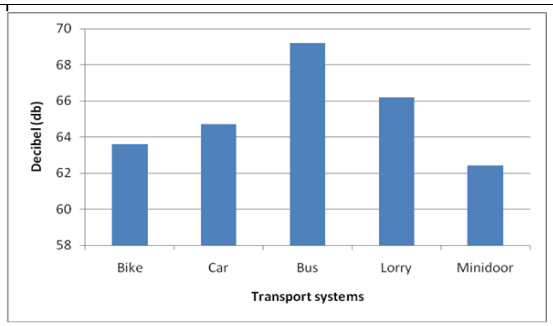


Fig.6.Sound recorded around 15 km distance from Karavalli

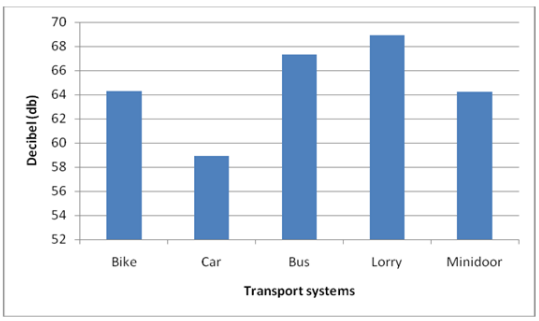


Fig.7.Sound recorded around 20 km distance from Karavalli

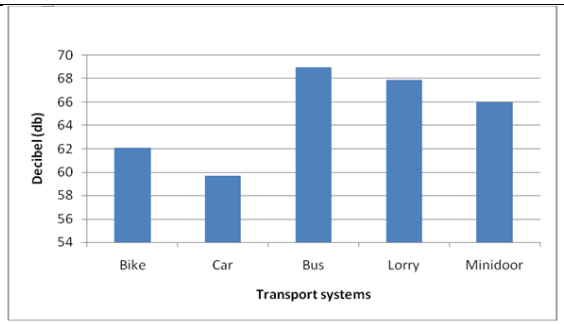


Fig.8.Sound recorded around 25 km distance from Karavalli

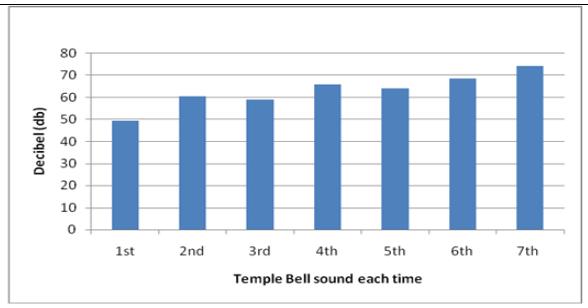


Fig.9. Sound recorded in Nachiamman Temple at the middle of Kolli hills

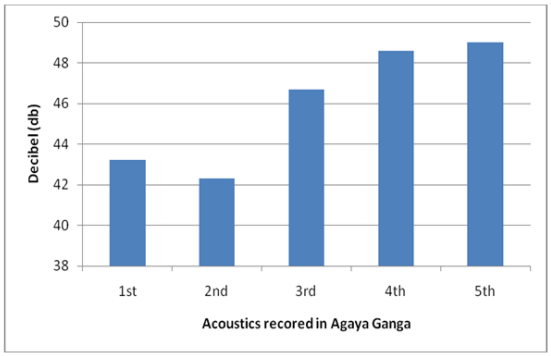


Fig.10. Sound recorded in Agaya Ganga waterfalls, Kolli hills top

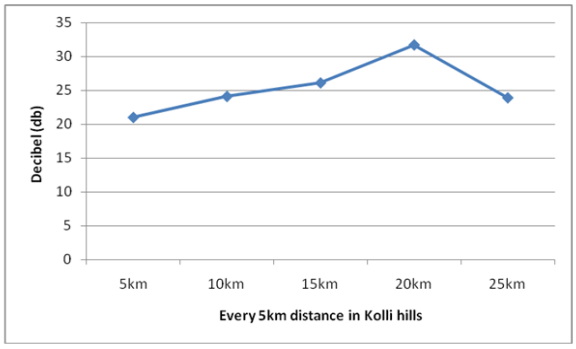


Fig.11.Normal sound measured at without the disturbance of vehicles in Kolli hills

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REVIEW ARTICLE

Protein and Peptide Based Drug Delivery System: A Review

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ABSTRACT

Protein and peptide is a novel drug delivery mechanism and is a novel approach to the drug delivery system. Protein and peptides are the most abundant material of the living organism and biological cell. The successful delivery of pharmaceuticals based on peptides and proteins is determined primarily by their ability to overcome the multiple barriers encountered in the biological environment. Oral route is the most common distribution route from the patient's perspective. This route has the key advantages of ease, acceptability and high patient compliance. Buccal membrane contains numerous elastic fibers in the dermis, another barrier for drug diffusion across the buccal membrane. Particles that enter the alveoli will be absorbed into the systemic circulation, avoiding the metabolism and harsh intestinal conditions first pass. Due to their poor absorption and metabolic instability when provided by other alternative routes, parenteral mode of drug delivery has been the key route of choice for protein / peptide.

Keywords: Protein and Peptide, delivery, Route, Enzyme, Systems

INTRODUCTION

Proteins are amino acid chains, each joined to its neighbor by a specific form of covalent bond. The structural structure of proteins is formed by the polymerization of L-α amino acids by peptide bonds. The term protein refers to molecules made up of m re than 50 amino acids. The term peptide is applied to molecules containing fewer than 50 amino acids[1]. The chemical and structural complexities involved require an efficient delivery system in which the physicochemical and biological properties, including molecule size, confirmation stability, solubility, light sensitivity, humidity and heat, biological half-life, immunogenicity, dosage requirements, susceptibility to breakdown in both physical and biological environments, special requirement [2].

Protein and Peptide Structure

It is important to have an idea about protein and peptide structure in order to resolve various problems encountered during the development of drug delivery systems. The proteins are relatively large, complex structured molecules. The peptide chains in peptides and proteins are rarely linear and follow a number of complex patterns and conformations of the three dimensional folds.

- Primary structure: It refers to the number and unique amino acid series.
- Secondary structure: arrangement of individual amino acids around the backbone of a polypeptide.
- Tertiary structure: 3D configuration of a single protein molecule.
- Quaternary structure: Proteins containing two or more non- covalent polypeptide chain [3].

PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

Protein and peptide is a novel drug delivery mechanism and is a novel approach to the drug delivery system [4]. [Protein and peptides are the most abundant material of the living organism and biological cell [5, 6]. Its function involves hormones, enzymes, structural element and immunoglobulin [7]. It is also important to participate in a variety of metabolic processes, immunogenic defense and to participate in many biologies [8]. First used, Berzelius [9,10,11]. Protein exists in all living cells to provide dietary function for body building capacity [12]. Essential molecules for plant proteins are one of the most abundant organic molecules in the biological system, the word protein and animal cells. In Protein is primarily act has enzyme for the catalysis of biochemical reactions [13].

It acts in order to give a definite shape, strength to the cell and tissues [14]. It has one of the most important applicability to control the metabolic processes, pH, osmotic pressure and temperature [15]. Protein insulin controls the amount of blood sugar [16]. It is essential for muscle development and mechanical work [17,18]. In the case of peptide, the two amino acids are reduced to dipeptide [19- 22]. The so-called 100 polymers and more than 100 amino acids have proteins [23]. Proteins are first categorized into two groups based on protein solubility and another is ambiguity of protein structure. In the first example, they are categorized into two groups of globular protein and fibrous proteins on the basis of solubility. The proteins are soluble in water or common salts known to contain globular proteins and the proteins are insoluble in water and common solvents [24]. Secondly, on the basis of complexity Proteins are categorized into three forms Fist is a simple protein that can contain only one amino acid, second is a conjugated protein that can contain amino acids and non-protein components, and third is a hydrolysis substance produced by the action of physiological agents such as fire, chemical agent and enzymatic activity on the protein molecules [25].

The structure of Protein is mainly categorized into four categories First is primary protein structure The primary protein structure is referred to as the number, existence and sequence of amino acids along with polypeptide chain, In this structure the N amino acid terminal often shown at the left end of Polypeptide and C amino acid terminal shown at the right side, The best example of Building is an Insulin Molecule [26]. Secondary protein structure where the Long Polypeptide Chain is folded or entangled in a particular geometric arrangement. The two types of Protein Alpha Helical Structure secondary structure arrangements and the Beta Pleated sheet [27]. In tertiary protein structure are the three-dimensional coiling and folding of the chain, stabilized by the interaction of the amino acid sequences, this folding results that the (R-) group is side chai amino acids, these interactions are primarily (H-) bonded interactions [28]. The ultimate form of the tertiary protein structure is an elapsed, globe, and any other irregular shape.In Protein's Quaternary structure are the two or more polypeptide chain held together by non-covalent bond to give the protein's quaternary structure, Hemoglobin has Example of Protein's Quaternary structure.Proteins and peptide are endogenous working essential to sustain the biological environment [29]. the discovery of numerous hormones and peptides is applicable to pharmaceutical and biopharmaceutical products, applicable in human disease pathophysiology, significant application in medical practice of protein and peptide, drug discovery processes and research activities [30]

Barriers to Peptide and Protein Delivery [31, 32]

The successful delivery of pharmaceuticals based on peptides and proteins is determined primarily by their ability to overcome the multiple barriers encountered in the biological environment. There are different obstacles faced:-

- Enzymatic Barriers
- Intestinal Epithelial Barriers
- Capillary Endothelial Barrier
- Blood Brain barrier (BBB)

NEED OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. Protein and peptides in biological cells and organic molecules are very important [33]
2. Diseases such as Diabetes mellitus are caused in the absence of proteins and peptides. (Caused by lack of INSULIN protein) [34]
3. R-DNA development and hybridoma methods are now being used in pharmaceuticals based on proteins and peptides [35]

ADVANTAGES OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. The erythropoietin is used primarily for RBC development [36]
2. The plasminogen activator protein Tissue is used for Heart Attack, Stroke [37].
3. Oxytocin is used in occupational pain management [38].
4. Bradykinin enhances circulation at the periphery [39].
5. Somatostatin reduces gastric ulcer bleeding [40]
6. Gonadotropin stimulates ovulation [41].
7. Insulin keeps blood sugar level [42]

DISADVANTAGES OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM [43]

1. Large molecular size
2. Short plasma half life
3. Ion permeability
4. Immunogenicity
5. Aggregation
6. Denaturation
7. Susceptibility to enz. Degradation

FUNCTIONS OF PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

1. Transport and storage of small, biological molecules and molecules [44].
2. Coordinated movement by muscle contracture [45].
3. Mechanical Fibrous Protein Support [46].
4. Nerve Impulse Generation and Transmission [47].
5. Catalysis enzymatic in biochemical reactions [48].
6. The defense of the Immune by antibodies [49].
7. Development regulation and Hormone differentiation [50].

DELIVERY OF PEPTIDE AND PROTEIN DRUGS

Different routes include

1. Oral route
2. Buccal route
3. Nasal route
4. Transdermal route
5. Pulmonary route

6. Rectal route
7. Parenteral route

ORAL ROUTE [51- 59]

Oral route is the most common distribution route from the patient's perspective. This route has the key advantages of ease, acceptability and high patient compliance. The key obstacles to effective oral delivery of protein and peptides are similar to conventional drug candidates except in the case of peptide / protein moieties these are more pronounced. The main barriers to effective oral delivery are-

- Low intrinsic peptide / protein permeability across biological membranes.
- Intestinal proteases and peptidases are susceptible to enzymatic attack.
- Simple, post-absorbent clearance.
- Physical weakness, such as adsorption and aggregation.

BUCCAL ROUTE [60- 63]

Buccal membrane contains numerous elastic fibers in the dermis, another barrier for drug diffusion across the buccal membrane. The barriers for efficient drug absorption are:

- Coat of mucus forming an oral epithelium.
- Barriers to the epithelium.
- Peptidases and microbial flora in the saliva and mucus layer.

The absorption of the buccal peptide is believed to be by passive absorption process. Molecular weight, polarity, conformation, dissociation, and enzymatic and chemical stability are the specific parameters that affect the degree of Buccal peptide absorption. Conventional methods include aqueous solutions, Buccal tablets and capsules, or sublingual ones. The inherent issue with these dosage types, however, is the possibility of drug loss through accidental swallowing, or salivary washout. Often they don't allow to drink and patient is also a handicap for speaking. With these formulations, the administration time is reduced and so controlled release cannot be achieved. Self-adhesive systems have been developed to address such limitations and are capable of being in close contact with the mucosa, i.e.the different adhesive polymers include water-soluble and insoluble polymers of both ionic and nonionic forms. Sodium carboxy methyl cellulose, hydroxyl propylmethyl cellulose, polyvinyl pyrrolidone, acacia, calcium carbophil, gelatin, and polyethylene glycol are some of those polymers.

NASAL ROUTE [64- 68]

The intranasal route is usually appropriate for the sporadic delivery of highly potent peptide / protein drugs with low molecular weight. It is documented that peptidal drug moieties such as calcitonin, ACTH, insulin, and interferon have substantial absorption through the nasal mucosa. Nasal route is used mainly to distribute protein medication. The nasal route after the parenteral route is the most effective one to achieve a systemic effect. Barriers to systemic absorption through nasal route:

- The absorption rate varies with the mucus production and the turnover of the mucous.
- Peptidases and proteases found in the mucus or connected with the nasal membrane serve as enzymatic barriers in the absorption of proteins / peptides.
- Modification of the absorption profile in illnesses such as allergic disorders and chronic rhinitis and upper respiratory tract infections.

Enhancers of penetration and preservatives can damage the mucosal cell membrane, and can even be ciliotoxic.

TRANSDERMAL ROUTE [69]

Advantages of Transdermal Route for peptide/protein Delivery are:

- Improved and better communication with patients.
- Elimination of the first-pass hepatic syndrome.

- Controlled administration and therefore prevention of toxic effects. Drugs can also be prescribed with a shorter half-life.
- Medications with low therapeutic index can be administered.

Limitations of Transdermal Route for peptide/protein Delivery are:

- Poor permeation levels for most protein drugs due to their broad molecular weight and hydrophilicity and lipophilic character of stratum corneum.
- High intra- and interpatient variability.

PULMONARY ROUTE [70, 71, 72]

Particles that enter the alveoli will be absorbed into the systemic circulation, avoiding the metabolism and harsh intestinal conditions first pass. Particle characteristics such as aerodynamic diameter may be designed to carry particles to different areas of the lung. The aerodynamic diameter da derives from the law of Stoke, and is determined by:

$da=(p_p/p_0)^{0.5}d_g$

Where p_p is the particle density, p_0 is standard particle density (1 g/cm3) and dg is the geometric diameter of the particle.

- Pulmonary Pathway Benefits for peptide / protein supply are.
- Offers a direct route to circulation.
- Dose requirement reduction up to 50 fold and thus a cost-effective option.
- Absorption is rapid.
- Secure drug entry road, also in lung disease patients.
- No immune-function cause.
- Improved patient compliance with reduced pain and discomfort.

Dry powders are used for pulmonary delivery of peptide / protein products, aerosols with or without penetration enhancers. Example insulin is given by aerosol while calcitonin is given as dry powder.
There are also few drawbacks associated with existing pulmonary delivery systems.

- Most of the medications are administered to the upper lung, which is a region with poor systemic absorption.
- Just a limited amount of available medicines.

Both basic diffusion and carrier-mediated transport mechanisms work in the lungs, close to the intestine.
The lung is a permanent organ, as a requirement. An average person inhales air everyday along with all the dust and all the particles that float in it. In terms of immunogenicity, the safety issue regarding the pulmonary route for peptide / protein administration should be considered.

RECTAL ROUTE [73, 74, 75]

Advantages of Rectal route are:

- Highly vascularised.
- The first step, or presystemic metabolism, is generally avoided.
- It is ideal for oral administration medications that can induce nausea / vomiting and irritate the GI mucosa.
- Drug absorption can be disrupted in the event of an adverse reaction or a drug overdose.
- A large dose of the medication may be given.
- Drug can be intended for the lymphatic system.

Various factors affecting absorption from the rectal route are:

- Amount of fluid found in the rectum.
- The pH and the rectal fluid buffer capacity.
- Surface stress and rectal fluid viscosity;
- Rectal rectal wall luminous pressure which increases rectal absorption.
- Solubility, coefficient of partition, product pKa;
- The composition of the particles and their surface properties.

The traditional dosage types were used for peptidal delivery including gels, liquids, and suppositories. Among these, gels have been found to provide an optimal balance between administration site retention and peptide release rate. Many of the peptide / protein drugs need absorption enhancers to achieve a fair absorption level.

PARENTERAL ROUTE [76]

Due to their poor absorption and metabolic instability when provided by other alternative routes, parenteral mode of drug delivery has been the key route of choice for protein / peptide. The efficient design of these moieties demands that they target particular receptors so as to enhance a drug's therapeutic index. There is the risk of inducing immune responses and other possible deleterious side effects and interactions when peptides are administered at high dosage rates. Consequently, targeting protects both the medication and the body from these contraindicating manifestations. Intravenous, intramuscular, subcutaneous, intraperitoneal, intrathecal application involves the parenteral drug delivery system.

OCULAR ROUTE [77- 81]

Mechanism of Drug Absorption by Ocular Route

Barrier to ocular route is;

- Tear dilution
- Lachrymal drainage
- Protein binding

The systemic delivery of peptide / protein drugs was tried through the ocular route. The idea behind the delivery of ocular drugs to systemic circulation takes advantage of the robust dynamics of the lachrymal system, which exports the drug to the nasal cavity from where substantial systemic absorption occurs. Attempts to administer insulin have been made through this route. Tangible movements and tears however wash away the insulin solution quickly. To fix this issue, sodium hyaluronic acid has increased the viscosity of the insulin solution. The feasibility of ocular peptide / protein delivery using eye drops as a delivery method is limited. The eye drops exhibit poor bioavailability, poor therapeutic effectiveness and limited period of action. Face extensions may be employed to overcome these limitations. Another system based on absorbable gelatin sponge was successfully employed to strengthen the above limitations. The tool is produced by perforating a gelatin disk. The solution for the medication is sorbed into the disk and the wet matrices are dried under vacuum. The system was used to deliver insulin. The benefits of this device are:

- The fabrication process is fairly simple and inexpensive.
- The tool is soft and pliable on hydration and therefore comfortable.
- The procedure can simply be terminated by removing the tool from the eye.
- Tried to give the retinopathic drug because of diabetics.

PHARMACEUTICAL APPROACHES

The protein and Peptides are having Five Approaches they has Follows

1. Chemical modification
2. Enzyme inhibitors
3. Mucoadhesive polymeric system

4. Absorption enhancers
5. Formulation vehicle

Chemical modification [82, 83, 84]

The Chemical Modification of Drug Delivery System for Protein and Peptide Drugs is critical in improving both enzymatic stability and membrane permeations. This is relevant to the immunogenicity reduction.
The Chemical Alteration is included as follows in two forms of Change:

1. Amino acid Modification
2. Hydrophobization

Amino acid Modifications: Amino acid modification is one of the important approaches in which D-amino acid and L-amino acid substitution is important to alter the physiological properties of protein and peptide drug delivery systems. Hydrophobicisation: The Lipophilic Moieties have an important approach.

Enzyme inhibitors [85]

The enzyme (protease) inhibitors are the enzymatic mechanism used by drug delivery systems for Protein and Peptide.With the aid of the variety of Proteolytic Enzymes, GIT and Liver play an important role in metabolizing the protein and peptides into smaller fragments of the two to ten amino acids.These protease inhibitors are given CO-with protein and peptide to alter the enzyme stability environment in order to suppress photolytic activity.

Mucoadhesive polymeric systems [86]

The mucoadhesive polymeric system is essential to prevent and maintain the therapeutic efficacy of the problem associated with Presystemic Metabolism or first pass metabolism. At the site of action the residence time of this drug delivery mechanism and the rate of drug clearance increasing or decreasing.

Absorption enhancers [87, 88]

In order for therapeutic agents to exert their pharmacological effects, they must cross into the systemic circulation from the biological membranes and enter the place of action.Absorption enhancers are the components of the formulation that temporarily disrupt the intestinal barrier to increase the permeation of these drugs. The action of absorption enhancers should preferably be immediate and should correlate with the presence of the drug at the site of absorption. Numerous groups of chemical substances, including detergents, surfactants, bile salts, Ca2 + chelating agents, fatty acids, glycerides of the long chain, acyl carnitine, alkanoyl cholines,N-acetylated α-amino acids, N-acetylated non-α-amino acids, chitosans, mucoadhesive polymers and phospholipids have been reported to improve the absorption of large polypeptide drugs by the intestines.

Formulation vehicles [89]

A primary purpose of oral delivery systems is to protect protein and peptide drugs in the GIT against acid and luminal proteases. Several formulation strategies are being investigated to overcome those barriers. Here we discuss the use of dry enteric-coated emulsions, microspheres, liposomes, and nanoparticles for oral peptide and protein delivery. Emulsions in the intestinal lumen shield the drug from chemical and enzymatic degradation. Drug absorption enhancement depends on the emulsifying agent type, distributed phase particle size, pH, drug solubility, lipid phase type used etc.The lipid step of microemulsions consists of medium-chain fatty acid triglycerides that increase the bioavailability of analog muramyl dipeptides.

APPLICATION

1. CVS acing drugs Protein and Peptides (Angiotensin 2 antagonist, Bradykinin, Captopril) is essential for the Lowering blood pressure and improving peripheral circulation for Heart failure management [90].

2. For suppressing appetite and relieving pain, CNS active protein and peptides (Cholecystokininin, B-endorphin) is essential [91].
3. GI-active protein and peptides (gastrin antagonist, pancreatic enzymes) are effective in reducing gastric acid secretion, and are essential for digestive supplement [92].
4. Protein and peptide immunomodulation (Bursin, Cyclosporine, and Interferon) is essential for selective B-cell differentiatingharmone Inhibits T-lymphocyte functions enhancing killer cell activity [93].
5. Protein and peptide modulating metabolism (insulin, vasopressin) is essential in the treatment of diabetes mellitus and diabetes insipidus [94].

CONCULSION

Pharmaceuticals based on proteins and peptides are increasingly becoming a very significant class of therapeutic agents, and are likely to replace many current conventional pharmaceuticals in the very near future. Biotechnology technologies can produce peptide and protein pharmaceutical products on a wide scale and become commercially available for therapeutic use. Different drug delivery systems are developed which can protect them from proteolytic degradation, enhance permeation through the absorptive epithelia, and prolong the dose retention at the administration site.

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REVIEW ARTICLE

Vaccine Delivery Systems - A Review

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ABSTRACT

A vaccine is a biological preparation which enhances immunity to a specific disease. The traditional types of vaccines that have been used to date clinically are vaccines that contain either dead or live-attenuated microorganisms, inactivated toxins, protein subunits, and polysaccharide antigens or conjugates. Vaccine delivery systems are generally particulate e.g. microparticles, nanoparticles & liposomes and mainly function to target associated antigens into antigen presenting cells. Appropriate vaccine administration/delivery is the key element to ensure successful vaccination. This paper reviews carrier systems such as liposomes, microspheres, nanoparticles, which are now being investigated and developed as vaccine delivery systems. This paper also describes to administer the vaccine delivery systems through different routes into the human body.

Keywords: Vaccine delivery system, Vaccine, Liposomes, Microparticles, Nanoparticles.

INTRODUCTION

Vaccination is the most effective means of controlling infectious disease-related morbidity and mortality. The World Health Organization (WHO) estimates that vaccination prevents over 2.5 million child deaths each year worldwide. A vaccine is a biological preparation which enhances immunity to a specific disease. The traditional types of vaccines that have been used to date clinically are vaccines that contain either dead or live-attenuated microorganisms, inactivated toxins (Toxoid), protein subunits, and polysaccharide antigens or conjugates. A number of innovative vaccines are in development such as recombinant vector and DNA vaccines. These agents resemble a disease-causing microorganism and stimulate the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily challenge these microorganisms upon subsequent

encounters. Appropriate vaccine administration/delivery is the key element to ensure successful vaccination. Typically, most vaccines are administered via the subcutaneous (SC) or intramuscular (IM) routes. Hypodermic injections are associated with pain and distress that might lead to poor patient compliance and require highly trained personnel for administration. They are associated with a risk of disease transmission due to the possibility of needle-stick injuries or reuse of contaminated needles. Insufficient vaccine supply or limitation of vaccine production may also prove problematic in instances when mass vaccination is necessary [1, 2].

VACCINE DELIVERY SYSTEMS

Delivery of antigens from oil-based adjuvants such as Freunds adjuvant leads to a decrease in the number of doses of the vaccine to be administered, but such adjuvants are not commonly used due to toxicity issues such as inductions of granulomas at the injection site. Aluminum hydroxide and aluminum phosphate in the form of aluminum are approved adjuvants by FDA for human use. Other reason driving the development of vaccines as controlled drug delivery systems are immunization failure with conventional immunization regimen involving prime doses and booster doses, as patients neglect the latter [3].

LIPOSOMES AS DRUG AND VACCINE DELIVERY SYSTEMS

Liposomes are effective delivery systems as they render soluble protein antigens into a particulate form thereby lengthening their half-life in vivo. Furthermore, liposomal formulations composed of cationic lipids have been shown to be immune stimulatory [4, 5]. Liposome formation is an energy-dependent process that is heavily affected by the geometry of the lipid monomers, which can be quantified by the critical packing parameter (CPP) of the lipids. Lipids expressing large head groups and double hydrocarbon chains have a CPP of < 1, which is required for the formation of bilayered vesicles [6]. The bilayered nature of liposomes makes them attractive delivery systems as both hydrophilic and lipophilic substances can be encapsulated within the liposomes. By applying post-formation techniques such as sonication, extrusion or freeze-drying, the natural heterogeneous multilamellar structure of liposomes can be altered to favor a higher encapsulation ratio [7].

The main advantages of using liposomes as drug delivery systems are [8-10];

- ✓ Their ability to entrap and therefore protect drugs from degradation.
- ✓ Deliver drugs with otherwise poor solubility.
- ✓ Improve the therapeutic index of drugs by shielding and therefore reducing toxicity.
- ✓ Alter the specific targeting/ distribution of the drug.

Many of the properties leading to successful liposomal drug delivery systems are also valid for vaccine delivery [11]. First, liposomes efficiently protect small peptide/protein antigens from enzymatic breakdown by host cells [12, 13]. The bilayered nature of liposomes allows these hydrophilic components to be either encapsulated in or adsorbed onto the liposomal surface while the lipophilic part of the liposomes allows other lipids or apolar polymeric components to be included in the membrane bilayer. Second, depending on the structure, charge and size of the liposome, it can have specific pharmacokinetics and may be designed to achieve maximum retention and vaccine antigen presentation [14]. Third, owing to their particulate nature, liposomes are avidly taken up by antigen-presenting cells (APCs). This ability probably represents the most important characteristic of liposomes to be used as vaccine delivery systems [15] because it allows for simultaneously ingested antigen to be processed and presented on Major histocompatibility complex (MHC) molecules.

Oil in water liposomal formulations were recently described in which mineral oil was emulsified in the presence of liposomes, which donated phospholipids as stabilizers [16]. Clearly, this is a very complex formulation, which would need to show a dramatic improvement over alternative approaches before it can be accepted as a significant advance in the field. Modified liposomal structures termed ‘cochleates’ are also being evaluated as systemic and mucosal adjuvants in small animal models [17]. The development of polymerized liposomes, which show enhanced stability in the gut, also offers potential for the development of mucosal vaccines [18].

MICROPARTICLES AS VACCINE DELIVERY SYSTEM

Poly lactic-co-glycolic acid (PLG) polymers are the primary candidates for development of microparticles as vaccine adjuvants. PLG polymers are biodegradable, biocompatible and have been used widely in humans for many years as resorbable suture material and as controlled release drug delivery systems [19, 20]. Several research groups have demonstrated the adjuvant effect achieved through encapsulation of antigens into PLG Microparticles in the early 1990s [21-24]. However the potential microparticles as vaccine adjuvants has been limited because of the denaturation and degradation of proteins during the encapsulation process [25]. Therefore new approaches to circumvent this problem of protein degradation were developed. One such process involved adsorption of the antigen onto the surface of charged (cationic or anionic) microparticles [26]. The approach allowed to the induction of significantly enhanced antibody titers in mice with an adsorbed recombinant p55 gag antigen from HIV-1 [27]. Anionic PLG microparticles with adsorbed recombinant antigens have also been used to induce potent antibody responses against *Neisseria meningitides* serotype B, including functional titers able to effectively kill the bacteria in-vitro [28].

A number of studies have been undertaken to evaluate the potential for microparticles with encapsulated antigens for the development of single dose vaccine through controlled release of the antigen. Controlled release PLG microparticles had already been developed for a variety of entrapped drugs, including a recombinant protein [19, 20]. Hence there was considerable optimism that PLG microparticles might also prove successful for controlled release of vaccines. However, although microparticles with entrapped antigens were able to induce potent long lasting immune responses in rodent models, it was clear that bacterial toxoid vaccines in particular had significant stability problems following microencapsulation [29]. A variety of approaches have been undertaken to stabilize toxoids entrapped within microparticles, these have generally met with limited success [30-33]. It has been reported that HBsAg entrapped in controlled release PLG particles could induce potent immune responses following a single immunization [34], an observation consistent with earlier work on the same antigen [35]. HBsAg microspheres displayed higher serum anti-HBsAg IgG titers consistently, demonstrating that alum combined with antigen has a strong impact in enhancing the magnitude of immune responses to PLA microspheres, possibly due to a synergistic immune stimulatory adjuvant effect between the PLA microspheres and alum adjuvant [36].

In considering the optimal approach for microencapsulation of vaccines, it is expected that each protein will present its own distinctive challenges and specific approaches need to be adapted to allow the development of a stable product. A universal formulation approach that could be applied to be all vaccine antigens is unlikely. Although vaccines entrapped in PLG microparticles have induced responses of considerable duration in small animal models, early studies showed that alum formulations could also induce small long-lasting responses [37, 38]. Hence, the slow-decay kinetics of antibody responses in rodents make them less than ideal to evaluate the potential of controlled-release microparticle formulations, which are designed to provide 'pulses' of antigen release to mimic booster doses of vaccines. Overall, there is little evidence that microparticles can provide in vivo boosting of responses following a single immunization, although, many studies confirm that responses can be maintained for extended periods (3-4 weeks) [39].

An additional problem in the development of microparticles as single-dose vaccines relates to the uncertainty over what is the optimal release profile of antigens to induce long-term responses in larger animals, including humans. Although, there have been many claims that a 'pulses' of antigen release is preferred, rather than a continuous release profile, the earliest studies on control-led release of vaccines actually showed that continuous antigen release induced higher antibody titers [40]. Some other studies indicate that both continuous and discontinuous antigen release can induce potent responses in small-animal models [39]. It seems unlikely that the question on optimal release profile of antigens can be addressed adequately in small-animal models [41]. Given these various problems, we cannot find any studies in which PLG microparticles with entrapped vaccines have been evaluated in non human primates or humans. In contrast, PLG microparticles with adsorbed DNA vaccines are currently undergoing clinical

evaluation and have already been evaluated in non-human primates for delivery of a recombinant protein vaccine [42].

NANOPARTICLES AS VACCINE DELIVERY SYSTEM

Nanoparticles offer the potential to protect antigens and adjuvant from premature enzymatic and proteolytic degradation. Nanoparticle delivery systems offer the added strength of multi-component loading which is of considerable significance particularly in immunotherapy where simultaneous delivery of antigens, immune adjuvants, and targeting ligands is ideal. Additionally, due to their large surface area, nanoparticles can be readily surface-engineered with proteins, peptides, polymers, cell-penetrating moieties, reporter groups, and other functional and targeting ligands. The ease of design and use combined with multi functionality makes using nanoparticles a versatile and useful delivery strategy for vaccines and immune therapies [43]. Traditional vaccines include live attenuated microbes, killed microbes, or components of microbes. While several of these vaccinations have been crucial in preventing infectious disease, others do not provide sufficient disease protection. However, some live vaccines are not safe for use in community in the increasing population of immune compromised individuals. There is also a large range of infectious diseases for which there are no approved vaccines. A number of vaccines are being designed to overcome these challenges based on isolated proteins or polysaccharides, or naked DNA encoding a protective antigen. While these may be healthier, more defined, and less reactogenic than other current vaccines, they are also weak immunogens that require adjuvants to improve their effectiveness. Aluminum is the most widely used adjuvant, but it may cause local reactions and does not yield effective cell-mediated immunity [44, 45]. As a consequence, there is a great need to develop novel adjuvants and delivery systems for the next generation of vaccines.

Recently attention has been focused on the utility of Nanoparticles (NPs) as vaccine delivery vehicles. The vaccine antigen is either encapsulated on the surface of the NPs. And it provide a method of administering antigens by encapsulating antigenic content that can otherwise degrade rapidly upon injection or cause a short-lived, localized immune response. Conjugation of antigens onto NPs will cause the immunogen to be introduced to the immune systems in much the same way as the pathogen would present it, thus producing a similar response. In addition, NPs made from some composites not only allow the site-directed delivery of antigens, but also the sustained release of antigens to optimize immune system exposure. The potential for NPs to deliver vaccines through non-traditional methods such as topical, inhalation, or optical delivery, as well as adding multiple antigens to the same particle to protect against more than one disease, is also being explored.

Characterization of Nanovaccines

If synthesized, characterizing the structure and composition of the NP formulations is important in order to avoid any difference between (or within) batches. Variation could arise from contamination, a poly disperse population of NPs, the accumulation of toxic components or incomplete particle formation. In order to maintain a homogenous population, several methods are employed to measure uniformity within colloidal solutions. Spatial uniformity among NPs is important as the spherical volume may influence how much antigen is encapsulated or conjugated to the surface, and may vary the vaccine's immunizing dose. Consequently, the size and shape of particles is characterized using a variety of methods including electron microscopy, dynamic light scattering, and density gradient centrifugation [46, 47]. The amount of antigen-present is then quantified using one or more of the following techniques: Lowry and Bradford assays, enzyme-linked immune sorbent assay, dot-blots, density gradient centrifugation, sodium dodecyl sulphate polyacrylamide gel electrophoresis, and Western blotting [48-52]. In certain cases, the compositional content of the NP may need to be calculated if any of the reagents are toxic at high doses. This is especially true of Quil A, a key component of ISCOMs that can have a haemolytic effect at appropriate concentrations and is measured in a rocket electrophoresis assay or by high-performance liquid chromatography in reverse phase [53, 54]. Certain components of ISCOMs such as cholesterol and phospholipids are measured respectively by gas chromatography and phosphorus assays. Quantification of metal (and non-metal) NPs, such as

gold, can be quantified using instrumental neutron activation analysis or inductively coupled plasma mass spectrometry [55, 56].

EMULSION DELIVERY OF VACCINE

Emulsions are heterogenous liquid systems may be water-in-oil emulsions, oil-in-water emulsions, or more complex systems such as water-in-oil-in-water multiple emulsions, micro-emulsions, or nano-emulsions. Antigens are dissolved in a water phase and emulsified by an appropriate emulsifier in the liquid. Factors such as viscosity of the oil phase, oil-to-water phase ratio and emulsion droplet size decide the controlled release characteristics of an emulsion. For example, high oil content can cause unnecessary irritation at the injection site, and too large a droplet size will result in a physically unstable product there by reducing its shelf life. Squalene O/W emulsion containing influenza vaccine was approved in Italy in 1997 and in several additional countries in 2000. [57]

Huang et al.,[58] developed a novel emulsion-type vaccine delivery systems of the amphiphilic bioresorbable polymer poly(ethylene glycol)-block-poly(lactide-co-epsilon caprolactone) (PEGb- PLACL) using ovalbumin as model antigen. Results from physicochemical characterization studies and in vitro release studies have shown that PEG-b-PLACL-emulsified formulations consist of homogeneous fine particles and are stable, reproducible and therefore advantageous over conventional adjuvant-prepared vaccines. In vivo studies in mice have shown that antigen-specific antibody titers and T-cell proliferative responses, as well as the secretion of IFN-gamma, were significantly enhanced for ovalbumin- PEG-b-PLACL based emulsions.

MUCOSAL DELIVERY OF VACCINES

Mucosal vaccination offers protection against microorganisms which gain access to body via mucosal membranes. Patient compliance, ease of administration, reduction in possibility of needle-borne injections, stimulation of both systemic and mucosal immunity are some of the advantages. Coadministration of antigens with adjuvants like aluminium hydroxide, complete Freunds adjuvant, incomplete freunds adjuvant, cholera toxin, heat labile enterotoxin of E. coli, etc., potentiated immune response of antigen. For example, Freunds adjuvant when administered subcutaneously to neonatal mice induced mixed T helper1 and 2 responses with interferon-y component against Helicobacter pylori infection. Delivery systems like PLG microspheres, PLGA microparticles carrying immunogenic agents etc are taken up by Peyers patches. Particles of <5 μm further move into lymph nodes and spleen stimulating specific IgG, IgM responses. Chitosan, a bioadhesive polysaccharide discussed earlier is suitable for mucosal vaccination due to its ability to open up tight junctions and promote paracellular transport of antigen across mucosa.

Nasal mucosa delivery

Since nasal mucosa is the first contact site for antigen inhalation, systemic and local immunity can be stimulated by activation of T-cells, B-cells and dendritic cells present in nasal-associated lymphoid tissue underneath nasal epithelium in the form of IgG and secretive IgA. Hence, nasal delivery of vaccines can be used to treat upper respiratory tract infections and also to produce systemic immunity [59]. Intranasal vaccines include influenza A and B viruses, proteosoma-influenza, adenovirus vectored influenza, group B meningococcal native, attenuated respiratory syncytial virus, and parainfluenza 3virus.

INTRADERMAL DELIVERY OF VACCINES

Intradermal route is widely used only for the administration of Bacille Calmette-Guérin and rabies vaccines. However, there is renewed interest in the delivery of intradermal vaccines, driven by the fact that human skin dermis and epidermis are rich in antigen-presenting cells, which suggests that the delivery of vaccines to these layers, rather than to muscle or subcutaneous tissue, should be more effective and induce protective immune responses with smaller amounts of vaccine antigen [60].

Intradermal delivery clinical trials and dose-saving potential have been conducted with several different vaccines, with variable results. These have been reviewed in a recent report from the Program for Appropriate Technology in Health (PATH) and the World Health Organization (WHO) [61]. For some vaccines, there has been a clear demonstration of dose-saving by intradermal delivery; however, there are several knowledge gaps as well as developmental and operational challenges to overcome if the benefits of intradermal delivery are to be fully realized.

New delivery devices

New devices for easier, more reliable intradermal delivery as alternatives to the currently used Mantoux technique are being developed [61, 62]. Some devices, such as disposable jet injectors, are needle-free and thus could reduce or eliminate needle stick injuries and the costs associated with their treatment worldwide, estimated at US$ 535 million per year [63]. Other intradermal delivery devices, such as microneedle patches, are likely to occupy less volume than vials or prefilled syringes, thus reducing the demand for cold chain capacity.

Regulatory issues

Intradermal delivery of fractional doses of an current formulation of the vaccine intended for subcutaneous / intramuscular injection on a "off-label" basis will be needed. To allow official "on-label" use of fractional doses, a licensing agreement between the national regulatory authority concerned and the vaccine manufacturer will be required [64]. Alternatively, it will require marketing approval for the new intradermal formulation and vaccine presentation.

For several existing vaccines, there may not be a powerful enough commercial motivation for manufacturers to undertake the costly reformulation processes, production of new presentations, and marketing implementation of autho Mrizations for a new distribution path. Consequently, novel vaccines may be more likely candidates for intradermal delivery. This route should be examined early in research and development in order to prevent a later stage of costly retesting and reconstruction. Unless there are clear reasons for using the intradermal route, it is likely that vaccine manufacturers will continue to use traditional presentations and distribution routes even for new vaccines, thus reducing the risk from production.

Device development

For this route to be widely used, novel devices will be required for easy, effective and reproducible intradermal delivery of vaccines. There are several different types of intradermal delivery devices in development [60-62]. However they can not be designed in isolation; device inventors need access to vaccines and co-operation with vaccine manufacturers to allow device / vaccine combinations to be produced, tested and accepted. In the short term, delivery technologies may include simple adapters compatible with existing needles and syringes for monitoring delivery depth and angle, syringe-mounted arrays of hollow microneedles and needle-free disposable jet injectors [61]. Each of these methods is consistent with current liquid and lyophilized formulations and presentations, and would therefore be easier than any other innovations to be implemented into immunization programmes. Later developmental instruments involve skin patches coated in microneedles filled with or consisting of vaccine. Encouraging preclinical data have been obtained with several formats of this type of device [65-67]. The challenges remain in creating solid formulations of various types of vaccine antigens, developing methods for coating the microneedles with appropriate antigen and monitoring the reproducibility of antigen delivery. In addition to dose-saving, if these can be resolved, microneedle patches may have other advantages, including small packed volumes and ease of use.

CONCLUSION

These days, vaccine drug delivery devices are growing in popularity because of the advantages they offer. Vaccine drug delivery devices have also known to be patient safe as they eliminate the need to prescribe booster doses and

offer minimal doses of long-term therapy with the usage of liposomal, microspherical and nanoparticulaed carrier delivery system.

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REVIEW ARTICLE

Corticosteroids in Dental Impaction - A Literature Review

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ABSTRACT

The classic signs of inflammation, which includes edema, erythema, pain and loss of function, commonly occurs after a routine or difficult third molar surgical procedure. The inflammatory process is necessary for the healing to occur, but often excessive inflammation which causes the patient unnecessary pain, trismus and edema. Corticosteroids reduce inflammation via the inhibition of phospholipase A2, which is the first enzyme involved in the conversion of phospholipids into arachidonic acid, therefore blocking the synthesis of other products such as prostaglandins, leukotrienes and substances related to thromboxane A2. In essence, corticosteroids prevents the formation of these end products which is a broth of potent inflammatory mediators causing the signs and symptoms. This articles has focused to review on submucosal administration of corticosteroids in third molar surgery.

Keywords : corticosteroid, submucosal,pain,swelling,trismus.

INTRODUCTION

Corticosteroids (CS) are an important class of naturally occurring and synthetic steroid hormones that affect virtually every aspect of human physiology. The most important glucocorticoid derived from the adrenal gland is

cortisol (sometimes called hydroxycortisone). The adrenal cortex consists of three zones. The zona glomerulosa, located immediately beneath the capsule, synthesizes aldosterone, the most potent mineralocorticoid (MC) in humans. The zona fasciculata (middle zone) produces cortisol (hydrocortisone), the principle circulating glucocorticoid (GC). Adrenal-androgens are secreted by both zona fasciculata and zona reticularis (innermost zone). GC secretion is regulated by adreno corticotropic hormone (ACTH), produced in the anterior pituitary and released in secretory bursts throughout the day and night. ACTH production is in turn driven by corticotrophin releasing hormone (CRH) from the hypothalamus. Pulses of ACTH occur every 30-120 minutes. Varying amplitude of ACTH pulses leads to the normal diurnal rhythm of cortisol production.[1–3**]**

The surgical extraction of impacted mandibular third molars is one of the most commonly performed procedures in oral surgery. Patients experience a range of uncomfortable signs and symptoms after extraction including pain, trismus, facial oedema, and functional discomfort of the oral cavity, because of muscular oedema and spasm.[2,4] Corticosteroids exert an important anti-inflammatory action, reducing liquid transudation and oedema formation, decreasing cell exudates, inhibiting vascular dilatation and reducing fibrin deposit around the inflamed area. The mechanisms responsible for these effects include inhibiting the leukocyte chemotaxis to the inflammatory focus, inhibition of fibroblast function and endothelial cells, and suppression of the production of numerous chemical inflammation mediators.[5,6] Although corticosteroids are most effective during the first 24 hours post-surgery, their effect can also be noticed for 3 days. This articles has focused to review on submucosal administration of corticosteroids in third molar surgery.

REVIEW OF LITERATURE

The most researched outcome on the use of corticosteroids in oral surgery revolves around their effect in reducing post-operative pain, swelling and trismus in third molar surgery, orthognathic surgery and mandibular fracture. Over the last six decades, the use of corticosteroids for third molar surgery had been studied extensively in different formulations, dosings, routes and sites of administration (7)(8)(9). These corticosteroids include dexamethasone (per-oral/p.o.), dexamethasone acetate (intramuscular), dexamethasone sodium phosphate (intravenous and intramuscular), methylprednisolone , methylprednisolone acetate and methylprednisolone sodium succinate (both intravenous and intramuscular)[10-13]. Graziani et al in 2008, studied the effect of endo-alveolar and sub-mucosal administration of dexamethasone sodium phosphate to prevent inflammatory sequelae after surgical removal of lower third molars was studied. He included forty-three patients who underwent bilateral extractions of lower third molars and were randomly assigned to receive either dexamethasone 4 mg (group A) or 10 mg (group B) as endo-alveolar powder or 10 mg as sub-mucosal injection (group C) unilaterally. The controlateral site served as control and did not receive any steroid administration. Facial edema, trismus and pain perception were evaluated at the 2nd and 7th postoperative day. A multivariate analysis revealed that treatment and ostectomy time were both significantly positively associated with the degree of postoperative trismus and edema and found that both sub-mucosal and endo-alveolar administration of dexamethasone are effective in reducing postoperative sequelae of surgical removal of lower wisdom teeth.[14]

In a study by Majid et al in 2011, included thirty patients, each of whom required removal of a single impacted mandibular third molar under local anaesthesia, were randomly allocated to one of 3 groups of 10 each. The 2 experimental groups were given dexamethasone 4 mg submucosally or intramuscularly, and the control group had no steroid. Both dexamethasone groups showed significant reduction in swelling and pain compared with the control group at all intervals. Submucosal dexamethasone resulted in significantly less trismus than controls on day 1 postoperatively, but there were no significant differences among the groups at the other times [10]. Majid et al in 2011 evaluated the effect of a submucosal 4-mg dexamethasone injection on postoperative sequelae and QOL was measured after third molar surgery compared with an intramuscular 4-mg dexamethasone injection and a control group and stated that submucosal injection of dexamethasone 4 mg is an effective therapeutic strategy for improving

the quality of life after surgical removal of impacted lower third molars with a comparable effect on postoperative sequelae to intramuscular injection [15]. Antunes et al in 2011 conducted a prospective, controlled, randomized trial involving 60 lower third molar surgeries in 67 patients. The sample was randomly divided into three groups: group A (local injection), group B (tablets), and group C (control). Both the oral administration and local injection of dexamethasone proved effective in reducing pain, edema, and trismus compared to control group following lower third molar surgeries, achieving similar results [11]. Nair et al in 2013, included a total of 100 patients requiring surgical removal of a single mandibular third molar .The experimental group (50) received dexamethasone 4 mg as submucosal injection and control group (50) received no drugs. None of the patients developed wound infection or any serious postoperative complications. Postoperative edema tended to be less severe on the second postoperative day in the experimental group and the result was statistically significant. There were no significant differences in the reduction of pain and trismus between the two groups studied [11].

In 2013, Warraich et al, included 100 patients in their study, requiring surgical removal of third molar under local anesthesia, were randomly divided into 2 groups, group I receiving 4mg dexamethasone as submucosal injection and the control group II received no steroid administration. Facial swelling was quantified by anatomical facial landmarks. Furthermore, pain and patient satisfaction, as well as neurological score and the degree of mouth opening were observed from each patient and found that patients receiving dexamethasone showed significant reduction in pain, swelling, trismus, a tendency to less neurological complaints and improved quality of life compared with the control group [16].

Majid & Mahmood in 2013, in their study he included a total of 72 patients (32 males and 40 females) were included in the study and were randomly divided into six equal study groups: five treatment groups received dexamethasone 4 mg as intramuscular injection, intravenous injection, oral tablets, submucosal injection and endoalveolar powder; and control group which received no dexamethasone. Swelling, trismus and pain were evaluated at the first, third and seventh day post-operatively. A modified questionnaire was used to measure different aspects of QOL. All dexamethasone groups showed statistically significant improvement in swelling and pain at all intervals and in trismus at day 1 and day 3 intervals as compared to control. QOL measures also showed significant improvement [17].

A randomised double-blind clinical trial was conducted by Bhargava et al in 2014, on 60 patients with class II position B impaction of mandibular third molars. Sixty transalveolar extractions were performed prospectively with ten patients randomly allocated to each of the six study groups (group T: intra-space injection of Twin mix; group S: submucosal dexamethasone; group M: intramuscular dexamethasone; group V: intravenous dexamethasone; group O: per-oral dexamethasone; group C: control group, no dexamethasone). Mean operative visual analogue scale scores did not show statistical variation, and post-operative visual scores indicated better patient comfort in the steroid groups with statistically significant difference between group T and the control group on the first, third and the seventh post-operative day. Mean increase in distances between tragus and soft tissue menton to assess facial swelling showed strong statistically significant difference between the first and the third post-operative day between the control group and group T (p value <0.0001). Association of trismus was found less with the steroid treatment groups when compared to the control group.[18]

Zerener et al in 2015 in their study, included a total of 78 patients (aged 18 to 35) were divided into three groups randomly (control, dexamethasone, and triamcinolone acetonide). In the experimental groups, dexamethasone and triamcinolone acetonide were injected into submucosa at about 1 cm above the surgical area submucosally. The control group of patients did not take any drug submucosally but the same surgical procedure was applied. There were statistically significant differences between the control and experimental groups on the different days of the postoperative period. The effect of triamcinolone acetonide on pain started on the first day postoperatively and the effect of triamcinolone acetonide on trismus and pain was better than other groups at the third and seventh days. However, there was no statistically significant difference between the effects of dexamethasone and triamcinolone

acetonide regarding postoperative complications [16]. Ibikunle AA et al in 2016, conducted a randomised controlled trial in which subjects were randomly distributed into three groups of 62 subjects each, Group A consisted of subjects who received 40 mg oral prednisolone; Group B consisted of subjects who received 40 mg submucosal injection of prednisolone while Group C consisted of subjects who did not receive prednisolone. Measurements for facial width/facial swelling, pain, and mouth opening were recorded preoperatively and postoperatively. The postoperative evaluation points were postoperative days 1, 3, and 7. These measurements were compared with the preoperative values both within and among the groups. A considerable increase in the mean postoperative values for pain, facial width and trismus was observed. Notably, subjects who did not receive prednisolone showed comparatively higher values for the measured parameters throughout the postoperative evaluation period. Subjects who received submucosal injection of prednisolone showed overall lower values compared to those who received oral prednisolone [19].

Deo et al in 2016, in their study, included forty healthy adult subjects of either gender, underwent surgical removal of the lower impacted third molar under local anaesthesia and after being randomly assigned to receive either 8 mg dexamethasone submucosal injection or normal saline injection in proximity to surgical site. Facial swelling, trismus showed significant reduction immediate postoperative day in dexamethasone groups. Patient perception postoperative pain on VAS score was not significant but overall improvement in QOL was observed [8]. Saravanan et al in 2016, in their study, included 2 groups in which the group 2 (20 patients) is the study group in which all the patients had single dose of pre-operative sub mucosal dexamethasone of 4 mg/2 ml. The group 1 patients (20 patients) received single dose of pre-operative intra muscular dexamethasone of 4 mg/2 ml. The control group (20 patients) did not receive steroid in any form. The post-operative pain, swelling and trismus were assessed for all the groups. The submucosal dexamethasone group showed marked improvement in the mouth opening in the follow ups than the intra muscular dexamethasone group. In those five cases of bilateral impaction, in study groups 1 and 2, the mouth opening was very much significant when sub mucosal dexamethasone was given [12]. Navneet Singh et al in 2017, in their prospective study he included a total of 44 patients undergoing third molar surgery, who were divided in two groups – Group A who received 4mg of submucosal dexamethasone and Group B who received 4 mg of intramuscular dexamethasone during the extraction of third molars. Swelling, trismus and VAS scores were measured in both the groups on 3rd and 7th postoperative days and found that there was no significant difference in swelling, pain and trismus index between both the groups [20].

Daniel lim et al in 2017, in their prospective, randomized, double-blind study, included 65 patients who required surgical removal of impacted mandibular third molars with Class II or position B impaction (Pell and Gregory classification). Patients were randomly assigned to 1 of 3 groups: dexamethasone, methylprednisolone, or placebo (control) and found that both methylprednisolone and dexamethasone significantly reduced swelling and trismus whereas the methylprednisolone group had significantly less pain and consumed a lower amount of analgesics during the early postoperative days [21].

Mojsa et al in 2017, in their study, ninety patients were included and split randomly into three equal study groups (30 patients in each): the ‘before’ group received dexamethasone 15 min before surgery and placebo 15 min after surgery; the ‘after’ group received placebo 15 min before surgery and dexamethasone 15 min after surgery; the ‘placebo’ group received placebo 15 min before surgery and placebo 15 min after surgery. Postoperative pain was recorded by the patients using a visual analogue scale, numerical rating scale, and the McGill Pain Questionnaire at 1, 2, 4, 6, 8, 12, and 24 h after surgery. The patients also recorded the total number of analgesic doses consumed during the 24 h after the procedure. Swelling (determined using linear measurements of the face) and trismus (determined through measurement of maximum mouth opening) were assessed at 48 h, 72 h, and 7 days following surgery. Better control of pain, swelling, and trismus was demonstrated for dexamethasone in comparison to placebo. Postoperative dexamethasone provided better pain control than preoperative dexamethasone. There was no difference in total rescue analgesic intake between the preoperative and postoperative dexamethasone groups [22]

Khalida et al in 2017, in their randomised control study, included 50 patients requiring surgical removal of an impacted third molar and divided in to two group I patients received one regimen single dose of 4 mg dexamethasone sub mucosally, group II received no drug. The postoperative sequelae were assessed and statistically significant reduction in pain and swelling was noted in dexamethasone group [23]. Chug et al in 2018, in their study, allocated the participants randomly to three groups: the placebo group received normal saline injection (control), while the 8 mg dexamethasone group and 40 mg methylprednisolone group received submucosal injections of these steroids preoperatively. Each participant was assessed for postoperative pain, swelling, and trismus, along with a subjective assessment of QOL through a structured questionnaire. The participants administered dexamethasone showed significant reductions in pain and trismus compared to the control group ($P < 0.05$). Submucosal injection of dexamethasone was found to be superior to methylprednisolone only in terms of the reduction in swelling. QOL was minimally affected in patients administered dexamethasone as compared to methylprednisolone and control subjects [24].

Daniel lim et al in 2017, in their study, included 60 patients and were randomly assigned to three different groups, namely the saline control group, the (4 mg) dexamethasone group and the (40 mg) methylprednisolone group where the agents were administered as a preemptive submucosal injection. Postoperatively, patients were prescribed with standard analgesic and antibiotic. Pain was assessed on postoperative day one, two, five and seven based on visual analogue scale and the amount of analgesic consumed. The methylprednisolone group experienced significantly less pain and consumed less analgesic on postoperative day one and two when compared to control group [25]. Arora et al in 2018, in their prospective randomized study, included 45 patients requiring surgical removal of an impacted third molar. Selected patients were divided randomly into three groups of 15 patients each: group I patients received one regimen single dose of 4 mg dexamethasone sub mucosally, group II received one regimen single dose of 8 mg dexamethasone sub mucosally, and group III (control group), no dexamethasone was given but only received injection of normal saline sub mucosally after establishing local anaesthesia. The postoperative sequelae were assessed on the second and seventh postoperative day. As compared to group III, groups I and II showed statistically significant reduction in pain and swelling whereas no statistically significant difference was found between the test groups [9].

CONCLUSION

The submucosal route of corticosteroid administration is a viable alternative to the other routes. Indeed, it exhibited significant comparative advantages over other route of administration. In addition, it offers a safe, simple, cost-effective method, which produces a high concentration of the drug at the operative site, thereby lessening the systemic effects.

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RESEARCH ARTICLE

The Impact of Phorate on the Protein Levels of the Fresh Water Fish, *Catla catla*

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ABSTRACT

The extensively used organophosphate pesticide, phorate is a potential toxicant, polluting the aquatic system is experimented at its sublethal concentration. The selected animal for the current study was *Catla catla*. The 96 hrs LC_{50} was derived as 0.306 mg/l using the log-probit graphical analysis method. From this value, the sub acute concentrations (0.076 and 0.038 mg/l) were selected for the experimentation where there was a dose dependent decrease in the protein levels in the muscle, liver and kidney form the samples of the commercially important fish, *Catla catla*

Keywords : *Catla catla,* phorate, Protein.

INTRODUCTION

One of the main problems we face at present is 'Water pollution'. The addition of pollutants changes the nature of water (Voltz *et al;* 2005). These uncontrollable release of pollutants from industrial and agricultural operations reach out to the open areas like ponds and rivers and contaminate the water and correspondingly affects the aquatic organisms (Kamble and Muley, 2000, Bhachandra *et al.,* 2001; Madhab Prasad *et al.,* 2002 and Sindhe *et al.,* 2007). This contaminated water disturbs the ecological balance of the environment and a good quantity of aquatic organisms (Vosyliene and Jankaite, 2006). Arunachalam *et al.,* (1980) reported that the pesticides affect the growth and nutritional value of fish when their concentration in water exceeds the maximum limit. These pesticides affect the reproductive potential of the fish (Moore and Waring, 2001). Abhilash and Prakasam (2005) reported about the changes in the cellular morphology of pesticide exposed fish. The physiological functions of fish get changed upon the exposure to different pesticide concentrations (Gupta and Saxena, 2006). The fish serves as bio- indicator of water quality and the impact of pesticides can be clearly understood by analyzing the biochemical parameters of different tissues of it. Since the protein budget of a cell can be taken as an important diagnostic tool in the evaluation of its

physiological state, the present work has been done to assess the alterations of protein content in the various tissues of the Phorate treated fresh water fish, *Catla catla.*

MATERIALS AND METHODS

Live *Catla catla* were procured from hatcheries. Healthy fishes were carefully packed in a medium sized polythene bag with sufficient oxygen which would help them to carry on their normal processes of metabolic activities during their period of transportation. On arrival, the fishes were carefully transformed into large plastic tubs. They were left for acclimatization in the normal laboratory conditions for a period of ten days. From the stock, some well acclimatized *Catla catla*, approximately (10±2 g) were selected and exposed to various concentrations of phorate individually for the static bioassay test. Going by the recommendations of Florin-Muller (1970) for the maintenance of bioassay, the experiments were conducted in 10 litre tanks with 10 fishes each starved for 24 hours prior to the experiment. The medium of the experiment was renewed daily till the end of the experiment. The mortality of fishes at different concentrations was noted at LC_{50} values for 12, 48, 72 and 96 hours and the LC_{50} values of phorate were calculated using the software by converting mortalities (percentage values) into probit scale (Finney, 1971).

From the 96 hours LC_{50} values, two sub-lethal concentrations, viz 0.076($1/4^{th}$) mg/l and 0.038 mg/l ($1/8^{th}$) were chosen to expose the fish for protein estimation. The fishes were kept at each concentration for the period of 30 days. After 10, 20, and 30 days of exposure, the fishes were taken out and the following tissues, viz liver, muscle and kidney were dissected under aseptic condition. Using the wet sample, total protein was estimated as per the methods of Lowry *et al.,* (1951). The Bovine serum albumen was used as a standard. Four replicates were sustained for each set of experiments. Two-way analysis of variance (ANOVA) was applied to know the significance of variation caused by concentration of the pesticide and exposure

RESULTS AND DISCUSSION

Mortality response and relationship of selected fish to various concentrations of pesticides. An augmentation in the number of mortalities was observed as the concentration of insecticide. In control, there was no mortality found. The computed LC_{50} values for 12, 48, 72 and 96 hours were found to be 0.812, 0.527, 0.363 and 0.306 mg/l respectively. In the case of phorate, an increase in dose dependent and a decrease in time dependent were observed in the mortality rate with the increase in the exposure time from 24 to 96 h, i.e. LC_{50} was reduced. In the present probe, a relationship between the length of exposure period and the concentration of pesticide was shown by the acute toxicity test. As the exposure period went on increasing, the LC_{50} values of the fish gradually decreased. Acute toxicity served as the fastest acting mechanism in causing the damage to the organism. Vasait and Patil (2005) estimated the LC_{50} values of organophosphate pesticide to *N. botia* The result showed the decrease in LC_{50} values with the increase in the concentration and the duration of exposure.

In the current study, the LC_{50} doses for different exposures periods viz: 12, 48, 72 and 96 hours were found to be 0.812, 0.527, 0.363 and 0.306 mg/l respectively. The mortality rates were observed to be augmenting with the increase in exposure duration and with a decrease of LC_{50} value. Similar findings had been made for various pesticides by many authors (Das and Mukherjee, 2000). Acute toxicity studies are usually made to compare the sensitivities of different species to different potencies of the chemicals. Toxicity data for a variety of pesticides such as organophospate, organochlorine, carbamide and pyrethroid pesticides have been reported for number of fish species by various authors (Gurusamy and Ramdoss, 2000; Nishar Shailkh and Yeragi, 2004 and Visvanthan *et al.,* 2009). The calculated values for total proteins and percent changes over control along with standard deviation are graphically represented in Fig 1, 2 and 3. In the control fish, *Catla catla*, the total protein content is in the order of Muscle > Liver > Kidney. The total protein in the fish treated with Phorate decreased noticeably with increasing concentrations and exposure durations. The total protein level of the muscle tissue in the fish exposed to phorate was found to have

decreased to 7.84,6.28 and 5.15 mg/100mg wet tissue after the 10,20 and 30 days of exposure in 0.038 mg/l respectively whereas, in 0.076 mg/l, the amount of carbohydrate present was 6.24,5.18 and 4.20 mg/100mg wet tissue in the same exposure periods. Unlike the other tissues, the decrease in the total protein level in the liver was much higher during the prolongation of exposure period. The maximum decrease was 4.13 mg/100mg wet tissue in the 0.076 mg/l after the 30 days of exposure. The same trend was observed in Kidney too. To find out the effect of pesticide concentrations and the duration of exposure times on the protein changes in the tissues of *Catla catla.,* two - way analysis of variance (ANOVA) was carried out. The result showed In all the cases, pesticide concentrations have more influence than the duration of exposure.

Tripathi and Priyanka Verma, (2004a) noted the decrease in protein content in the tissues of brain, liver and muscle of fish *C. batrachus* in sublethal concentration of endosulfan and fenvalerate. Chezhian *et al*., (2010) observed that the decreased trend of protein content in various tissues of the fresh water fish, *L. rohita* may be due to the metabolic utilization of keto acids in the synthesis of glucose or for the osmatic and ionic regulation. Proteins are the important biomolecules involved in a wide spectrum of cellular functions (Prasanth, 2006). Rathakrishnan (2002) stated that the decrease in protein content in the fresh water fish, *Mystus vittatus* exposed to pesticide could be due to the excretion of proteins by kidney or kidney failure or impaired protein synthesis as a result of liver disorders.

The declined trend in the protein levels of tissues might be due to the metabolic utilization of ketoacids to gluconeogenesis pathway for the synthesis of glucose or due to the necessary proteins or for the maintenance of osmotic and ionic regulation which indicated the physiological adaptability of the fish to the pesticide stress (Venktramana *et al.,* 2006). Remia *et al.*,(2008) and Sathick *et al*.,(2019) reported that the reduction in the protein might be due to the proteolysis and increased metabolism under the toxic stress. In the present study, the pesticide, phorate induced changes in the level of protein in the different tissues of freshwater fish, *Catla catla* and affected the nutritive value of the fish.

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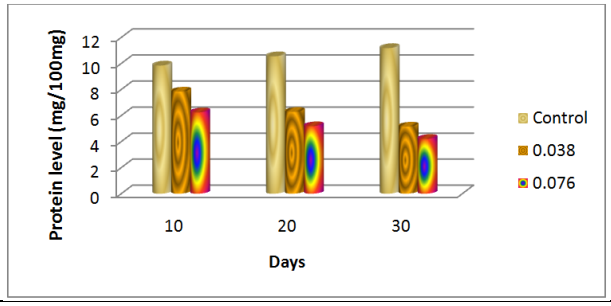

Fig. 1. Changes in the level of Protein content in the muscle tissue of *Catla catla* exposed to phorate

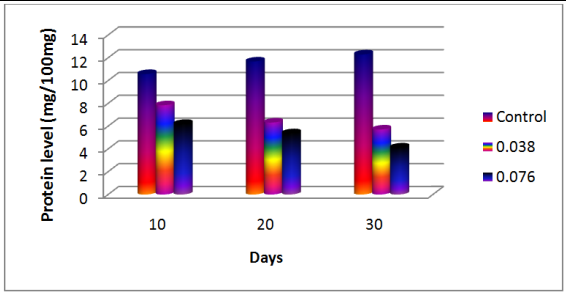

Fig. 2. Changes in the level of Protein content in the liver tissue of *Catla catla* exposed to phorate

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REVIEW ARTICLE

A Review on Nanoparticles and Its Properties

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ABSTRACT

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. Here, we review various aspects of nanoparticle formulation, characterization, effect of their characteristics and their applications in delivery of drug molecules and therapeutic genes.

Keywords: nanoparticles, drug delivery, Matrix, drug, therapeutic

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins,

peptides and genes [1-4]. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties [5, 6]. The advantages of using nanoparticles as a drug delivery system include the following: 1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration

They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects [3]. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity [4]. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance [5]. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc. In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particleparticle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. The present review details the latest development of nanoparticulate drug delivery systems, surface modification issues, drug loading strategies, release control and potential applications of nanoparticles. Preparation of Nanoparticles Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including [7]. (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) Drug release profile desired; and (f) Antigenicity of the final product. Nanoparticles have been prepared most frequency by three methods: (1) dispersion of preformed polymers; (2) polymerization of monomers; and (3) ionic gelation or coacervation of hydrophilic polymers. However, other methods such as supercritical fluid technology 8 and particle replication in non-wetting templates (PRINT) [9] have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in industry. Dispersion of preformed polymers: Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D, L-glycolide), PLG; poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA), [10-12]. This technique can be used in various ways as described below. Solvent evaporation method: In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration [13]. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed [14]. Spontaneous emulsification or solvent diffusion method: This is a modified version of solvent evaporation method [15]. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of

water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase. Polymerization method In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles [16;17]. Nanocapsule formation and their particle size depend on the concentration of the surfactants and stabilizers used [18]. Coacervation or ionic gelation method much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation [19, 20]. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.

Production of nanoparticles using supercritical fluid technology Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe [21]. A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure 21. Supercritical CO2 (SC CO2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1 °C, Pc = 73.8 bars), nontoxicity, non-flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles 8. Thote and Gupta (2005) reported the use of a modified SAS method for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose [22].

RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure [21] , Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles [23]. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive. Effect of Characteristics of Nanoparticles on Drug Delivery Particle size Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system [24]. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. Desai et al found that 100 nm nanoparticles had a 2.5 fold greater uptake than 1 µm microparticles and 6 fold greater uptakes than 10 µm microparticles in a Caco-2 cell line [25]. In a subsequent study

[26], the nanoparticles penetrated throughout the sub mucosal layers in a rat in situ intestinal loop model, while microparticles were predominantly localized in the epithelial lining. It was also reported that nanoparticles can across the blood-brain barrier following the opening of tight junctions by hyper osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors [27]. Tween 80 coated nanoparticles have been shown to cross the blood-brain barrier [28]. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles [29]. Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out [30]. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability. Polymer degradation can also be affected by the particle size. For instance, the rate of PLGA polymer degradation was found to increase with increasing particle size in vitro [31]. It was thought that in smaller particles, degradation products of PLGA formed can diffuse out of the particles easily while in large particles, degradation products are more likely remained within the polymer matrix for a longer period to cause autocatalytic degradation of the polymer material. Therefore, it was hypothesized that larger particles will contribute to faster polymer degradation as well as the drug release.

However, Panyam et al prepared PLGA particles with different size ranges and found that the polymer degradation rates in vitro were not substantially different for different size particles [32]. Currently, the fastest and most routine method of determining particle size is by photon-correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties [33]. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM). Surface properties of nanoparticles When nanoparticles are administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation [34]. Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This in turn influences the in vivo fate of nanoparticles [34, 35]. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system (MPS) such as liver, spleen, lungs and bone marrow. Indeed, once in the blood stream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the macrophages of MPS rich organs [36].

Generally, it is IgG, compliment C3 components that are used for recognition of foreign substances, especially foreign macromolecules. Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles in vivo. This can be achieved by (a) surface coating of nanoparticles with hydrophilic polymers/surfactants; (b) formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80). Studies show that PEG conformation at the nanoparticle surface is of utmost importance for the opsonin repelling function of the PEG layer. PEG surfaces in brush-like and intermediate configurations reduced phagocytosis and complement activation whereas PEG surfaces in mushroom-like configuration were potent complement activators and favoured phagocytosis [2, 37]. The zeta potential of a nanoparticle is commonly used to characterise the surface charge property of nanoparticles 38. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the nanocapsule or adsorbed onto the surface.

CONCLUSION

The foregoing show that nanoparticulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs. The core of this system can enclose a variety of drugs, enzymes, genes and is characterized by a long circulation time due to the hydrophilic shell which prevents recognition by the reticular-endothelial system. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering, is still required. Further advances are needed in order to turn the concept of nanoparticle technology into a realistic practical application as the next generation of drug delivery system.

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RESEARCH ARTICLE

Antibacterial Efficacy of Silver Nanoparticles against *Staphylococcus aureus*

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ABSTRACT

Aim: To assess the antibacterial efficacy of silver nano-particles against *Staphylococcus aureus*. Methodology: The antimicrobial efficacy of the 0.05%, 0.1%, 0.5% concentration of silver nano-particles was determined by the standard methods of Clinical and Laboratory Standards Institute (CLSI). The agar diffusion test (ADT) was used. The data was analyzed by ANOVA and Tukey's post hoc test using SPSS software. Results: 0.05%, 0.1%, 0.5% concentration of silver nano-particles has antibacterial effect against *Staphylococcus aureus*. Conclusion: Antibacterial efficacy against *S. aureus* increases as the concentration of silver nano-particles increases.

Keywords: *Staphylococcus aureus*; Endodontic infections; Silver nano-particles.

INTRODUCTION

Staphylococcus aureus is responsible for a variety of human infections including superficial lesions in the skin, localized abscesses, central nervous system infections, osteomyelitis, invasive endocarditis, septic arthritis, septicemia, pneumonia, and urinary tract infections [1]. *S. aureus* can infect the leaves and roots of *A. thaliana* , ultimately killing the plant. The pathogenicity of *S. aureus* shows characteristics typical of a plant pathogenic bacteria, including entry through stomatal openings, initial colonization of the substomatal lumen, multiplication, production of water-soaked lesions, and, ultimately, the rotting of infected plants [2]. *S. aureus* is one of the important resistant micro-organism frequently isolated from recurrent root canal treatments [3]. It plays a key role in causing primary and secondary root canal infections [4]. *S. aureus* is a facultative anaerobic, immobile, non-sporulated, gram-positive coccus which is source of periodontal, periapical, and endodontic infections[5,6]. Silver ions or salts are known to have a wide antimicrobial effect and they have been used for years, in different fields in

medicine, including wound dressings, catheters, and prostheses [7]. Silver ions are potent antimicrobial [8]. Silver has many advantages, such as long-term antibacterial activity due to sustained ion release, and low bacterial resistance, low toxicity and good biocompatibility with human cells. With the advent of nanotechnology, silver nano-particles have been synthesized, and they have shown potent antimicrobial properties. The aim of the present study was to assess the antibacterial efficacy of silver nano-particles against *S. aureus*.

MATERIALS AND METHODS

Ampoule of *S. aureus* (ATCC:25923) was revived by adding 0.3 to 0.4 ml of the brain heart infusion broth under sterile condition. The organisms were prepared by culturing on nutrient agar overnight. Following incubation, a standard inoculum of each bacterial strain was prepared in sterile water to a concentration of 1.5 x 10^8 CFU/ml (0.5 McFarland Standard). A sterile swab was dipped into the suspension and then streaked onto a pre prepared nutrient agar plate in order to provide a uniform coverage of bacteria on the surface of the plate. Silver nanoparticles used in the present study were obtained from nano Composix San Diego, USA. Particle size of these nanoparticles was 5nm. 0.05%, 0.1%, 0.5% concentration of silver nano-particles were evaluated. (Figure-1). Sterile paper disks with a diameter of 6 mm and a thickness of 1.5 mm were coated with silver nano-particles. The disks were then placed on top of the surface of the plate. All disks and plates were labeled and incubated for 24 hours at 37^0 C. The diameter of the halo formed was measured in millimeters. The mean of each sample was calculated and data was statistically analyzed.

RESULTS

After 24 hours of incubation under aerobic condition at 37ºC, 0.5% silver nanoparticles showed larger zone of inhibition followed by 0.1% silver nano-particles and least by 0.05% silver nano-particles.

DISCUSSION

Staphylococcus aureus is found in cases of septicemia, infective endocarditis, pneumonia, ocular infections, and central nervous system infections. *S. aureus* can be found, causing oral pathologies such as angular cheilitis, mumps and staphylococcal mucositis. [9,10]. Under favorable conditions the potentially lethal animal and human pathogen *S. aureus* could infect plants in nature [11]. *S. aureus* plays a major role in etiology of primary endodontic infection and persistent infection. It is one of the most resistant micro-organisms frequently isolated from recurrent root canal treatments, although less frequently than *Enterococcus faecalis* [12]. Cohen et alreported the presence of *S. aureus* in 67% cases of deciduous molars [13].*S. aureus* is a source of periodontal, periapical and endodontic infections [14].

In the present study, the agar diffusion test was used for testing all the samples which is one of the most frequently used in vitro methods to test the antimicrobial activity of dental materials. The advantages of this method is simplicity, low cost and the creation of direct comparisons of the silver nanoparticles against test microorganisms and the visual indication of which concentration has the potential to eliminate microorganisms in the local micro environment of the root canal system [15]. In earlier studies, it was found that nano-particles of less than 10 nm or lesser size are likely to have more antimicrobial effect[16]. Hence, 5nm silver nano-particles was used in this study. Bactericidal effect of silver nano-particles is in direct proportion with its concentration. 0.5% silver nanoparticles showed larger zone of inhibition followed by 0.1% silver nanoparticles and least by 0.05% silver nanoparticles. (Figure 2,3,4) Mean zone of inhibition for *S.aureus* differs significantly with different concentrations of silver nano-particles. (Table 1)

Bacteria cannot develop resistant to silver nano-particles easily [17]. Antimicrobial nano-particles such as silver nano-particles, chitosan exhibit significant antimicrobial ability in disinfection of root canal [18].The microorganisms are not likely to develop resistance against silver as compared to antibiotics. The silver nano-particles interacts with multiple targets in the microbial cell, such as cell membranes, enzymes and plasmids, concurrently providing the bacterial least ability to gain resistance. The bactericidal mechanisms can be summarized as follows: (i) silver nano-particles increasing the membrane permeability; (ii) intracellular penetration of silver nano-particles; (iii) silver nano-particles induced cellular toxicity triggered by the generation of reactive oxygen species (ROS), damaging the intracellular micro-organelles (i.e., mitochondria, ribosomes, and vacuoles) and biomolecules including DNA, protein, and lipids; and (iv) modulation of intracellular signal transduction pathways towards apoptosis.

It is necessary to recognize the limitations of in vitro antimicrobial testing per se and the difficulty in correlating in vitro results with the in vivo activity. Further studies are recommended, to address the efficacy of silver nano-particles against endodontic pathogens, to elucidate the interactions of silver nano-particles in the dentinal structures, its cytotoxicity, mutagenicity and other potential long-term effects of silver nano-particles.

SUMMARY

Strengths and limitations of this study:

- 0.05%, 0.1%, 0.5% concentration of silver nano-particles has antibacterial effect against *S. aureus* .
- Antibacterial efficacy of silver nano-particles increases as the concentration increases against *S. aureus* .
- Incorporation of silver nano-particles into the intra-canal medicaments, root canal sealers and also in irrigating solution can eradicate *S. aureus*; needs further evaluation.
- Further studies are recommended for cytotoxicity, mutagenicity and other potential long-term effects of silver nano-particles.

CONCLUSION

The results obtained from this study suggest that 0.05%, 0.1%, 0.5% concentration of silver nano-particles is effectively bactericidal against S. aureus. The use of silver nano-particles as an antimicrobial agent against *S. aureus* is possible. Incorporation of silver nano-particles into the intra canal medicaments, root canal sealers and also in irrigating solution can eradicate *S. aureus* from the root canal and thereby success rate of root canal treatments can be increased. Furthermore, in-vivo studies are needed to imply this effectively.

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Table 1: Mean zone of inhibition for S.*aureus* differs significantly with different concentrations of silver nano particles

Silver Nanoparticles	Mean zone of inhibition(in mm)	S.D.	ANOVA	Tukey's post hoc test
0.05%	12.5	1	150.5 p-value >0.05	3 > 2 > 1
0.1%	15.75	0.5		
0.5%	25.25	1.5		

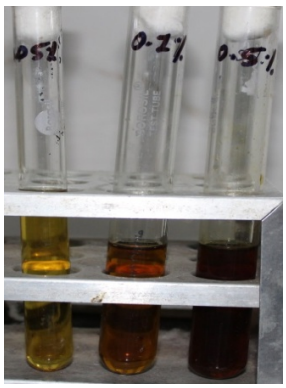

Figure 1: Test tubes containing 0.05%, 0.1%, 0.5% concentration of silver nanoparticles particles

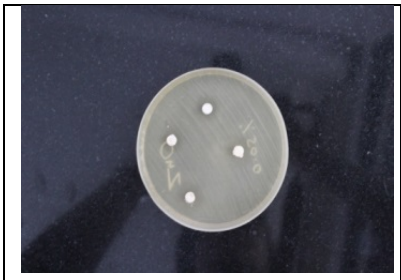

Fig.1. 0.05% Silver Nanoparticles

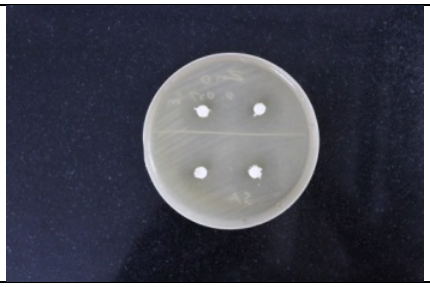

Fig.2. 0.1% Silver Nanoparticles

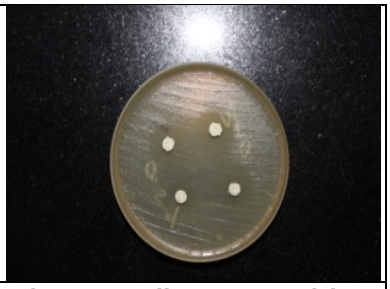

Fig.3. 0.5% Silver Nanoparticles

Figure 1,2,3: Zone of inhibition for 0.05%, 0.1%, 0.5% concentration of silver nanoparticles particles against *S. aureus*