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RESEARCH ARTICLE

Effect of Bromochloromethane Supplementation and Dietary Energy Restriction on Rumen Fermentation Pattern and Blood Biochemical Profile in Muzaffarnagari Rams

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ABSTRACT

The present study was conducted to assess the effect of bromochloromethane (BCM), a potent antimethanogenic compound, as feed additive and dietary energy restriction on rumen fermentation pattern and blood biochemical profile in rams fed on diet containing maintenance (NP_1) and sub-maintenance level of energy (NP_2) with adequate protein. Sixteen adult Muzaffarnagari rams of comparable bodyweight (40.25±0.65 kg) were divided into four equal groups. Both the diets were supplemented (BCM_1) with or without (BCM_0) BCM capsules, altogether 4 experimental diets (NP_1BCM_0, NP_1BCM_1, NP_2BCM_0 and NP_2BCM_1) were tested using 2 X 2 factorial design. BCM supplementation did not influence nitrogen fractions (Total N, TCA ppt N and ammonia N) and protozoal count but lowered ($P<0.01$) the pH of SRL. Ammonia N in rumen liquor was higher ($P<0.05$) in the rams fed on lower plane of energy. Plasma urea ($P< 0.05$), creatinine ($P< 0.01$) and triglycerides ($P< 0.05$) were lower in BCM_1 than BCM_0. Results of the present study revealed that BCM, a potent antimethanogenic compound may added in the diet of rams without affecting performance, however, its effect on long term feeding as an antimethanogenic feed additive needs to be tested with respect of its effect on vital organs.

Keywords: Bromochloromethane, energy, rumen fermentation, blood biochemical profile.

INTRODUCTION

Different chemicals are used as feed supplement or additive in animal nutrition research for optimizing the nutrient utilization in animals. Experimental trials to study the adverse effect, if any on long term feeding of such chemicals have been done extensively with some chemicals viz urea (Naik *et al.*,2004) as source of supplemental N and for chemical treatments of poor quality roughages to reduce the methane production in ruminants. Likewise many experimental trials have been conducted with Bromochloromethane (BCM) as potent antimethanogenic feed additive to reduce GE loss through combustible methane gas and to increase the efficiency of utilization of energy (Saravanan, 2000) maintenance status of animals and growth (McCrabb *et al.,* 1997). But most of the experiments have been conducted for short period. Beneficial result though have been reported, but its effect on blood parameters, rumen fermentation pattern and a rumen microbial population have been studied but limited researches (Vashistha, 2002).There may be some adverse effect of such chemicals as long term feeding to the animal. Keeping into account of its LD_{50} as 5000mg/kg (Deichman and Gerade, 1969) using this chemical in animals in very low doses (0.04mg/d) may be beneficial in reducing the environmental pollution out of methane production in one hand and no adverse health hazards in other hand due to saving of energy and metabolism within the body. But its effect of blood biochemical and rumen profile, whether adverse or beneficial have not been studied in rams fed on diets along with the BCM additive for large duration of feeding Moreover its effect may vary on different plane of nutrition. Therefore in the present experiment, an attempt has been made to study in detail the effect of feeding BCM capsule on rumen fermentation pattern and blood parameters in rams

MATERIALS AND METHODS

Selection of animals and experimental grouping

Sixteen healthy Muzaffarnagari rams of four years of age and of 40.25±0.65 kg body weights were selected and dewormed. They were fed on a balanced ration for about 15 days before beginning of the experimental feeding. Rams were randomly allocated on the basis of their live weight to four groups of four animals each following completely randomized design. The animals in groups 1 and 2 were fed on maintenance diets (NP1) as per NRC (1985) standard, whereas the animals in group 3 and 4 were fed on diets containing sub maintenance (NP_2) level of energy (21.3% lower than NRC) maintaining the CP level of the diets similar to in group 1 and 2 (Table 1). The animals in group 2 and 4 were fed daily one BCM (bromochloromethane) capsule (containing 0.04 ml BCM) whereas the animals in group 3 and 1 received no BCM capsule. Thus, four experimental diets (NP1BCM0, NP1BCM1 and NP2BCM0 NP2BCM1) were tested in a 2 X 2 factorial design.

Feeds and feeding

Jowar hay was fed as roughage and deoiled soybean meal along with crushed maize was used as concentrate supplement for feeding the rams. Adequate amounts of mineral mixture, salt and vitamins (vitablend $AB_2D_{3)}$ were supplemented along with the concentrates. BCM capsules were fed with daily quota of concentrate mixture of the animals in treatment 1 and 3

Sampling and analysis of rumen liquor

Rumen fluid samples (40 ml) were taken by a stomach tube at 0 and 4 hr post feeding for 3 consecutive days. Rumen fluid was filtered through four layers of cheese cloth and the pH was measured in the strained rumen liquor (SRL). These samples were acidified with 0.4ml of 10 N H_2SO_4 and stored at -20^{O}C for later analysis. SRL was analysed for Total N (AOAC, 1990), TCA precipatable N (AOAC, 1990), and ammonia N (Conway, 1957). For protozoa counting 1

ml of rumen liquor was pipetted with wide orifice pipette mixed with 1 ml of formalized physiological saline containing methyl green dye (10 ml formaldehyde, 0.85g sodium chloride, 0.06g methyl green and 90 ml distilled water) and allowed to stand overnight at room temperature, in air tight glass vials. If necessary further dilution was done in 30 microscopic fields in a heamocytometer counting chamber, under a magnification of 100X as described by Kamra *et al* (1991).

Blood Collection and analysis

The blood samples were collected from all the rams on third and fifth fortnight of feeding trial through jugular venipuncture into anticougulated tubes for separation of serum. The separated serum was stored at -20^{O}C till analysis for glucose (Hultman, 1959), urea (Rahmatullah and Boyde, 1980), Cholesterol (Wybenga *et al*., 1970) creatinine (Bones and Toussky 1945) and triglycerides (enzymatic method) adopting standard procedures.

Statistical Analysis

The data obtained during the study was analyzed as per standard methods (Snedecor and Cochran, 1989) following a 2X2 factorial design**.**

RESULTS AND DISCUSSION

Rumen fermentation pattern

Ammonia N in rumen liquor was higher ($P < 0.05$) in the rams fed on lower plane of energy (Table 1). After 4 hrs of feeding the ammonia N concentration was increased in rams on both the plane of nutrients; however the values differed nonsiginficantly.Ammonia N concentration depended on deficiency of energy for utilization NH_3 produced due to degradation of protein. Total-N and TCA precipitatable-N did not differ significantly between NP_1 and NP_2. Supplementation of BCM along with NP_1 or NP_2 could not make any significant difference of the N-fractions including the NH_3-N in rumen liquor. BCM supplementation might have drastically reduced the methane energy which in turn might have increased the availability of carbon skeleton (energy) for utilization of NH_3 in the rumen, as a result of which the difference in N fraction became non significant particularly after 4 hrs of feeding. Plane of energy could not affect other rumen parameters viz. protozoal count and pH in SRL also, even if there was supplementation of BCM. All these N-fractions and the protozoal population remained to be comparable between the rams in BCM supplemented and BCM non supplemented groups (BCM_0). Only the pH of SRL was found to be significantly ($P<0.01$) lower when BCM was supplemented with the diets. pH reduction in rumen fluid is one of the recent methods of practice to reduce methane production in rumen. Difference ($P <0.01$) in NH_3-N, protozoal count and pH of SRL between the 0 hr and 4 hr was obvious as the 0 hr collection was made after watering before feeding. Overall it could be inferred that no adverse effect was noticed on any of the rumen parameter on dietary supplementation of BCM capsules. Almost similar was the findings of Saravanan (2000) and Vashistha (2002). Moreover 21.3% difference in dietary energy concentration might have any adverse effect on rumen fermentation pattern.

Blood Biochemical Profile

On perusal of the data on blood biochemical profile (Table 3) it could be inferred that the rams fed on higher plane (NP_1) of nutrition was having higher ($P<0.01$) blood glucose level than that of rams fed on diets containing submaintanance level of energy (Table 3 and 4). However, the values did not differ significantly during 5th fortnight.

Supplementation of BCM along with the respective plane of nutrition, have an additive effect (P <0.05) on blood glucose level (Table 3) particularly in rams fed on lower plane of energy. However, the overall values and the values during 5th fortnight did not differ significantly when compared among NP_1 BCM_0, NP_1BCM_1 NP_2 BCM_0 and NP_2 BCM_1. The overall blood glucose level (mg/dl) was 28.11% higher in NP_2 BCM_1 than that of NP_2BCM_0 (Table 4). However, the values between NP_1BCM_0 and NP_1BCM_1 were almost comparable (Table 4). But it was 26.28% higher in NP_1BCM_1 than NP_1 BCM_0 during 5th fortnight; the values was also higher (P<0.05) in BCM_1 than that of BCM_0. However, the overall values and the values during 3rd fortnight between BCM_0 and BCM_1 did not differ significantly. The higher blood glucose level in higher plane of nutrition is obviously due to higher intake of energy which intern might have produce higher quantity of glucogenic fatty acid (propionic) in the rumen. Additive effect of BCM may be due to diversion of H from methanogenesis towards the production of propionic acid as an alternative H sink. Higher proportion of propionic acid in rams fed on BCM supplemented diet was reported in previous studies (Sarvanan, 2000; Vashistha, 2002).

Energy deficiency has been reflected well in the animals in lower plane of nutrition (NP_2) as the plasma glucose significantly (P<0.01) lower in NP_2 along with the significantly higher plasma urea (P<0.05), creatinine and triglyceride values and cholesterol being numerically higher. Higher triglyceride values indicate the higher rate of mobilization of depot fat.Supplementation of BCM has reduced the overall plasma urea (P<0.05) creatinine (P<0.01) and triglyceride (P<0.05) values in rams, which indicate the supplementary additional source of energy out of significant reduction of methane in group BCM_1, than that of BCM_0. As in the case blood glucose the effect of BCM supplementation has also been reflected in the plasma urea, creatinine and triglyceride values in NP_1 BCM_1 and NP_2 BCM_1. The values were numerically lowest in NP_1 BCM_1 followed by NP_1 BCM_0 (control) and NP_2BCM_1 and highest in rams fed on non-BCM supplemented diets containing lower plane of nutrition (NP_2 BCM_0). However, all the blood parameters were found to be varied within the normal range without any adverse effect due to supplementation of BCM.

CONCLUSION

Results of the present study revealed that bromochloromethane, a potent antimethanogenic compound may be added in the diet of rams at very low dose (0.04ml) without adversely affecting rumen ecosystem and blood biochemical profile however, its effect on long term feeding as an antimethanogenic feed additive needs to be tested with respect of its effect on vital organs.

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RESEARCH ARTICLE

Semisynthesis of New Antibacterial Triterpenoids Derivatives from the Natural α-Euphorbol

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ABSTRACT

The chemical hemisynthesis of new triterpene derivatives through chemical modifications of one of the major components of *Euphorbia resinifera* (α-euphorbol) and the *in vitro* antibacterial activity has been reported. The structures of the compounds were elucidated on the basis of 1D and 2D NMR experiments, as well as mass spectrometric data. Their antibacterial activity was tested against four gram negative: *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae* and four gram positive*: Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes* using micro dilution method.Results showed that all compounds had moderate (64 μg/ml) to strong (6.4 μg/ml) antibacterial activity (MIC values) against both gram-positive and gram-negative bacteria. The most active compounds were 3 and 5 for all the gram positive stains. The compounds 3 and 6 had also demonstrated interesting activities against *Pseudomonas aeruginosa,* when compound 4 was efficient against *Escherichia coli.* The isoxazolin derivative 7present moreselectivity for inhibiting the growth of gram negative bacteria.

Keywords: *Euphorbia resinifera* Berg., α-Euphorbol, epoxide, cleavage, antibacterial activity, isoxazolin

INTRODUCTION

Bacteria and fungi continue to develop new strategiesin order to adapt and survive the lethal and/or biostatic effects of antimicrobials through different mechanisms. Despite the great efforts from the world health organization and pharmaceutical industry to deal with this problem.The resistance toward available antimicrobial drug have increased, inducinga major world wide problem and threatening the global health safety [1].The discovery and development of new compounds to dead with resistance problems has become one of the most important aspect of antibacterial and antifungal research fields. So the discovery of novel and potent antibacterial as well as antifungal agents from natural products has gained a great interest. In the past three decades only two antibiotics; linezolid and daptomycin have been discovered, demonstrating the difficulty of this task [2].

Euphorbia is the largest genus of the family *Euphorbiaceae* and includes approximately 1600 species. *Euphorbia Resinifera* Berg. is a native Moroccan succulent shrub with latex that is locally used as a traditional medicine in north Africa to treat numerous diseases such as hypertension, antipyretic and anti-inflammatory activities [3]. The major constituents of the latex are the α-euphol and α-euphorbol described by their anti-inflammatory [3], antitumor [4], antileishmanial [5] and moderate with no selective antimicrobial activity against human pathogens gram positive or negative [6].

Euphorbia resinifera has been a subject of intense phytochemical examination and a source of many isolated compounds, including pentacyclic and tetracyclic triterpenic compounds such as α-euphol and α-euphorbol [7].The tetracyclic triterpenes have attracted the attention of several researchers by chemical studies of extraction, separation, structural elucidation and their chemical reactivity [1-8]. Natural triterpenoids have become a key source of new drugs in the last years [9-10] and several studies reported that hemisynthesis of new natural products is takes advantage of the high number of active compounds we can obtain, howeverit has been shown that modification of triterpenes through enzymatic or chemical reactions often resulted in enhanced biological activities [8]. In connection with our study on drug discovery, we report herein the semisynthesis of new active compounds by the cleavage of the epoxide in the side chain of α-euphorbol natural product obtained from *Euphorbia resinifera* latex.

MATERIALS AND METHODS

Plant material

Latex from *Euphorbia resinifera* Berg., was collected in the south area of Morocco, and identified by Dr. F. Msanda (Facutly of sciences, University Ibn Zohr, Morocco). Latex was obtained as described [12].

Extraction and purification

The latex of *Euphorbia resinifera* Berg., is *strongly* irritant to skin andmucous membranes. Handling of these substances should be carried out wearing latex gloves and face protection, and avoiding contact with the skin. The natural triterpene α-Euphorbol, one of the major constituent of *Euphorbia resinifera* Berg. latex,was extracted and isolated as described in the literature [12-13].

Antibacterial activities

The new triterpenic compounds 1–7, were tested for their antimicrobial activities. Those compounds were evaluated against four gram-negative: *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia*, and four gram positive bacterias*: Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis and Listeria monocytogenes* using micro-dilution method. Briefly, the microorganisms were incubated in 96 well plates with a liquid medium and

extracts for 18–30 h at 37°C. Sample 1:10 or 2:25 dilutions were used depending on the target strains. Those activities were measured by monitoring the absorbance differences at 600 nm between the initial and the final incubation systems [14].

RESULTS AND DISCUSSION

Extraction and Purification

The natural triterpene α-Euphorbolhas been obtained as described in the literature [12]. The structure of this compound has been confirmed by the comparison of his ^{1}H and ^{13}C NMR spectrum with the spectral data reported in the literature [14]. The ^{13}C NMR spectra showed 30 carbon signals suggesting that the compound could possess a triterpenoid skeleton. The ^{1}H NMR spectra exhibited signals mostly concentrated in the high field region, which are typically triterpene signals. The more peculiar signals in the proton NMR included two olefinic protons (δH 4.66 and 4.72, 2H, 2d) and an axial proton on an oxygen-bearing carbon (δH 3.22, dd, J=11.4, 4.2 HZ). It further exhibited seven singlets characteristic of tertiary methyl groups (δH 1.66, 1.58, 0.98, 0.93, 0.85, 0.78, 0.73) and a secondary methyl group (δH 0.84, d, J= 6.0), which forms the eighth methyl groups in the skeleton. The ^{13}C-NMR spectra exhibited four signals (δc 134.27, 133.78, 105.89 and 156.90) characteristic of olefinic carbons indicating that the compound is unsaturated and contains two double bonds. The carbon signal at 79.29 ppm was characteristic of carbon bearing a hydroxyl group.

Synthesis

Epoxides owe their importance to their high reactivity, which is due to the ease of opening of the highly strained three-membered ring. They are a useful building blocks for an increasing variety of chemical products because a large number of compounds can be obtained from them by simple chemistry [15]. Epoxides can be prepared by a classic reaction widely used in organic synthesis that is the reaction of peracids on olefins. Usually, it leads to a mixture of stereoisomeric epoxides when the unsaturated system is asymmetrically substituted. The most commonly used peracids are meta-chloroperbenzoic acid (m-CPBA), peracetic acid, perbenzoic acid, trifluoroperacetic acid and para-nitroperbenzoic acid [16-17].

In this type of reaction, organic peracids act as electrophilic reagents. Therefore, they react faster with alkenes bearing electron donor groups on the double bond thus making the electron density on the double bond larger. In the presence of hydrochloric acid, the opening of epoxides in ethylenic alcohols is a frequently observed reaction in the chemistry of natural substances (ref), whereas the treatment of epoxides catalysed by trifluoride diethyl etherate (BF_3-Et_2O) may also provide hydrocarbons and tertiary alcohols, aldehydes or ketones depending on the substitution of the starting epoxide. Obtaining these products is mainly due to rearrangements that accompany this kind of reactions [17].

The synthesis of triterpenoids 2-7 has been accomplished as outlined in Figure 1. We have prepared five compounds with triterpene as a principal squeleton in accordance with the starting material α-euphorbol. The structure of the triterpene α -Euphorbol contains a tetrasubstitutedin tracyclic double bond and another disubstituted exocyclic more reactive.The first step of our synthesis consisted in preparing the epoxide 2 from the natural α-Euphorbol1 using meta chloroperbenzoic acid (*m*cpba) in methylene chloride at room temperature. The epoxide 2 obtained corresponds to the attack of the exocyclic double bond in position 24 with the peracid. The intracyclic double bonds in position 8 remain intact since it is protected by the methyl groups 18, 19 and 30. The structure of compound 2 was obtained by analysis of their NMR spectral data and mass spectrometry. The Mass spectrum (EI +) reveals a peak at m / z = 456 corresponding to the molecular peak M^{+}. ^{1}H NMR spectra showed a multiplied at δ 2.55 ppm assigned to the oxiranic protons (H31) and a doublet of doublet at δ 3.21 (J1=11.5 Hz, J2=4.5 Hz) due to the resonance of H-3. The ^{13}C NMR

spectra confirmed the proposed structures from the disappearance of the signals corresponding to the exocyclic double bounds and the appearance of signals at δ 50.96 and 62.75 ppm assigned respectively to the oxiranic carbon C24 and C31.

Therefore, we synthesized the following compounds: 3-7, however, the epoxide 2 was smoothly converted to the corresponding allylic alcohols 3 and 4 after purification through silica gel column with hexane-ethyl acetate 9:1 as eluent.The structures of the newly prepared products 3and 4were fully characterized from their spectroscopic data (NMR) and mass spectrometry.The ^{1}H NMR compound 3spectrum exhibited specially two signals with high chemical shifts values; the first one resonated in the olefinic region and the other one was observed a little up field region. The signal at δ 5.33ppm appeared to be characteristic of olefin, and it was assigned to the proton in position 23 as doublet doublet. The second signal observed at 4.24 ppm has been assigned to protons at position 31. The ^{13}C NMR spectra revealed the presence of a signal at 59.63 ppm attributed to carbon at position 31 characteristic of a primary alcohol. Whereas the new signals at 126.10 and 145.13 ppm corresponding to a double bond were assigned respectively to C-23 and C24. The structure was confirmed using mass spectral data (*m/z*): 439 [M-OH].

Compound 4 was identified using ^{1}H and ^{13}C spectra and confirmed using mass spectral data with a strong signal at (*m/z*): 439 corresponding to the [M-OH]-. ^{1}H NMR spectra of compound 4 showed at 4.13 ppm assigned to the two protons in position 31. The ^{13}C NMR spectra showed a signal at 62.79 ppm attributed to carbon at position 3 with two signals at 142.1 and 131.6 ppm corresponding to C-24 and C25. The chemistry of boron trifluoride diethyl etherate (BF_3-Et_2O) is defined by its exceptional Lewis acidity, which is generated by the intrinsic electron deficiency of the boron atom, a reason for which (BF_3-Et_2O) is used extensively as a catalyst in organic chemistry [18]. Lewis acid-catalyzed rearrangement of epoxides to carbonyl compounds is a well-known reaction in organic chemistry, and has been studied extensively in the synthetic laboratory as well as computationally [19].

During our experiments on the epoxide ring-opening of epoxy-α-euphorbol, the reaction of epoxide 2 with boron trifluoride diethyl etherate (BF_3-Et_2O) was carried out in different solvents, when Compound 2 was treated with hydroborate ether (BF_3-Et_2O) in Toluene as an inert solvent we obtain the corresponding aldehydes 5 and 6. ^{1}H NMR spectra showed a singlet at δ 9.51 ppm for compound 5and at 9.43 ppm for compound 6 corresponding to the aldehyde proton. The formation of compound 6 can be explained by the methyl migration as described in Figure 2. When the reaction was performed in Acetonitrile at -10 ºC conditions, we found the surprising result of the heterocyclic compound dihydroisoxazoline 7 formation and no trace of the expected carbonyl product has been detected (Figure 3). There is only limited information on the role of the boron trifluoride diethyl etherate (BF_3-OEt_2) complex in the ring-opening of epoxides [20-21] to afford heterocyclic compounds due to the participation of solvent in the synthesis as described in Figure 3.

The structure of this compounds was identified using ^{1}H and ^{13}C spectra and confirmed using mass spectral data (*m/z*): 498 $[M]^{+}$.Signals in the ^{1}H NMR spectrum were observed mainly in the up field region. The spectra exhibited specially two new signals, the first one resonated in the blended methyl field 1.9 ppm corresponding to the CH_3 on the isoxazolinring and another one at a high chemical shifts values 3.73 to 3.94 ppm as four doublets appeared to be characteristic of the CH_2 heterocyclic proton at position 31.The ^{13}C NMR spectra exhibited 32 carbon signals confirming the proposed structure. The spectra exhibited specially three new signals, one at 163.15 ppm corresponding to the resonance of the carbon C=N, another signal at 93.51 ppm assigned to CH_2 at position 31 and a signal at 66.68 ppm corresponding to Carbon at position 24.

Biological Activities

Our results showed that several of the tested compounds had moderate to good antibacterial activities, in choice tests when tested at an initial dose of 64µg/ml (table 1 and 2). For the Gram negative stains. The compound 7 was the most efficient with IC50 values between 6.4 and 8.0µg/ml. The compounds 3, 4, 5 and 6 had moderate activities againt

Proteus mirabilis with values between 26 and 34µg/ml. The compounds 3 and 6 had also demonstrated interesting activities against *Pseudomonas aeruginosa* with 36 and 28µg/ml respectively, when compound 4 was efficient against *Escherichia coli* with an IC50 values of 34µg/ml. For the gram positive stains. The most active compounds were 3 and 5 with IC50 values less than 19 µg/ml for all the gram positive stains. The compounds 2 and 4 had also demonstrated very interesting IC50 against *Enterococcus faecalis* with 12 and 16µg/ml respectively, and 11.8µg/ml from compound 4 only against *Listeria monocytogenes* (Figures 4, 5)

The natural α-euphorbol show no activity on gram positive and gram negative bacteria at the initial concentration of 64 µg/ml. Overall, among the tetracyclic triterpenes, the most active compounds were highly oxygenated with ketone/OH substituents at C-24 and C-31.Compound 7 with a heterocycle at the lateral chain showed to be selective for gram negative bacteria. The compounds 3 and 5 had shown the same efficiency pattern against the gram positive bacteria, but more investigations had to be done to establish those homologies. The semisynthesis consists on the chemical modification of an initial compound by operating modifications on the molecular weight or bychanging the molecular structure to form new compounds with a more interesting bioactivity, this approach had been used for natural products and proved its efficiency [22-24].

One of the applications of this technique for the development of antimicrobial drugs is the semisynthesis of tetracycline, doxycycline, and tigecyclinefromthe naturally isolated antibiotic chlortetracycline, this drug have shown interesting antibacterial activities against *Staphylococcus aureus*, *Acinetobacter baumannii,* and *Escherichia coli* [23].

CONCLUSION

Among the semi-synthesis of the six new triterpenes, one of them with a heterocyclic on the lateral chain. The resulting isoxazolin compound was obtained by the participation of the solvent in the opening epoxide reaction by trifluoride diethyl etherate (BF_3-Et_2O). All these newly prepared triterpenic compounds were fully characterized using spectroscopic data. Furthermore, their antibacterial activities were determined, the introduction of an isoxazolin heterocycle increase the antibacterial activity and the selectivity againt gram negative strains.

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Table 1. IC50 OF DIFFERENT COMPOUNDS AGAINST GRAM NEGATIVE STAINS

	Pseudomonas aeruginosa	***Escherichia coli***	***Klebsiella pneumoniae***	***Proteus mirabilis***
Compounds	**IC50 µg/mL**	**IC50 µg/mL**	**IC50 µg/mL**	**IC50 µg/mL**
1	>64	>64	>64	>64
2	58.0	52	48	48
3	36.0	48.0	50.0	28
4	44.0	34.0	54.0	32.0
5	54.0	50.0	46.0	34.0
6	28.0	38.0	44.0	26.0
7	8.0	6.4	6.8	7.2

Table 2. IC50 OF DIFFERENT COMPOUNDS AGAINST GRAM POSITIVE STAINS

	Enterococcus faecalis	*Staphylococcus aureus*	*Bacillus subtilis*	*Listeria monocytogenes*
Compounds	**IC50 µg/mL**	**IC50 µg/mL**	**IC50 µg/mL**	**IC50 µg/mL**
1	>64	>64	>64	32.0
2	12.0	35.0	44.0	48.0
3	12.0	15.6	17.6	12.4
4	16.0	28.0	33.0	11.8
5	8.0	16.0	9.2	18.4
6	34.0	42.0	20.6	38.0
7	>64	>64	>64	>64

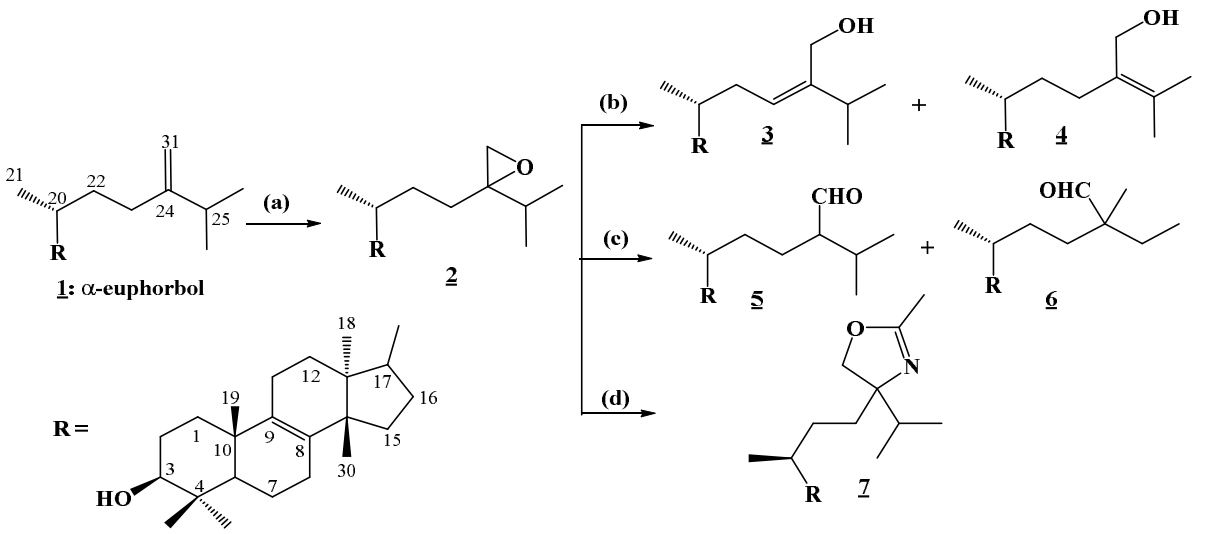

Figure 1. Hemisynthesis of triterpenes derivatives

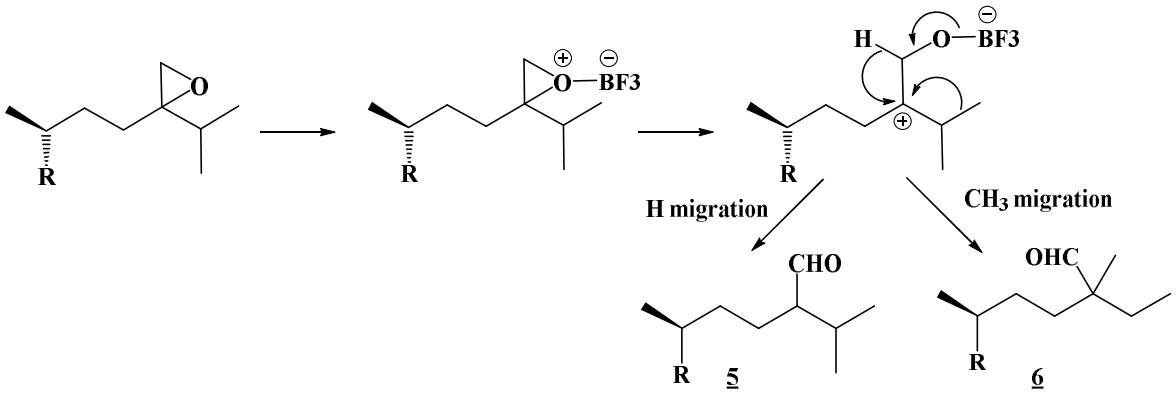

Figure 2. Mechanism of the epoxydecleaveage by BF_3-Et_2O in Toluene

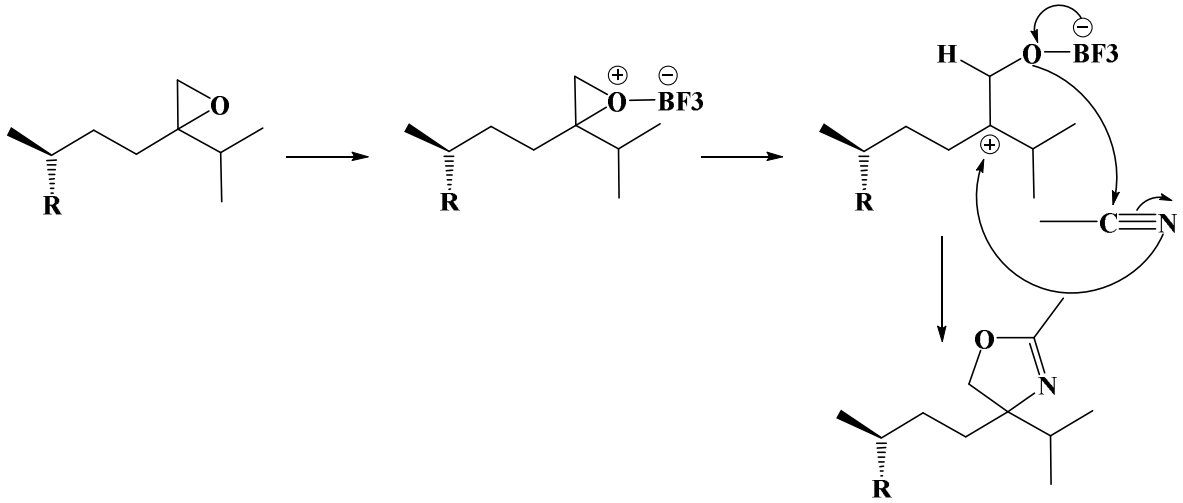

Figure 3. Synthesis mechanism of compound 7

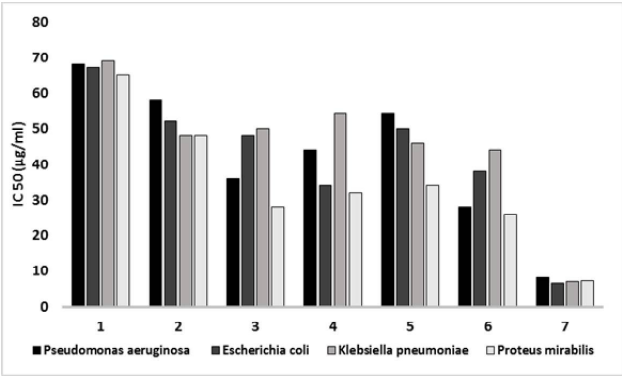

Figure 4. IC50 of different compounds against Gram negative stains

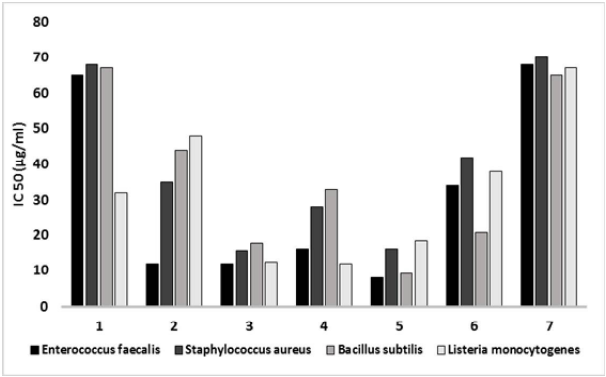

Figure 5. IC50 of different compounds against Gram positive stains

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RESEARCH ARTICLE

Investigation of the Characteristics of Hydrogenated Amorphous Silicon for Application in IR Image Sensors

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ABSTRACT

Hydrogenated amorphous silicon (a-Si:H) is a promising active element for the application of sensing membrane in image sensors used for Infrared imaging. Due to the specific electromagnetic spectrum in which the IR image sensor operates, the sensor membrane requires high optical absorption coefficient, high TCR (temperature coefficient of resistance) and specific sensor resistance. In this paper, these crucial properties of the image sensor is investigated for the inherent a-Si:H deposited by PECVD (Plasma Enhanced Chemical Vapor Deposition). The experimental study shows a satisfactory choice of a-Si:H as a sensing membrane in IR image sensors.

Key Words: Hydrogenated amorphous silicon, Image sensor, residual stress and TCR.

INTRODUCTION

Hydrogenated amorphous silicon (a-Si:H) is one of the promising active elements in many electronic devices, including, solar cell, active matrix displays, thin film transistors and image sensors. The demand for low cost infrared (IR) image sensors has increased enormously in the recent years, giving thrust to search for new and efficient materials. The incident radiation absorbed in an image sensor is converted to heat and the consequent temperature change is measured. For high sensitivity, the sensing membrane should have high absorbing efficiency combined with a small thermal mass (Laamanen et al.2010). In the mid 1990s, scientists started using a thin layer of amorphous silicon as sensing membrane in image sensors. The most significant properties of amorphous silicon is that it possess high optical absorption coefficient, high TCR at room temperature and that it can be prepared with a range of

resistivity to meet sensor resistance specification. Even though, amorphous silicon can be deposited by many methods like physical vapor deposition (PVD), chemical vapor deposition (CVD) and sputtering, deposition by PECVD has many advantageous features. It can be deposited at relatively low temperatures and the deposited film exhibits good adhesion, step coverage and is also of high electronic quality (Ong et al.,2006).

The thickness, optical and electrical properties of the deposited film, determines the operating efficiency of the electrical device. In PECVD, these properties can be easily controlled by the process parameters of deposition. Since the operation of image sensor in the IR region is restricted to 3-5 μm and 8-12 μm window of the electromagnetic spectrum (Almasri et al., 2006), a number of films were deposited and the result of the most optimized film was taken and analysed for its suitability in the application of image sensor.

METHODS AND IMPLEMENTATION

A number of undoped a-Si:H thin films (ATF) were organized by 13.56 MHz RF excited parallel plate plasma improved chemical vapour deposition system (*Plasmalab 100*, Oxford Instruments). The samples were deposited on glass and Silicon substrates and the thickness of the films were kept between 100 to 300 nm. For the optimized sample, the reactive gases employed were Silane (SiH_4) and Argon (Ar) at a steady stream of 25 and 475 sccm, respectively. The substrate was heated to 250°C and pressure in the reactive chamber was maintained at 1 Torr. A stylus profilometer measured the thickness and measured the deposition rate of the film was calculated. The deposition time was adjusted to get the optimized film thickness of 100 nm. The deposition of a-Si:H takes place by incorporation of SiH_n precussors into the deposited film by the dissociation of silane (SiH_4) as,

$$SiH_4 \Leftrightarrow SiH_2 + H_2$$
$$SiH_4 \Leftrightarrow SiH_3 + H$$
$$SiH_4 \Leftrightarrow Si + 2H_2$$

Operation

IR spectrum was obtained by an FTIR (*Perkin-Elmer*) spectrometer on oxide-coated silicon substrate. The deposited films are analysed optically using the transmittance spectrum of the ATF residued on pyrex glass using a UV-VIS-NIR spectrophotometer (*Jasco*). For the measurement of stress the ATF was deposited on a silicon substrate. The resistance of the ATF of thickness 110 nm, was too high to be measured by a four probe. So, two probe technique is employed to measure the resistance at different temperatures on a highly resistive glass substrate.

RESULTS AND DISCUSSION

The transitions between valence band and localized states in the forbidden gap is used to detect IR radiation. The FTIR spectra of hydrogenated a-Si (Figure. 1), clearly indicates that the IR absorbance is relatively high in the long wavelength infrared region (8 – 12 μm), corresponding to wave-numbers (1250 cm^{-1} to 833 cm^{-1}) and almost peaking beyond 1000 cm^{-1}. Thermal imagers, especially uncooled microbolometers are designed to be sensitive in this wavelength range. The four strong absorption peaks observed in the FTIR spectra are 461, 609, 814 and 1100. The peak at 461 cm^{-1} is due to vibrations of $Si-Si$, as referred from the work of E. Pascual et al. [4]. $Si-Si$ Stretching mode contributes to the peak at 609 cm^{-1} (Orduña-Diaz et al.,2010). The presence of $Si=H_2$ can be inferred from the absorption line at 814 cm^{-1} (Waman et al., 2011) where the range of wave numbers between 800 to 900 cm^{-1} is attributed to the presence of $Si=H_2$. According to (Vásquez et al., 2007) absorption peaks at 1100 cm^{-1} is due to $Si-O-Si$ asymmetric stretching mode and the intensity of the peak is high, because of the oxide coated substrate.

The minute broad shoulder between2000 to 2100 cm^{-1} is because of the presence of $Si \equiv H_3$ and the intensity difference between peaks at 814 and 2000 – 2100 cm^{-1} suggest abundance of $Si = H_2$ rather than $Si \equiv H_3$ in the thin film.

Hydrogenated amorphous silicon has higher optical absorption than crystalline silicon. The absorption coefficients are calculated from the UV transmittance spectra (Figure. 2). The absorption coefficient varies from 5.8 x 10^5 cm^{-1} at 400 nm, reaches a maximum of 6.9 x 10^5 cm^{-1} at 452 nm and reduces to 9.8 x 10^4 cm^{-1} at 600nm (Fig 3). These values meet the stringent requirements for device quality a-Si:H enshrined by (Poortmans et al.,2006). The absorption spectrum of hydrogenated amorphous silicon can effectively make use of the peak of the AM 1.5 solar spectrum. Sensors designed in the region of 400 to 600 nm have the best absorbance efficiency and usually, thin film solar cells use this region for maximum absorbance of the solar radiation. The Refractive index and thickness of a thin film can be calculated from the transmittance spectrum using the Swanepoel's method (Swanepoel et al.,1983). The refractive index (*n*) in the regions of medium and weak absorption can be estimated using the following equations [9],

$$n = [N_1 + (N_1^2 - s^2)^{1/2}]^{1/2} \quad (1)$$

and N_1 is given by

$$N_1 = 2s\frac{t_M - t_m}{t_M t_m} + \frac{s^2 + 1}{2} \quad (2)$$

where *s* is the index of refraction of the substrate , t_M is the maximum transmission and t_m is minimum transmission for a particular wavelength, obtained on the envelop enclosing the transmission spectrum using computational procedure.The deviation of index of refraction with wavelength is given in Figure. 4. It has a value of 3.7 at about 600 nm and decreases with increase in wavelength which is consistent with what is reported in literature. The thickness (Swanepoel et al., 1983) of the deposited film is computed from

$$t = \frac{\lambda_1 \lambda_2}{2(\lambda_1 n_1 - \lambda_2 n_2)} \quad (3)$$

Where *t* is the film thickness and λ_1 and λ_2 are the wavelengths corresponding to adjacent maxima and minima, respectively, and n_1 and n_2 are the refractive indexes at wavelengths λ_1 and λ_2, respectively. The thickness of the film calculated using the above equation is 109.6 nm which is consistent with the value of 110 nm measured using a profilometer. The study of optical absorption throws light on the microstructure, disorderliness and the optical quality of the films. The absorption coefficient α is computes from the energy of a photon and is expressed as,

$$\alpha\hbar\omega = A(\hbar\omega - E_g)^{\gamma} \quad (4)$$

Where *A* is a constant and E_g is the optical band space. The type of transition is determined by the values γ takes, *0.5* for direct allowed transition, 1.5 for forbidden direct transition 2 for allowed indirect transition and 3 for forbidden indirect transition. Using the absorption coefficients calculated from the transmittance spectra, a Tauc plot is generated. The optical band gap energy obtained is 1.71 eV. The Urbach energy E_U characterises the tailing of the band edges that have an exponential energy distribution (Müllerová et al., 2010).

$$\alpha(\hbar\omega) = \alpha_o \exp\left(\frac{\hbar\omega}{E_U}\right) \quad (5)$$

The Urbach energy is E_U and the constant is α_o. The tails states gives information about the temperature induced structural disorder and the defect density of hydrogenated amorphous silicon. The complementary of the slope in the undeviating region of the low-level light particle's energy plot between ln (α) and $\hbar\omega$ gives the Urbach energy. (Poortmans et al.,2006), has stated, for device quality a-Si:H the Urbach energy is around 50 meV. The Urbach energy for the film deposited for sensing membrane is 69 meV. The Figure. 5 plots the x-ray deflection pattern of ATF residued on glass substrate. It clearly exhibits the characteristic "hump" of amorphous silicon centered at $2\theta = 24^o$. The high background produced by the scattering of the x-rays confirms the amorphousness of the deposited film. Any imaging sensor would undergo different processes after the deposition of sensing membrane and it must be mechanically strong enough to withstand the strain from such processes. Hence, the residual stress of the sensing medium must have a low value. The deposition of thin films onto substrates introduces residual stress and causes the bending of substrates. This stress is of tensile nature and depends on the deposition parameters and should be optimized. The Stoney's formula (Cai et al., 2007), dealt in Eq. 6 gives the residual stress

$$\sigma = \frac{\mathrm{E}_s}{6(1-\mathrm{V}_s)} \frac{h_s^2}{h_f}\left(\frac{1}{R_2} - \frac{1}{R_1}\right) \quad (6)$$

where σ gives the stress and h_s is the thickness of the substrate and h_f is the film's thickness, E_s the Young's modulus and V_s the Poisson's ratio of the substrate, R_1 is substrate radii of curvature before accumulation and R_2 after accumulation on the thin film. The measured stress of the ATF is 49.36 MPa which is excellent and agrees with values obtained for the case of microbolometer .In case of amorphous silicon, an intrinsic semiconductor the property of conductiveness can be expressed as (Orduña-Diaz et al.,2010),

$$\sigma(T) = \sigma_o \exp(E_A / kT) \quad (7)$$

where E_A is the activation energy, σ_o the conductivity prefactor, k Boltzmann's invariant and T the substantiative temperature. In the Arrhenius plot's slope between ln σ versus 1/T (Figure.6), the activation energy is determined. The TCR $\alpha(T)$ is related to activation energy (Orduña-Diaz et al.,2010) as

$$\alpha(T) = -\frac{E_A}{kT^2} \quad (8)$$

The presence of impurities in the film can be measured not only by the activation energy but also the optical space can be included. In addition it gives a better estimation of the position of Fermi level. TCR and sheet resistance of the films depends to a large extent on the deposition temperature, pressure and gas flows (Syllaios et al., 2000).The conductivity is found to be 4.11 x 10^{-8} Ω-cm^{-1} at a temperature of 30 ^oC. The activation energy calculated from the Arrhenius plot is 1.1 eV and the corresponding TCR is -13 % /K^{-1} at 30 ^oC. The values are consistent with that mentioned in the references (Street et al., 1991) and (Pankove et al.,1984). Both the activation energy and TCR reduces for p- and n-doped hydrogenated amorphous silicon. The ability to get a broad range of TCR values by changing the process parameters and doping levels makes a-Si:H, a highly versatile material in the area of image sensors.

CONCLUSION

In this paper, intrinsic ATF deposited PECVD is analysed for their optical absorbance spectrum with regard to image sensors. The optical parameters and thickness are calculated from the transmittance spectrum and the estimated values of optical band gap, Urbach energy and refractive index are 1.7 eV, 65 meV and 3.7 at 600 nm, respectively. The residual stress obtained is 49.3 MPa. The operating wavelength range of an image sensor along with optical absorption coefficient is discussed. The values obtained for optical energy gap, Urbach energy and residual stress suggests that the quality of the film to be consistent with that required for an electronic device. TCR is calculated at room temperature from the resistivities measured at different temperatures. The study presented in this paper shows that intrinsic ATF deposited by PECVD forms a satisfactory choice as sensing membrane in IR image sensors.

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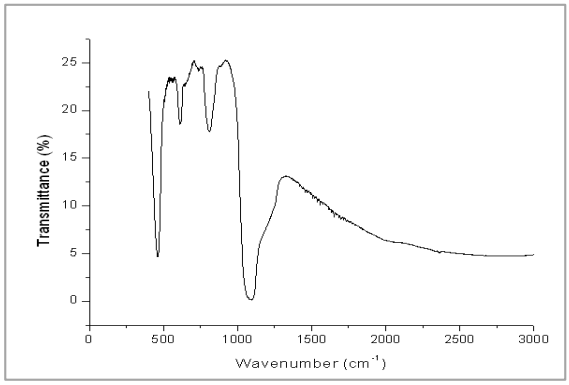

Figure 1. FTIR spectral transmittance of undoped ATF.

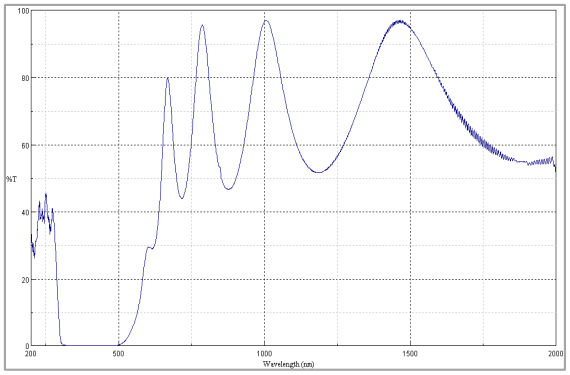

Figure 2. Transmittance spectrum of undoped ATF in the wavelength region 200 to 2000 nm.

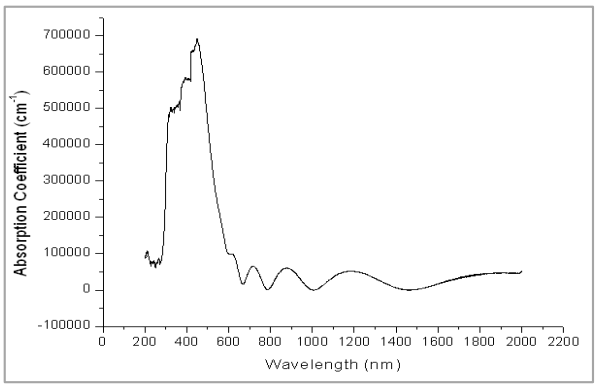

Figure 3. Optical coefficients of ATF versus wavelength.

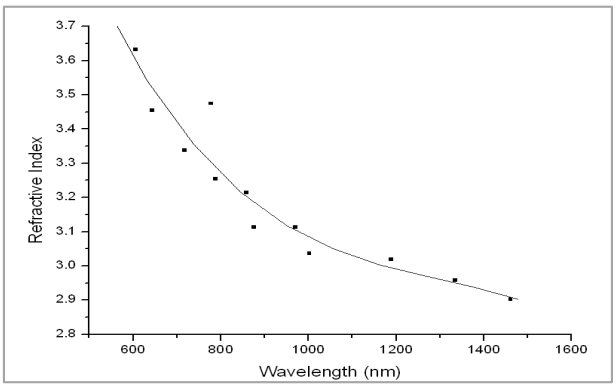

Figure 4. Spectral refractive index of hydrogenated amorphous silicon determined from transmittance spectra.

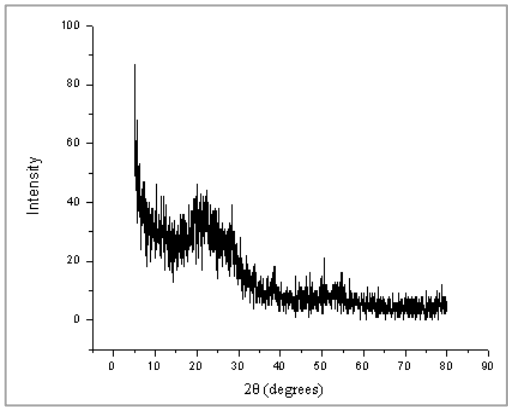

Figure 5. X-Ray diffraction scan of the ATF on glass substrate

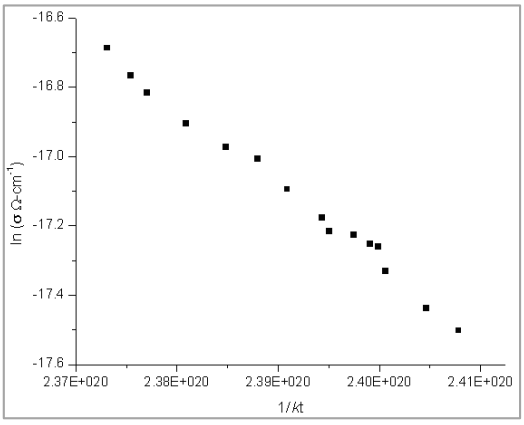

Figure 6. Conductivity versus inverse of temperature for undoped a-Si:H.

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RESEARCH ARTICLE

Nanostructure ZnO:CuO Papered by Pulse Laser Deposition For Gas Sensing

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ABSTRACT

Pulse laser deposition was used in this research by Nd:YAG laser with λ=1064 nm average frequency 6 Hz and pulls duration 10 nm) to deposit an ZnO:CuO with different composition ratio (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%) with thickness 100 nm to study the effect of nanostructure properties of ZnO:CuO thin films. X-ray diffraction pattern for Zinc oxide films with different composition ratio with CuO show these films have polycrystalline hexagonal structure. From atomic force microscope of prepared samples show a decrease in average diameter with increase etching time. The operation temperature of gas sensor was studied for different temperature and found the maximum sensitivity (49%) for 15 min porous silicon time.

Key Words: ZnO:CuO, laser, structure, ratio, microscope, polycrystalline hexagonal .

INTRODUCTION

Metal oxide semiconductors such as ZnO [1], In2O3 [2], WO3 [3], and TiO_2 [4]. Have attracted significant attention towards gas sensing due to their simple implementation, low cost, and good reliability for real-time control systems with respect to other gas sensors. The gas sensing properties of metal oxide semiconductors are influenced by many factors such as their operating temperatures, morphology and chemical composition of the films [5]. In such gas sensors the change in the electrical conductivity is due to the interaction of the targeted gas molecules (chemi or physisorption) with the surface of the metal oxide grains. Consequently, metal oxide sensors show changes in the resistance under exposure to oxidizing or reducing gases. [6] Since the majority of these sensitive layers are n-type, p-type semiconductors sensitive to gases are highly demanded for gas sensing applications such as sensor arrays for electronic nose. These p-type semiconductor gas sensors have much different sensing pattern from their n-type counterparts. In addition, it is also reported that p-type semiconductors are more appropriated for detecting oxidizing gases such as NO_2 [7]. At elevated temperatures, the presence of chemically adsorbed molecules such as

NO2 can cause electron depletion at the surface of the metal oxide grains; and consequently, the electrical resistivity of the thin films increases. The competition between chemisorptions of NO_2 and atmospheric oxygen O_2, at the same active surface sites of the metal oxide layer, plays an important role in determining the specific NO2/metal oxide interaction. The diffusion of Cu into ZnO can cause the formation of complex centres (CuZn, Cui). It is possible that Cu atoms can replace either substitutional or interstitial Zn atoms in the ZnO lattice creating structural deformations [8]. CuO significantly affects the electrical, chemical, structural and optical properties of ZnO, and the study of the electronic state of Cu in ZnO has been the subject of interest for a long time[9].

EXPERIMENTAL

Zinc Oxide powder with different Mixing concentrations for CuO at (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%) CuO pressing it under 5 Ton to formed a target with 2.5 cm diameter and 0.2 cm thickness. It should be as dense and homogenous as possible to ensure a good quality of the deposit with different mixing rate (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%) ZnO:CuO thin films were prepared by PLD technique. The pulsed laser deposition experiment is carried out inside a vacuum chamber generally at (10^{-3}Torr) vacuum conditions. The focused Nd:YAG Q-switching laser beam coming through a window is incident on the target surface making an angle of 45° with it. The substrate is placed in front of the target with its surface parallel to that of the target. Porous silicon prepared by electrochemical Etching wasthe silicon wafer serves as the anode. The cathode is made of platinum or any HF-resistant and conducting material. Si wafer p-type was used as a starting substrate in the photochemical etching. The samples were cut from the wafer and rinsed with acetone and methanol to remove dirt. In order to remove the native oxide layer on the samples, they were etched in diluted HF acid (1:1) with constant current 40 mA and different time (10and 50) min. In order to study the structural properties, the crystal structure is analyzed with a SHIMADZU 6000 X-ray diffractometer system which records the intensity as a function of Bragg's angle. The source of radiation is Cu (kj) with wavelength λ=1.5406Å. The morphological surface analysis is carried out employing an atomic force microscope (AA3000 Scanning Probe Microscope SPM, tip NSC35/AIBSfrom Angstrom Ad-Vance Inc. Modification of the deposition technique is done by many investigators from time to time with the aim of obtaining better quality films by this process. These include rotation of the target, positioning of the substrate with respect to targetas shown in figure (1).

Porous silicon prepared by electrochemical etching were The silicon wafer serves as the anode. The cathode is made of platinum or any HF-resistant and conducting material. Thecell body itself is, in general, made of highly acid-resistant polymer such as Teflon. Si waferp-type (111) was used as a starting substrate in the photochemical etching. The samples werecut from the wafer and rinsed with acetone and methanol to remove dirt. In order to removethe native oxide layer on the samples, they were etched in diluted HF acid (1:1) with constanttime (15)min and different current (15) mA as shown in figure (2).

RESULTS AND DISCUSSION

Figure (3) shows the X-ray diffraction patterns for the ZnO with different composite ratio of CuO as (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%) on a glass substrate, where , the thin films prepared by pulse laser deposition (PLD) technique.Can be noticed from X-ray pattern that the peaks at (31.9403◦, 34.5121◦, 36.3949◦, 47.6923◦and 57.0149◦) referred to (100), (002), (101), (012) and (110) direction, respectively. The x-ray diffraction data of thin films coincides with that of known hexagonal structure according to the International Centre for Diffraction (card No. 96-900-4182). The preferred peak for ZnO thin film appear at 2θ =36.3949 for (101) plane and appear peak of CuO located at 2 θ=38.4021 for hkl (111) at high ratio of CuO (70%-90%). according to (card No. 96-110-0029). These results coincide with M.Rahmani Table (1) show the experiment of standard peaks from International Centre for Diffraction (card No. 96-410-5683) as we can note that the grain size and d_{hkl} decrease with increasing CuO ratio and FWHM of

the (101) peak became wider and the orientation poorer. Increasing of FWHM with CuO mixing concentration suggests degradation in crystal quality [10].

Atomic Force Microscopy (AFM)

An atomic force microscopic (AFM) allowed to get the microscopic information of the surface structure and to plot topographies of the surface relief. This technique offered the digital images, which gave quantitative measurements of surface features, such as the average diameter and the average roughness. From Figures (4 to 8) shows the Atomic force microscopy (AFM)images for ZnO:CuO at different ratio (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%). which is deposited on glass substrate .AFM parameters contain average diameter, average roughness and peak –peak value for these samples have been shown in table (2) .This Table illustrates an increment in average diameter with mixing ratio.From tables (2) we observed disagreement in crystal size result calculated from X-ray diffraction and AFM. This result can be described according to the fact that, the broadening in X-ray peak is not only due to crystal refining, but also due to the strain formed in the crystal [11].

Figure (9) show the variation of sensitivity as a function operation temperature in the range (25-300^{o}C) of the ZnO:CuO thin films with different lasing deposition energy (600 mJ) which deposited on porous silicon.The sensing test was done using 3% NO_2: air mixed ratio and bias voltage (6V) were applied on the electrodes of all samples. The sensitivity of ZnO: CuO composite sensor was measured to 60 ppm of NO_2. The maximum sensitivity to NO_2 sensor is about 49% at around, were , the sensor displays a great enhancement of sensitivity to NO_2, especially at 200°C where the sensitivity to 60 ppm of NO_2 reaches as high as 49%. It should also be noted that the optimum sensing temperature required for the maximum sensitivity is at around 200°C for ZnO: CuO sensor. Furthermore, it is well known that the conductivity of a semiconductor gas sensor is mainly determined by the presence of singly-charged oxygen vacancies and the surface adsorbed oxygen species plays an important role during the sensing process. The higher sensitivity may be attributed to the optimum surface roughness, porosity, large surface area and large rate of oxidation.

Figure (9): The variation of sensitivity with the operating temperature for different ZnO: CuOgas sensor with NO_2 test gas. The response time and recovery time of the pure ZnO: CuOtowards 3% NO_2 :Air gas mixing ratios has been explored.The successive testswere performed at a bias voltage of 6V and a 25-300^{o}C operating temperature. The results are shown in figure (10). This figure indicate here both the response and recovery times of the ZnO: CuO gas sensor with (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%) concentration ratio. The response and recovery time decrease with increasing AgO concentration to minimum value at 50% ZnO:50% CuO concentration and increased after that. Figure (10): The response and recovery times of the ZnO: CuO gas sensor. Figure (11) show the variation of resistance with time of ZnO:CuO as exposed to 3 % (60l/h) NO_2 in the air ambient injected into testing chamber and bias voltage is keeping at (6V), at optimal operating temperature of each sample. The resistance is measured directly with time and the sensor resistance initially reaches the steady state before gas opening, in this time we open the gas to allow mixing with air inside the chamber. The resistance increases abruptly to reach a steady state then we switch the gas off. Then current returned to initial case. The ability of a sensor to sense the presence of gas depends on the nature of the interaction between the gas molecules and the surface atoms of the sensing film. The reactivity of the surface is critically dependent on its mixing and the defect structure. This figure shows a rapid decrease of the resistance with time for doped ZnO:CuO to reach a saturation state when the gas on, this may be due to a saturation of adsorption of NO_2 gas on the surface. The resistance returns to the initial state when the gas off.

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Table 1. Illustrates the Structural parameters: 2θ, dhkl, (hkl), FWHM and G.S of deposited ZnO:CuO different ratio (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%).

ZnO:CuO	2θ (Deg.)	FWHM (Deg.)	d_{hkl} Exp.(Å)	G.S (nm)	d_{hkl} Std.(Å)	Phase	hkl	card No.
	31.7100	0.4000	2.8195	20.6	2.8174	Hex.ZnO	(100)	96-900-4182
	34.4220	0.3400	2.6033	24.5	2.6037	Hex.ZnO	(002)	96-900-4182
90:10	36.2140	0.4700	2.4785	17.8	2.4780	Hex.ZnO	(101)	96-900-4182
	47.5120	0.4000	1.9122	21.7	1.9122	Hex.ZnO	(102)	96-900-4182
	56.5200	0.4000	1.6269	22.5	1.6266	Hex.ZnO	(110)	96-900-4182
	31.7121	0.4080	2.8193	20.2	2.8174	Hex.ZnO	(100)	96-900-4182
	34.4241	0.3468	2.6032	24.0	2.6037	Hex.ZnO	(002)	96-900-4182
70:30	36.2161	0.4794	2.4784	17.4	2.4780	Hex.ZnO	(101)	96-900-4182
	47.5141	0.4080	1.9121	21.3	1.9122	Hex.ZnO	(102)	96-900-4182
	56.5221	0.4080	1.6269	22.1	1.6266	Hex.ZnO	(110)	96-900-4182
	31.7142	0.4162	2.8191	19.8	2.8174	Hex.ZnO	(100)	96-900-4182
	34.4262	0.3537	2.6030	23.5	2.6037	Hex.ZnO	(002)	96-900-4182
50:50	36.2182	0.4890	2.4782	17.1	2.4780	Hex.ZnO	(101)	96-900-4182
	47.5162	0.4162	1.9120	20.9	1.9122	Hex.ZnO	(102)	96-900-4182
	56.5242	0.4162	1.6268	21.7	1.6266	Hex.ZnO	(110)	96-900-4182
	31.7163	0.4245	2.8190	19.5	2.8174	Hex.ZnO	(100)	96-900-4182

30:70	34.4283	0.3608	2.6029	23.1	2.6037	Hex.ZnO	(002)	96-900-4182
	36.2203	0.4988	2.4781	16.8	2.4780	Hex.ZnO	(101)	96-900-4182
	38.4320	0.4700	2.3404	17.9	2.3395	Mno. CuO	(111)	96-410-5683
	35.5600	0.5245	2.5226	15.9	2.5221	Mno. CuO	(11-1)	96-410-5683
10:90	38.4400	0.4608	2.3399	18.3	2.3395	Mno. CuO	(111)	96-410-5683
	49.0340	0.4988	1.8563	17.5	1.8555	Mno. CuO	(20-2)	96-410-5683

Table 2. AFM parameters for for ZnO: CuO ratio deposited on glass.

ZnO:CuO	Average diameter (nm)	Average roughness (nm)	Peak-peak (nm)
90:10	99.86	4.64	18.6
70:30	79.94	2.95	14.4
50:50	78.72	3.04	13.4
30:70	65.49	2.74	14.4
10:90	59.30	2.71	15.1

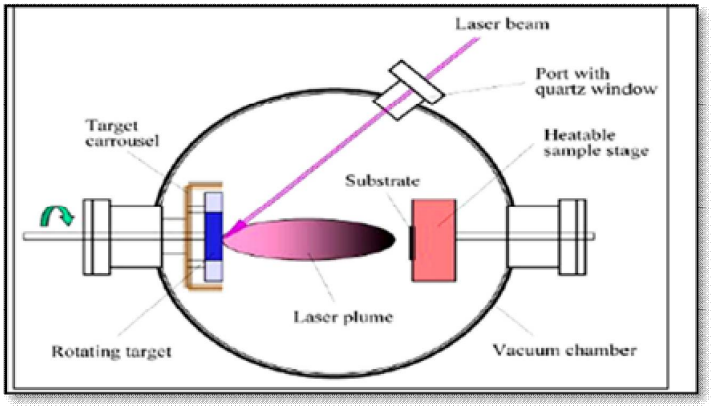

Figure 1. Schematic of a PLD chamber

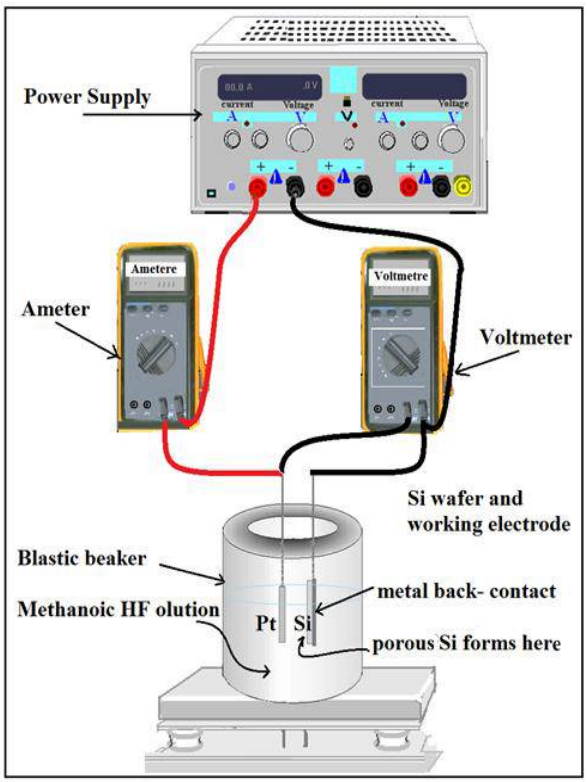

Figure 2. a photo of the porous silicon testing system

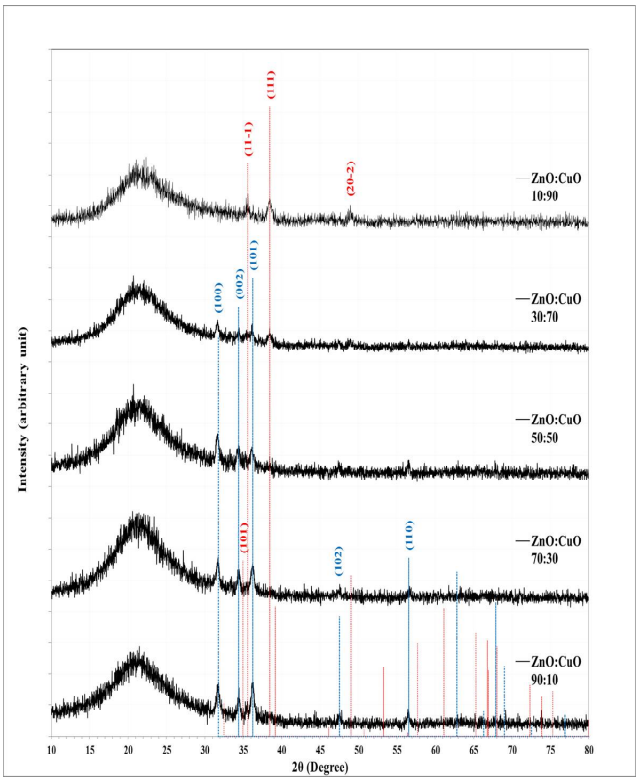

Figure 3. X-ray diffraction patterns of deposited ZnO : CuO in different ratio (90%:10%, 70%:30%, 50%:50%, 30%:70%, 10%:90%).

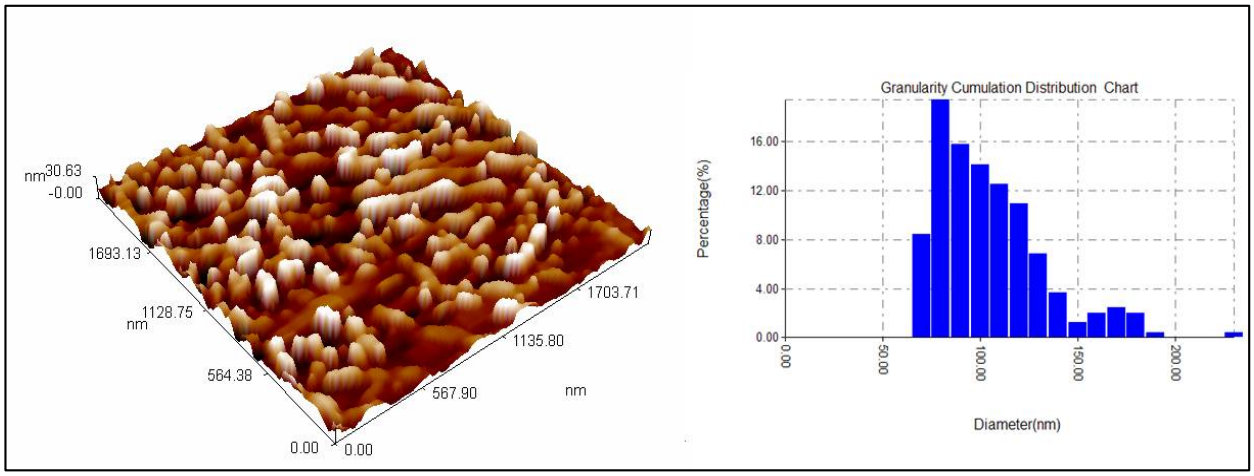

Figure 4. The Atomic force microscopy (AFM) image for ZnO: CuO(90%:10%).

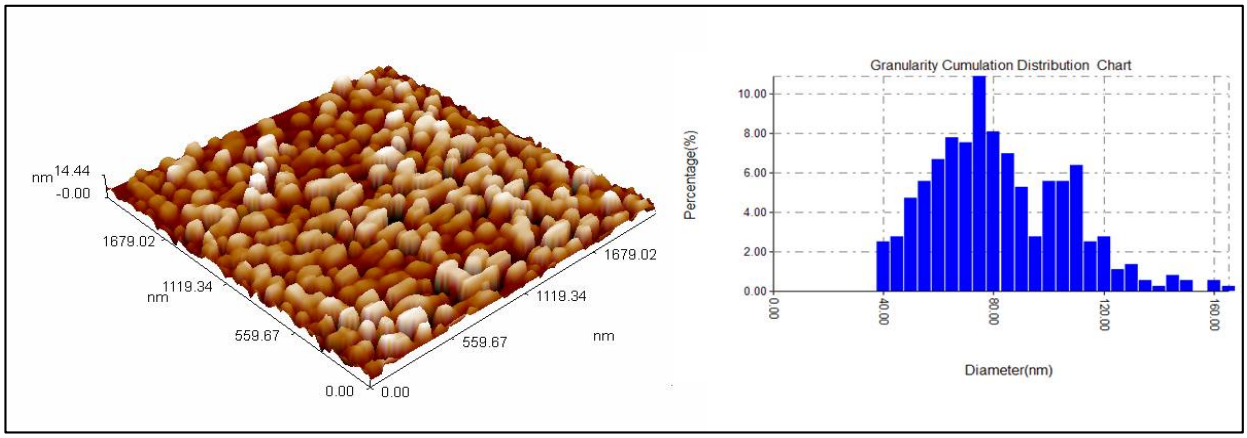

Figure 5. The Atomic force microscopy (AFM) image for ZnO: CuO (70%:30%).

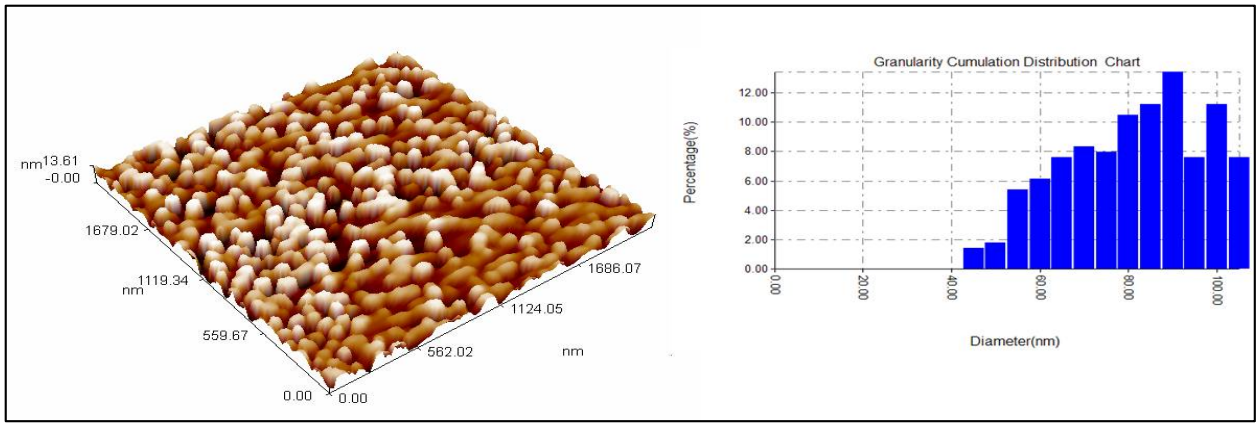

Figure 6. The Atomic force microscopy (AFM) image for ZnO: CuO (50%:50%).

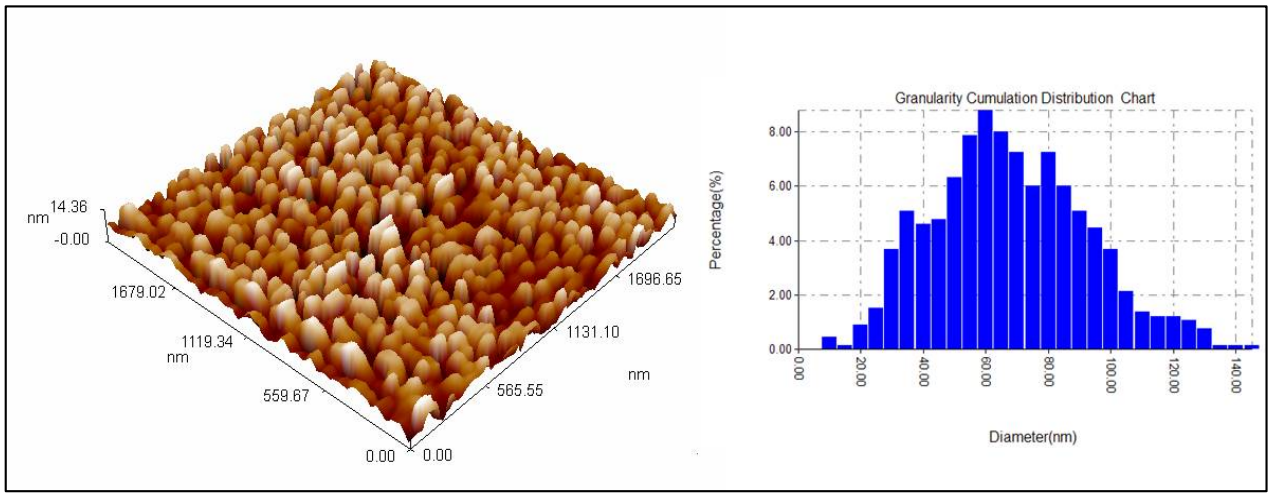

Figure 7. The Atomic force microscopy (AFM) image for ZnO: CuO (30%:70%).

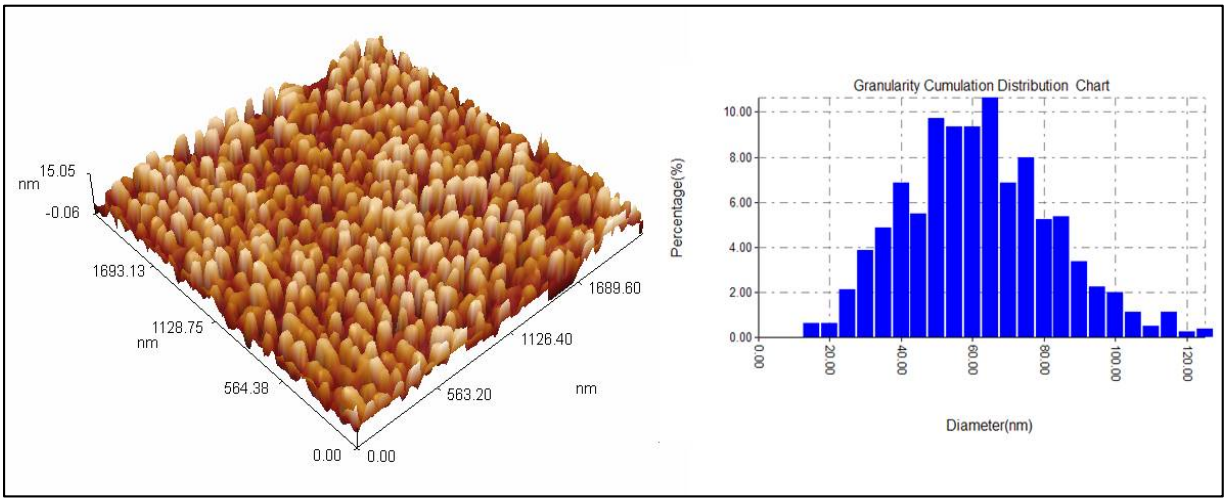

Figure 8. The Atomic force microscopy (AFM) image for ZnO: CuO(10%:90%).

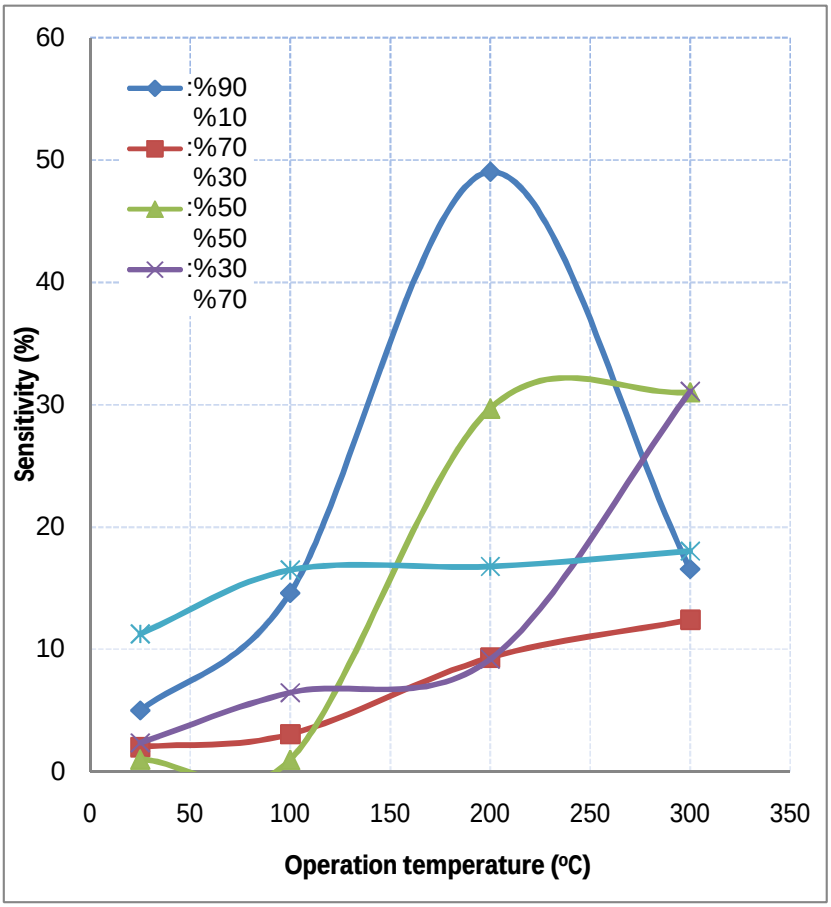

Figure 9. The variation of sensitivity with the operating temperature for different ZnO: CuO gas sensor with NO_2 test gas

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RESEARCH ARTICLE

In vitro Studies on Degradability of Dry Matter, Organic Matter, Ether Extract and Crude Fibre of Rumen Protected Rapeseed Oil Mixed Dairy Cattle Ration

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ABSTRACT

The degradability of dry matter, organic matter, ether extract and crude fibre of experimental rations (control group (RI),treatment groups (RII and RIII) which supplemented with rumen protected fat prepared from rapeseed oil was studied by in vitro rumen stimulation technique. The rumen protected fat was prepared as Calcium salts of rapeseed oil (CaRSO) and encapsulated rapeseed oil and that was mixed with concentrate and roughage based on milk yield of the animals (20 g/kg of milk). The in vitro dry matter and organic matter degradability was similar among all the three groups GI, GII and GIII. In vitro ether extract and crude fibre degradability was less in treatment groups (G II and G III) when compared to control group (G I).

Keywords: *In vitro* degradability, Dry matter, Organic matter, Rumen protected rapeseed oil.

INTRODUCTION

There has been considerable interest in recent years in those factors that improves energy density of high yielding dairy cattle ration by incorporating high levels of grains and vegetable oils. But, high level of cereal grains cause acidosis and other digestive disturbances. The incorporation of supplemental fat in dairy. Cattle was limited up to 3 per cent of dry matter intake (DMI) and above that nutrient digestibility were impaired due to extensive changes in microbial fermentation in rumen. In dairy animals, energy density of ration could be enhanced by addition rumen protected fat up to 6-7 per cent of DMI without any impact on rumen fermentation and digestive disturbances to the animals (Naik *et al*., 2009). Moreover, different approaches are recommended to prepare rumen protected fat rich in poly unsaturated fatty acids. The rumen protected fat prepared by calcium salts of long chain fatty acids of oils and encapsulated vegetable oils as rumen protected fat could be used to increase energy density of ruminant rations without affecting the DMI and nutrient digestibility (Gawad *et al*., 2015). This present study was conducted to study the effect of calcium salts of rapeseed oil fatty acids and encapsulated rapeseed oil on *in vitro* nutrient degradability.

MATERIALS AND METHODS

Preparation of rumen protected fat

The calcium salts of rape seed oil fatty acids (CaRSO) was prepared as rumen protected fat (RPF) following the procedure given by Naik *et al.*(2007) with modifications suggested by Perez (2009) and another form encapsulated rape seed oil fatty acid was also prepared as per method given by Gawad *et al.* (2015).

Preparation of experimental rations

The experimental ration RI was prepared as basal diet by mixing compounded cattle feed and finely chopped CO_3 grass mixed at 45:55 ratio on dry matter basis and in experimental ration RII, CaRSO was mixed at 1 per cent of total basal diet prepared with compounded cattle feed and finely chopped CO_3 grass mixed at 45:55 ratio on DMB. Meanwhile, in experimental ration RIII, encapsulated rape seed oil fatty acid was mixed at 1 per cent of total basal diet prepared with compounded cattle feed and finely chopped CO_3 grass mixed at 45:55 ratio on DMB.

Rumen degradability studies

Apparatus

In vitro experimentation was conducted to determine DM, OM, EE and CF degradability of different experimental rations following the method of Czerkawski, and Breckenridge (1977) in equipment called as RUSI-E-TEK. The apparatus is a semi-continuous culture system patented equipment of Tamil Nadu Veterinary and Animal Sciences University, Chennai for rumen simulation studies. It consists of four 850 ml capacity reaction vessels with perforated container immersed in water bath maintained at 39º C. The apparatus were designed in such way to simulate rumen motility by moving perforated container inside the reaction vessel in anaerobic condition. The infusion of artificial saliva maintained at a constant ratio of 0.04 mL per 6 seconds into the reaction vessels by a peristaltic pump. The effluent and fermentation gases were collected in effluent collection vessels and gas collection bags respectively. Artificial saliva was prepared by adding 9.8 g of sodium hydrogen carbonate, 4.97 g of disodium hydrogen ortho phosphate, 0.57 g of potassium chloride, 0.47 g of sodium chloride, 0.123 g of magnesium chloride and 0.04 g of calcium chloride in one litre of distilled water and kept at 39^0C.

Nylon bags used in this study were made up of woven monofilament polyester cloth, with a specified pore size of 100 μm. The sample size to bag ratio was 15mg/cm2 with specific size of 12.5 x7.5 cm to hold ten gram of sample (Carro *et al.,* 1995).

Experimental design

In reaction vessel I, five nylon bags were packed in perforated container. Nylon bag tied with white rubber band contain 80 g rumen cud, where as other four nylon bags tied with four different colour bands (yellow, blue, black and red) filled with 10 g of control ration (GI) were placed in reaction vessel. Other reaction vessels II, III were charged with 80 g rumen cud and four nylon bags with 10 g experimental rations (GII, GIII) to be tested were placed into container and the assembly was put into the respective reaction vessels. The entire container was filled with distilled water up to the brim to make total volume of the container to one liter.

Incubation Procedure

Rumen liquor and cud was collected from slaughter house in specially designed collection flask maintaining temperature at 39 C and anaerobic condition which used as source of rumen microbes. The rumen liquor was strained through a double layered muslin cloth and filled in reaction vessel. The rumen liquor was purged with carbon dioxide to maintain anaerobic condition. Rumen liquor was checked for protozoal motility at regular intervals. The reaction vessels –I, II and III was filled with 500 mL strained rumen liquor and 200 mL artificial saliva. The samples were incubated for 6, 12, 24 and 48 hours. After six hours incubation period the marked nylon bag (for example yellow – GI sample for six hours incubation period) was taken out and simultaneously one nylon bag from each of three perforated feed container were taken out. Similarly, after 12, 24 and 48 hours of incubation period one nylon bag was taken from perforated container of each reaction vessel. The removed nylon bags were allowed to drain, squeezed and washed in artificial saliva followed by further washing in water and dried at 60 C for 24 hours.

Analytical procedures

All the experimental rations and *in vitro* digesta were dried in hot air oven at 60°C for 24 hours and analysed for proximate analysis (AOAC 2016).

In vitro degradability calculation

In vitro dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD), *in vitro* ether extractand *in vitro* crude fibre degradabilityof control and experimental rations were calculated using the following formula and expressed as per cent on DM basis.

$$\textit{In vitro}\text{ dry matter degradability} = \frac{\text{Weight of bag with sample before incubation) – (Weight of bag with samples after incubation)}}{\text{Weight of sample (g)}} \times 100$$

$$\textit{In vitro}\text{ degradability of other nutrients} = \frac{\text{(Weight of nutrient present in sample before incubation) – (Weight of nutrient present in samples after incubation)}}{\text{Weight of nutrient present in sample before incubation}} \times 100$$

RESULTS AND DISCUSSION

Chemical composition of experimental rations

The chemical composition of control ration G I and treatment rations G II and G III are presented in Table-1. The in vitro degradability values of experimental rations are presented in Table 2 and Table 3. The *in vitro* dry matter degradability values of experimental rations similar in all the three groups GI G II and G III (78.47, 78.02 and 79.61) which were in accordance with findings of Naik *et al.* (2009 b) and Saijpaul *et al.* (2010). The *in vitro* organic matter degradability was similar among all the three groups G I G II and G III (78.84, 78.28 and 79.76) indicating that RPF had no effect on IVDMD and IVOMD. Like that, the *in vitro* ether extract and crude fibre values of experimental rations were less in treatment groups (G II and G III) when compared to control group (G I) indicating that RPF had decreased the *in vitro* degradability of ether extract and crude fibre. The supplementation of encapsulated rapeseed oil in ration had shown decreased ether extract and crude fibre *in vitro* degradability when compared to supplementation of CaRSO.

CONCLUSION

It was concluded that supplementation calcium salts of rapeseed oil fatty acids and encapsulated rapeseed oil in the ration of dairy cows at a level of 1 per cent of ration had shown no effect on IVDMD and IVOMD, but they decreased the *in vitro* ether extract and crude fibre degradability. Encapsulated rapeseed oil had decreased *in vitro* degradability of ether extract and crude fibre than that of calcium soaps of rapeseed oil fatty acids.

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Table 1. Chemical composition of experimental ration (% Dry matter basis)

			Experimental ration		
Attributes	**Concentrates**	**Green grass**	**G I**	**G II**	**G III**
Dry matter	86.87 ± 0.43	26.55 ± 0.38	47.93 ± 0.6	51.84 ± 0.17	52.45 ± 0.13
Crude protein	22.03 ± 0.13	10.52 ± 0.15	14.36 ± 0.05	15.48 ± 0.22	15.69 ± 0.15
Crude fibre	13.27 ± 0.51	36.15 ± 0.26	31.11 ± 0.23	27.79 ± 0.29	29.25 ± 0.33
Ether extract	2.34 ± 0.10	1.43 ± 0.036	2.07 ± 0.05	3.57 ± 0.021	3.14 ± 0.061
Total ash	12.31 ± 0.14	8.19 ± 0.18	5.04 ± 0.01	6.13 ± 0.039	6.07 ± 0.03
Nitrogen free extract	50.06 ± 0.69	43.71 ± 0.34	47.42 ± 0.13	47.03 ± 0.64	45.86 ± 0.28
Organic matter	87.69 ± 0.14	91.81 ± 0.18	94.95 ± 0.01	93.87 ± 0.04	93.93 ± 0.03

Table 2. *In vitro* dry matter and Organic matter degradability (IVDMD) of experimental rations (Per cent)

Incubation time	**Experimental rations**		
	Group I	**Group II**	**Group III**
	Dry matter degradability(IVDMD)		
6 hours	67.68	66.87	58.13
12 hours	70.07	63.79	68.66
24 hours	77.44	72.07	75.09
48 hours	78.47	78.02	79.61
	Organic matter degradability (IVOMD)		
6 hours	67.77	67.14	58.02
12 hours	70.48	64.00	68.69
24 hours	77.57	72.20	75.15
48 hours	78.84	78.28	79.76

Table 3. *In vitro* Ether extract and Crude Fibre degradability of experimental rations (Per cent)

Incubation time	**Experimental rations**		
	Group I	**Group II**	**Group III**
	Ether extract degradability		
6 hours	27.14	24.04	22.65
12 hours	39.13	32.39	30.22
24 hours	44.68	39.52	34.40
48 hours	52.55	45.23	42.81
	Crude fibre degradability		
6 hours	55.25	56.02	49.01
12 hours	61.64	54.29	56.65
24 hours	67.15	61.92	60.16
48 hours	69.003	67.74	62.56

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RESEARCH ARTICLE

Cost and Effort Estimation in the Early Stage Assessment of Object Oriented Software

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ABSTRACT

The most demanding activities in software development organizations are software quality and cost estimation Software companies are alert on minimizing software error, producing good quality software products within the estimated budget. Currently, the researchers are focusing on to predict the software development effort and cost. Predicting the software cost prior to beginning any software development is essential for the project managers and the key stakeholders. Major project target such as project schedules, budgeting, resource allocation, and project delivery dates are set on the effort and cost estimates. Thus, the reliability of the estimation is the desirable factor to find the success or failure rate of the project. The main aim is to work with early stage improvement while taking the decision in the design phase. Basically, size and cost is a deliberate element of the software project so, based on the size and other functionalities, the software manager estimate the total effort required to develop the project. From the effort and work schedule, the total cost can be estimated. This will retain the relations between the developer and the customer. In this paper, the proposed model implements the technique using java tool.

Keywords: Quality, Effort, Cost, Software Development, Metrics.

INTRODUCTION

Estimation of effort and duration of software development has become a topic of growing Importance. This is not surprising. It often happens that software is more expensive than estimated and completion is later than planned. The most remarkable things in estimation and control of software development projects are

- 35% of the participating organizations do not make an estimate
- 50% of the responding organizations record no data on an ongoing project
- 57% do not use cost-accounting
- 80% of the projects executed by the participating organizations have overruns of budgets and duration
- The mean overruns of budgets and duration are 50%.

MATERIALS AND METHODS

Estimation Techniques

This predicting process focuses on estimates of effort and time with the project activities, which starts from requirement phase and ends with maintenance phase. Two groups of methods are commonly used to predict the software cost such as Algorithmic and Non-algorithmic. In this section, various accepted estimation technique are discussed.

Algorithmic Models

Nowadays, many researchers [33]-[36] use these models. These are classified into different models and to find the estimation it uses the equation shown in"(1)":

$$Effort = f(x_{1,}, x_{2,} \dots x_{n,}) \quad \dots(1)$$

where, $(x_{1,}, x_{2,} \dots x_{n,})$ are the cost factors. s. The cost factors are as follows

- Product factors
- Computer factors
- Personnel factors
- Project factors

Source Line of Code

Source lines of code (SLOC or LOC) is a software metric used to assess the software size by counting the number of lines in the source code, excluding the instructions such as comments, blanks, and continuation lines. SLOC measures the size of the project only at the end of the development and not at the early stage due to the lack of information about requirements.

Function Point Size Estimates

Function Point metric has been proposed by Albrecht [36] to measure the functionality of project by using the following indicators

- User Inputs,
- User Outputs,
- Logic files,
- Inquiries,
- Interfaces

These indicators have the complexity degree ranges from 1(Simple) , 2 (Medium) to 3(complex) and weight for each indicator lies between 3 and 15.

Seer-Sem

SEER for Software (SEER-SEM) is an algorithmic project management software application designed specifically to estimate, plan and monitor the effort and resources required for any type of software development and/or maintenance project.

COCOMO

Cost models generally use some cost indicators for estimation and observe to all specifications of artifacts and activities. COCOMO 81 (Constructive Cost Model), proposed by Barry Boehm [5], [10] is the most popular method which is categorized in algorithmic methods. The model parameters are derived from fitting a regression formula using data from historical projects (61 projects for COCOMO 81 and 163 projects for COCOMO II). COCOMO II is the successor of COCOMO 81 and is claimed to be better suited for estimating modern software development projects; providing support for more recent software development processes and was tuned using a larger database of 161 projects. COCOMO II predicts the amount of effort based on Person-Month (PM) in the software projects.

It uses function point or line of code as the size metrics and composes of 17 Effort Multipliers and 5 scale factors. Some rating levels are defined for scale factors including very low, low, nominal, high, very high and extra high. A quantitative value is assigned to each rating level as its weight to get accurate results.

Putnam's model

Putnam model is an empirical software effort estimation model proposed by Putnam. It is based on size of the product, scaling factor and function of the project size, productivity and total schedule of the project in years.

Non Algorithmic Methods

These methods are based on analytical relationship and implications. So, the analysis of previous datasets are required in this model to perform the estimation process. Here, three most popular methods have been selected for the assessing the cost and effort. [21]-[29]

Analogy

In this method, the cost and effort estimation is done by assessing the results of similar project. The steps of this method are considered as:

i. Choosing of analogy
ii. Investigating similarities and differences
iii. Examining of analogy quality
iv. Providing the estimation

Expert judgment

Estimation based on Expert judgment is done by getting advices from experts who have extensive experiences in similar projects. This method is usually used when there is limitation in finding data and gathering requirements. Consultation is the basic issue in this method. One of the most common methods which works according to this technique, is Delphi. Delphi arranges an especial meeting among the project experts and tries to achieve the true information about the project from their debates. Delphi includes some steps

i. The coordinator gives an estimation form to each expert.
ii. Each expert presents his own estimation (without discussing with others)
iii. The coordinator gathers all forms and sums up them (including mean or median) on a form and ask experts to start another iteration.
iv. steps (ii-iii) are repeated until an approval is gained.

Machine learning Models

Most techniques about software cost estimation use statistical methods, which are not able to present reason and strong results. Machine learning approaches could be appropriate at this filed because they can increase the accuracy of estimation by training rules of estimation and repeating the run cycles. Machine learning methods could be categorized into two main methods, which are explained in the next subsections.

Neural networks

Neural networks include several layers which each layer is composed of several elements called neuron. Neurons, by investigating the weights defined for inputs, produce the outputs. Outputs will be the actual effort, which is the main goal of estimation. Back propagation neural network is the best selection for software estimation problem because it adjusts the weights by comparing the network outputs and actual results. In addition, training is done effectively.

Fuzzy Method

The system with fuzzy logic simulate human behavior and reasoning. The fuzzy systems are an efficient tool where decision making is very difficult and conditions are vague...

Parametric Models

The parametric modeling approach uses historical data to formulate one or more algorithms which produce estimates of software development effort. This approach makes use of an average ‘productivity factor’. This assumes productivity as the basic factor, whereas software factors and organizational variables such as project team composition are somehow incorporated in the calculated productivity rate. Most researchers agree that there is a need to modify the results of the models for the ‘software factors’ which are identified as potential amplifiers of effort. The quality of the parametric models is highly dependent on an expert estimation of the size and complexity of the individual components of the system to be built. The underlying assumption is that the components of a model can be estimated more accurately than the effort needed for the development of a system.

Parkinson’s law

G. Northcote Parkinson first described the phenomenon now known as Parkinson's Law or 'Work expands so as to fill the time available for its completion"[38].

Price-to-win

The software cost is estimated by the price what the customer has available to spend on the project.

Top-Down and Bottom-Up Estimating

Essentially, there are two approaches for estimating of the quantitative product and project attributes: the top-down and the bottom-up. Thus, estimating software development can be implemented in either or both of these ways.

Need for Metrics

The challenge is therefore to propose metrics that are firmly rooted in theory and which are relevant to practitioners in organizations. Hence, these needs are addressed through the development and implementation to verify a new suite of metrics for object-oriented design. Seven design metrics and two proposed metrics are analytically evaluated against the proposed Software Assessment Model. An automated Software Quality Indication was then developed and implemented to collect an empirical sample of these metrics to demonstrate their feasibility. These suggest the ways in which managers can use metrics to improve the quality of design. Also, the impact of the proposed metrics in the Software Assessment Model is highlighted.

Two object-oriented design metrics are proposed, namely Effort Estimate metrics and Cost Estimate metrics for object-oriented systems. Theoretical basis for the development of object-oriented metrics is shown in Fig. 1. When software complexity evaluation is performed before system building, it can be used for predicting the key aspects like development, testing and early maintenance costs**.** Moreover, on the basis of knowledge that is present in the early stages of the software life-cycle, for example the number of classes, main class relationships, number of methods, method interfaces, the process of system analysis and design allows for the defining and tuning of metrics for predicting effort. From the cognitive point of view, the observable complexity can be regarded as the effort required to understand the subsystem or class behaviour and functionalities. This complexity can usually be evaluated in the early phases of design and can be used for predicting the costs of reuse and maintenance [40] for estimating and predicting other features [6]

As a result, a model and metrics are proposed for the calculation of effort estimation or prediction of early design. The validation was performed by using real data from different projects. The validation presented shows that several metrics can be profitably employed for effort estimation or prediction can also be successfully used in the estimation or prediction of early Effort Estimate by using the model. The metrics presented in the work are an extension of a framework specifically defined for object-oriented systems [7], [8], [32]. The Effort Estimate metrics calculates the total size of the system S, by calculating the number of lines of code of each class C. Here component (C) implies the classes, use cases and the events. The research focuses on Events, State and Use Cases and verifies their efforts. The RUP (Rational Unified Process) of UML is based on the use case concept.

The Cost Estimate metrics identifies the key class or the supporting class for the redesigning effort intimated by the Software Assessment Tool. The key class has close attachment to most of the elements in the project than the supporting class and redesigning a key class will involve significantly more effort. The Cost Estimate also considers the real time issues for the Cost Estimate, the project type, the developer skills and the user interface type, which are also found to have an influence over the efforts in the development.

Effort Estimate Metrics (EE)

Effort Estimate metrics measures the size of design elements, typically by counting the elements contained within, for example, the number of operations in a class, the number of classes in a package and so on. Size metrics are good candidates for developing the cost or Cost Estimates for implementation, review, testing or maintenance purposes and activities. Such estimates are then used as input for project planning purposes and personnel allocation. In addition, large design elements are big classes or packages may suffer from poor design. In an iterative development process, more functionality is added to a class or package over the time. The risk is that, eventually, many unrelated responsibilities are assigned to a design element. As a result, it has low functional cohesion. This inturn negatively impacts the understandability, reusability and maintainability of the design element. Therefore interfaces and implementation of large classes or packages should be reviewed for functional cohesion. If there is no justification for the large size, the design element should be considered for refactoring, for instance, extract parts of the functionality to separate, more cohesive classes.

Practical Software Measurement (PSM) [41] defines a generic process for selecting software measures and using the resulting information to manage projects. The research is based on a broad survey of the related literature that can assess the state of the art and practice in object-oriented measurement along with modelling and also maps the information collected in the PSM framework. It proposes a metrics called the Effort Estimate Metrics which uses the events, states and Use cases, as those elements in the project decides the scope of the object-oriented programming under investigation "(2)".

Size (S) = Size (C) ……..(2)

Where Components (S) = $\{C_1 \ldots C_n\}$, such that if $C_i = C_j$ then i=j, where i, j = 1… n. The above equation calculates the total size of the System S by calculating number of lines in the code of each class C. Here, Components (S) implies the classes, use cases and the events. Various researches have been carried out on design metrics and researchers have developed Quality Model for Objected Oriented Design (QMOOD) [10]. It measures the functional, structural and relational details of the system based on high-level attributes. In the proposed model, the reusability is calculated based on the coupling, cohesion, Effort Estimate and messaging.

The Proposed Effort Estimate Metrics

The proposed work takes into consideration, the Effort Estimate metrics, comfortable for most of the OOPS project that are successfully proved in the real time, based on the quality control suggestions from the Company and has opted this metrics for testing. The Effort Estimate metrics can be calculated as follows "(3)"
Effort Estimate = No. of classes * No. of methods * No. of variables.....(3)

The resultant value for Effort Estimate should not exceed the standard, which is based on the experience study from the industry. The tool analyses the input sample classes for various metrics (Seven Metrics) and Effort Estimate metrics, which has the major influence for the design quality indications. The Software Assessment tool will intimate Java classes, which has a deviation from the standard and suggests for re Effort Estimates for those classes.The research focuses on Events, States and Use cases and has verified the efforts. The RUP (Rational Unified Process) part of UML is based on the use case concept. A use case captures a contract between the stakeholders, also called the primary actors of a system and its behaviour. The use case gathers the different sequences of behaviour, or scenario together. In short, an use case is a good way of eliciting requirements at the early stages of software development.

Number of Actors associated with a Use case (NAU)

This metric computes the number of actors which are associated with a use case and it is useful to measure the importance of the requirement expressed by the use case. The reason for this argument is that, the requirements that many actors concerned are likely to be important for the system to function properly as a whole. It does not count normal system classes for this metric, because this metric concerns the interactions between systems and its stakeholders.

Number of Messages associated with a Use case (NMU)

As explained before, a use case is further refined through its scenario. In UML, there are two scenario diagrams, which are the sequence diagram and the collaboration diagram. These two kinds of scenario diagram are completely isomorphic in meaning, where, one kind of diagram can be automatically replaced with another kind without any loss of information contained in it. The NMU metric counts the number of messages comprising the scenario of a use case. This metric is useful for tracing requirements into design-level elements.

Number of System Classes associated with a Use case (NSCU)

This metric counts the number of classes whose objects participate in the scenario of a use case. This metric does not include actors as this is done with the NAU metric. Like NMU, NSCU metrics are good for estimating the impact of a requirement change onto the system. Any change in the use cases, spread to classes, the interactions of their objects and vice versa. The Effort Estimate is calculated based on all the above parameters events, states and use case (NAU, NMU, NSCU) of the classes involved.

The Cost Estimate Metrics (CE)

Software project managers are responsible for controlling the project budgets and are responsible to estimate the effort required for software development in its total life cycle. The principal components of project costs are the computer component costs, required software and tools cost, travel expenses, cost of training employees and the cost of effort involved. The foremost cost is the effort cost, which is most difficult to estimate and control. It has the major effect on the overall costs. Many software systems built in the recent years have been developed using the object-oriented technology. In most cases, the estimation and prediction of effort is performed with difficulty due to the lack of metrics and suitable models. The work also covers OOPS software effort estimation which predicts the redesign or rework efforts incurred as a result of design imperfections. The estimation typically produces projections of the software size, effort, schedule and the quality required to complete the project. These estimates form the basis for initial project plans and subsequent re-plans. Generally, the same factors that get estimated need to be tracked in order to manage a successful project. While estimation models may provide from 15 to 100 adjustment factors, most organizations realize that their performance is only affected by a few of them. The survey of estimation models for object-oriented development shows that the two most commonly used adjustment factors are the team experience or expertise and the application type are complexity or difficulty. If complexity is considered as an adjustment during the software sizing step, then it should not be factored again.

Comparison of results from Nesi & Querci [6] and Lorenz & Kidd [42] also suggests that the effort to implement a class in Smalltalk is greater than the effort to implement a class in Java. Bucci et al. [43] notes that the productivity declines as teams get larger than four persons. Documentation still needs to be considered in planning object-oriented projects. Nesi & Querci [6] report that 36% of effort is spent on documentation for an industrial project.

The Proposed Cost Estimate Metrics

Estimating object-oriented projects has a great demand, even though people have been estimating object-oriented projects are implemented in many different programming languages for over a decade. The following are some of the things that have been identified. The simple estimates are based on the number and kind of classes contained in the object-oriented application. The experience and skill of the object-oriented developer is the second most important factor in this estimate. According to the industrial estimate, a skilled developer codes an OOPS project class in 10 days and a beginner will do it in 20 days. This difference in duration confirms that the developer's skill and effort is a significant factor towards the completion of the project. The project type is also an important factor, which ranges to any size. The number of developers and their effort is given as an input to the project type. Design wise, the proposed Cost Estimate identifies the key class or the supporting class in the redesigning effort intimated by the Software Assessment Tool. The key class has close attachment to most of the elements in the project than the supporting class and redesigning a key class involves significantly more efforts. The Cost Estimate also take into consideration the real time issues like the project type, developer skills and the user interface type which also are found to have an influence over the efforts in the development "(4)".

Cost Estimate = K * U * Ex……(4)

Number of key classes (K), Complexity created by the user interface (U) and Experience of the developer (Ex).

As suggested earlier during redesigning, the efforts depend on the subjects taken for redesigning. Also when the User Interface type involves drag and drop facilities, the system involves more complexity from real time exceptions. The experience of the developer is based on the time response of the developer to arrive at a Effort Estimate for a class. The Project manager carries a statistics of the developer profile. An industry procedure is taken and an attempt is made on a real time consideration regarding the cost per hour while calculating the redesigning efforts.

The Cost Estimate Calculation

In calculating the Cost Estimate for the OOPS project using the Design Phase Model, the input source is verified for the OOPS Metrics for any design compliance and imperfections. The model automatically indicates the classes that require redesigning efforts based on the practical standard. In addition, it accepts certain details from the user. The model considers the key classes, supporting classes, the anticipated complexity of user interface as well as the developer efficiency to arrive at the Cost Estimate.The estimation of effort is a complex task since it considers various parameters for its calculation. The four important parameters such as User Interface type, Project size, Expert skill set and Class type are taken as important parameters along with the per effort cost. Each of the parameters stated varies and takes values ranging from minimum to maximum is depicted in Fig. 2. The first key parameter, which helps in cost estimation, is the User Interface. The design of the user interface by the programmer can be simple or complex depending on the nature of the project. Hence, the Cost Estimate metrics can take the minimum or maximum value of the parameter for an application. The effort or cost estimate also depends upon the size of the projects. When the size of the project is small, it assumes a minimum value and in the case of others, it assumes either the medium or maximum value.

The next parameter taken for consideration is the expert skill set which according to many companies is difficult to obtain. The code efficiency of a project depends on the knowledge of the programmer in developing solution to it. Hence, the expertise or skill set required for a programmer plays an important role in cost or effort estimation. Depending on the programmer's expertise, the project may take values between low, medium or high and thereby affects the estimation of effort. The last parameter which helps in effort estimation is the class type. In designing the classes, the classes can be categorized as key classes or support classes. The type of classes indicated also forms a major factor in effort estimation as it indicates the attachment of classes to the project or application. All the

parameters are proposed to be equally important. These parameters are implemented for the effort estimation based on the per effort cost and produce the results shown in Fig. 3.

The Ranking Approach

A Ranking based performance is planned to perform annotation prediction that is taken into account as user's potential annotation for bugs. The method as in Fig 4. Take the information area unit obtained from Existing package modules with noted defect numbers as well as values of all package metrics which has lines of code, previous defects, and new lines side, and also the numbers of defects found. Once finishing ranking approach on defect modules, results area unit created in standout sheet.

System Analysis and Cost Estimation

Cost Estimate supports deciding is needed for the allocation of funds to program areas and/or specific comes details like techniques are accustomed estimate effort, metrics are accustomed live the accuracy of effort estimation methods/techniques .What effort predictors are utilized in computer code development method.

Reliability of Object Oriented Design

As changes are made to an object-oriented design, its structure or behaviour may be affected. Modifications made to one class can have ripple effects on other classes in the design. Ripple effects may or may not be desirable do not require additional changes. A good object-oriented design from stable standpoint should localize changes as much as possible to classes on which alterations are made. The reliability of an object-oriented design indicates its resistance to interclass propagation of changes that the design would have when it is modified. Class reliability is the likelihood that the class will not be change-prone as a consequence of changes made to other classes in the design. There are two aspects of design reliability. Logical reliability, that is concerned with the stability of the design structure and performance reliability, which is concerned with the reliability of design behaviour. The role of object-oriented design metrics are discussed in the development of software by measuring the key parameters involved in the project and a high level Software quality indicator is designed to indicate the quality of design at its early stages.

Case Study

A study has been performed on 6 different modules of various applications developed in an object-oriented environment, which are components of larger applications. Some of the technical characteristics of the application are distributed environment, C++ as the primary language, relational database, object-oriented, object persistence tier. The software is designed in UML and developed in different applications, which is used for the evaluation of object-oriented software. The chosen 6 systems reflects a general application and provides the real time metrics value. The subjects used for metrics verification are labelled as A, B, C, D, E and F for easy reference.

Data Collection

Subject A (Data Compression application using Huffman Run Length Encoding) focuses on the compression of data. It compresses large files for efficient memory management and implements new algorithms to compress data with respect to their format. The details of all the compressed files are recorded in the Operating System which also helps to add more files into a particular folder for the protection in the operating system. Subject B (Encryption application using Rivest-Code) can be implemented over network application, whenever confidential data has been transferred over the network by creating an API. It basically aims at implementing the standard encryption algorithm to support secure transmission of data. Subject C (Proxy server with advanced controls) serves as a middleman in serving the

client request and provides security and performance improvement in the network. Communication is done directly with the web server to service the request. It is also used to share the web resource in the internal network, without exposing the other machines in the network. Subject D (MP3 application) is designed to control a compact MP3 player with various advanced level controls to choose the songs from list, favourites and set advanced level volume control. Subject E (HTC) focuses on virtual reality for a classroom, which has all the facilities like video conferencing, FTP and messenger. Subject F (GRD) is developed to be a grid based search engine which needs to dynamically assign the searching task to various machines in the network based on the utilization.

Subjects A, B, C and D

The application was developed using object-oriented environment under Windows 2000 operating system, developed in Java programming environment, using Swings and Multi-threading. The system had a maximum of five classes and can therefore be considered as a small-scale for object-oriented system.

Subjects E and F

The application was developed using object-oriented software system under Windows 2000 operating system and developed with Java programming environment using Swings, Multithreading and Networking. The system had maximum of 15 classes and hence therefore considered to be a large scaled object -oriented system. The metric data collected for 12 metrics for the six applications is shown in Table I .

Metrics Standards

Influence of Effort Estimate Metrics

The Table II shows some of the Effort Estimate Metrics data for the subjects which has been considered for study Based on Lorenz and Kidds [42]. The optimistic Effort Estimate of a project is 45 per class taking into consideration the use case / classes / attributes and methods, which play the major influence in deciding the efforts for the project. The project B, D, E, and F shows careless efforts in designing the application as the Effort Estimate of the projects shows a major deviation from the proposed.

Influence of Cost Estimate

The Fig. 5 Shows the Cost Estimate is based on Assessment Tool quality indication taking into consideration important metrics that is deviated by the design. The Table 3 shows the Data Collection of Effort Estimate and Cost Estimation for a sample of 6 subjects. The Cost Estimates here indicate redesigning cost in efforts for the Subjects. The project has minimum interface (lack drag and drop) and the project size is medium and thing into consideration the Effort Estimate identification by the tool and the identification of the class as key class or the supporting class by the tool for project is developed with highly rated developers. The cost is the standard followed in the industry. The project redesigning efforts were also considered the type of efforts. The tool suggests that the redesigning cost depends on the key class / supporting class, type of user interface (full blown / minimum interface, project size, developer profile and the developer cost per hour. Fig. 5.

CONCLUSION

The Effort Estimate and Cost Estimate metrics were proposed and implemented in the design level before the development and coding stage. The model calculates the Effort Estimate by using the number of classes, the number of methods and the number of variables. Also, the working of design metrics in the model was explained. The need

for effort calculation in the design stage and working of the effort estimation by using the Cost Estimate metrics were discussed. The reliability of the model was also highlighted.

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Table .1.Data Collection of 6 Subjects Using the Software Assessment Model

Project Name	Data Compression	Encryption	Proxy Server	Video conferencing	HTC	GRID
No. of .Classes	5	4	5	32	13	12
No. of. Methods	19	43	14	314	55	67
Coupling factor	1.35	3.0833	3.55	0.717	1.57	1.16
Avg. No. of Parameter / Method	0.08947	0.93	0.5	0.82	0.78	0.552
WMC Optimal = 15	4,4,7,3,1	12,4,13,14	3,3,2,3,3	22,23, 4,21,5,11,11,2, 3,11,16,8,2,43.	0,11,4,10,6, 3,6,3,2,3,2, 1,4	6,3,8,3,1,5,6, 14,8,4
WAC Optimal=5	2,2,9,0,2	10,1,14,3	2,10,9,10, 3	26,8,3,11,1,7,8 ,2,17,32,11,18, 3,46	12,8,14,23, 13,16,3,9,1 6,14,4,28	18,3,10,9,6,0, 11,2,0,10,13, 7
RFC Optimal=35	10,9,21,3,1	24,28,27,26	19,27,15, 27,23	78,39,17,37,8, 27,36,18,28,34 ,34,29,5,94	0,33,21,47, 29,25,16,15 ,11,12,10,4, 28	29,7,27,25,6, 24,18,4,35,36 ,11
LOC Optimal=50	35,16,115,20,10	105,37,118, 129	36,74,57, 74,83	257,44,77,18,4 4,77,94,9,23,1 83,448	0,127,53,11 6,81,27 81,27,32,29 ,30,23,17,6, 33	127,53,116,8 1,27,32,29,30 ,23,17,6,33
CBO Optimal=8	6,5,9,5,2	9,6,12,10	9,19,13,1 9,11	34,25,18,21,15 ,15,17,7,11,30, 15,21,7,72	5,32,16,30, 23,4,1,13,13 ,17,9,9,4,34	19,6,15,12,16 ,3,19,11,15,2, 10,8
LCOM Optimal=1	1.0	0.9579	0.7011	0.9	1.0	1.0
DIT Optimal=6	0.987	1.0	2.0	1.0	6.0	7.0
Design Size Optimal=4.5/class	285	1204	476	525	7150	5628

Table 2. Data Collection of Effort Estimate

Subject	Effort Metrics
A	285
B	1204
C	476
D	1560
E	7150
F	5628

Table 3.Data Collection of Effort Estimate and Cost Estimation

Project Name	Effort Estimate	Cost Estimation
A	285	560
B	1204	720
C	476	840
D	1560	840
E	7150	560
F	5628	840

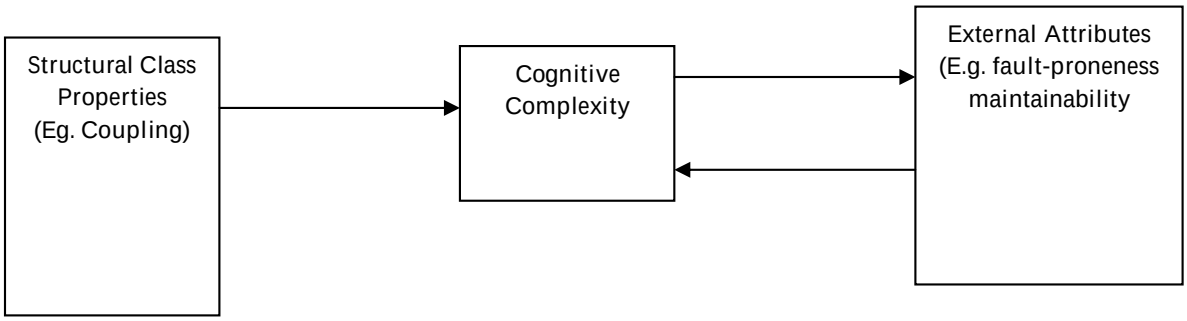

Figure 1. Theoretical Basis for the Development of Object Oriented Metrics

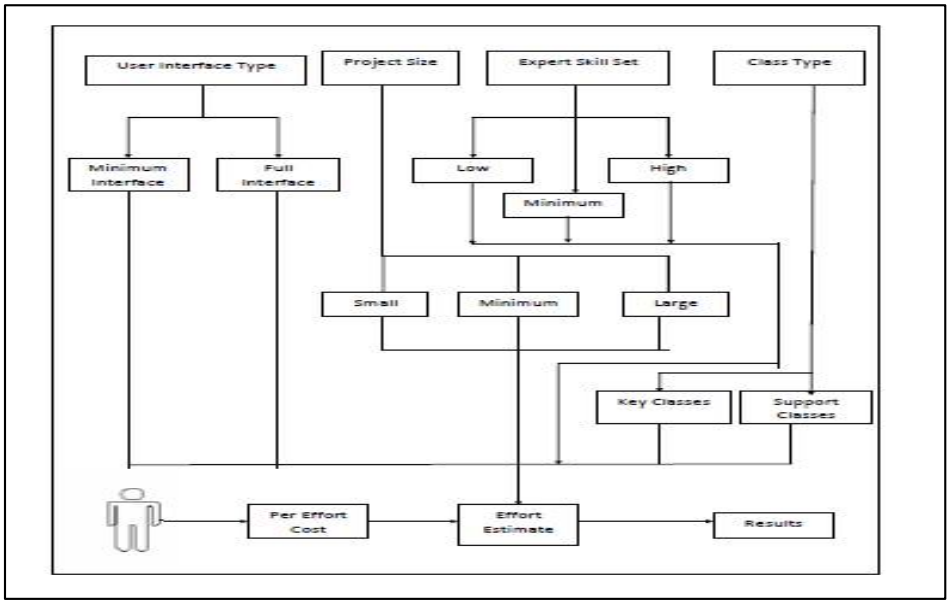

Figure 2. Cost Estimate Calculations

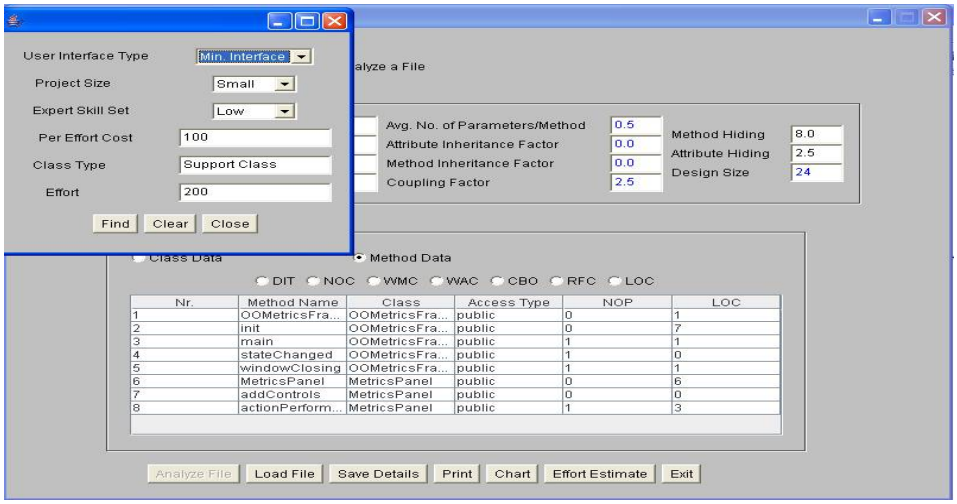

Figure 3. Cost Estimate calculating the redesigning efforts for a project

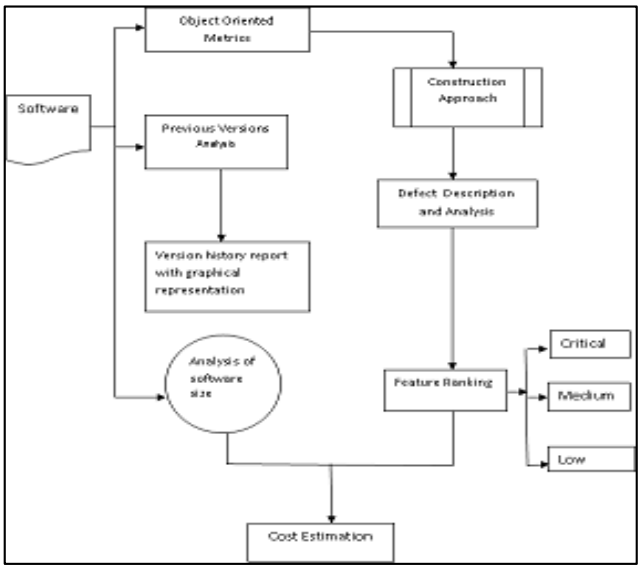

Figure 4. Ranking Approach

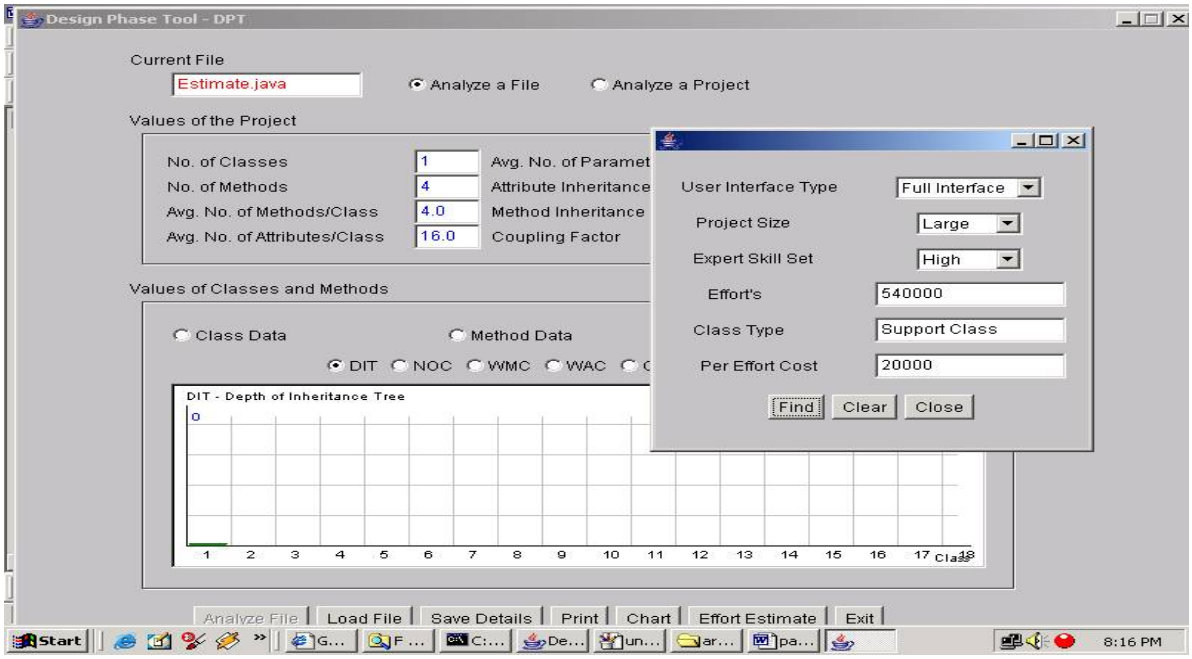

Figure 5. Cost Estimate calculating the redesigning efforts for a project.

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RESEARCH ARTICLE

Perpetration and Characterization of Silicon Carbide by Pulse Laser Deposition as Heterojunction Solar Cell

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ABSTRACT

Silicon Carbide (SiC) thin films have been prepared by using the pulse laser deposition technique (PLD). The deposition lasing energy and reputation rate have been varied to optimize the quality of the films. The samples have been characterized by X-Ray diffraction (XRD), atomic force microscopy (AFM), uv-visible spectroscopy, and the current-voltage characteristic curve. The results show that good quality silicon carbide films can be prepared by pulse laser deposition technique. The XRD of prepared films shows amorphous structure goes to polycrystalline when annealed to 400^{o}C. It can be seen from The I-V characteristics of Si/p-Si solar cell, that the photocurrent density increases with increasing of the bias voltage and the higher falling factor is (0.46) and efficiency (3.46).

Keywords: silicon carbide, Pulse laser deposition, solar cell.

INTRODUCTION

Silicon carbide is an important non-oxide ceramic which has diverse industrial applications. In fact, it has exclusive properties such as high hardness and strength, chemical and thermal stability, high melting point, oxidation resistance, high erosion resistance, etc[1]. All of these qualities make SiC a perfect candidate for high power, high temperature electronic devices as well as abrasion and cutting applications[2]. Quite a lot of works were reported on SiC synthesis since the manufacturing process initiated by Acheson in 1892. Several alternative methods have been previously reported for the SiC production. An overview of the most common used methods for SiC elaboration such as physical vapour deposition (PVD)[3], chemical vapour deposition (CVD)[4], sol-gel[5], liquid phase sintering (LPS)[6] or mechanical alloying (MA) will be detailed.The growth of SiC thin films by pulse laser deposition technique affects their properties. It has been noticed that the dopant incorporation allows controlling thin film properties such as optical bandgap and electrical conductivity, which are quite attractive because make possible to

obtain semiconductor or insulator SiC-based films [7]. This research contained the PLD technique to preparation of Silicon Carbide thin films. The Structural, morphological, and optical properties of SiC thin films were deposited by PLD at R.T. and then annealing at 400^{o}C. In addition, a current-voltage characteristic curve was measured to study fall factor and efficiency of SiC/P-Si solar cell.

EXPERIMENT SETUP

PLD technique is used to deposit SiC thin films shows in figure (1), where these techniques consist of vacuum chamber evacuated to pressure (10^{-3} mbar)and Q-switched Nd:YAG laser. The focused Nd: YAG laser beam at (600 mJ to 900 mJ), comes through a window and incident on surface of the SiC target with an angle of 45°. The substrate is placed in front of target and its surface parallel to it. Suitable gap is kept between substrate and target so that the substrate holder doesn't impede the incident laser beam. The films were deposited on glass at room temperature. A Q-switched Nd: YAG laser with (1064 nm) wavelength, (pulse width 10 ns) repetition frequency, (10 Hz), for 500 laser pulses used as deposition technique.

RESULTS AND DISCUSSION

Fig. (2) shows the X-ray diffraction for SiC thin films prepared by pulse laser deposition technique at different laser energy. This figure shows amorphous structure for thin film produced at 600 mJ and convert to poly-crystalline structure when annealing to 400^{o}C. The peaksappear at 2θ =35.61^{0}, 41.42^{0} and 60.10^{0} belongthe (111), (200) and (220) planes for cubic SiC structure, which identical with standard card number (JCPDS 29-1129).These results are found in a good agreement with[8]. Table (1) shows the Structural parameters for SiC thin films which contain Bragg angle (2θ),full width at half maximum (FWHM), experimental Inter-planar spacing (calculated using Bragg low), crystalline size (calculated using Sherrer formula) and the corresponding planes. Fig. (3) shows the atomic force microscopy images and their granularity accumulation distribution for pure SiC thin films deposited by pulses laser on glass substrate using two laser energies (800 and 1000 mJ) and different number of pulses (800 and 1500 pulses). AFM parameters (average diameter, average roughness and peak –peak) for these samples have been shown in Table (2). This Table illustrates that the average diameter and film roughness increase with increasing number of pulse, due to particles aggregation with increase film thickness, while decrease with increasing laser energy from 800 to 1000 mJ due to higher energy used to extract the particles from sample surface. These results are found in a good agreement with the [9].

From UV-Visible near IR absorption spectrum the optical measurements for SiC thin films prepared by pulse laser on glass substrates with different energy is carried out in the wavelength range 300–1100 nm. Fig.(4) shows the Absorption spectra for SiC thin films deposited on glass substrates using different laser energies(600, 700, 800, and 900mJ).In general, it can be observed that the Absorption decreases with increasing of λ for all prepared samples. Also,It can be observed that the Absorption increases with increasing of laser energy. There is a sharp fall in the absorbance of the films prepared at higher energy, which is due to enhancing thin films crystallinity.

Optical Energy Gap

The optical energy gap values (E_g^{opt}) for SiC films, deposited on glass substratehave been determined by using Tauc equation. This is used to find the type of the optical transition by plotting the relations $(\alpha h\upsilon)^{1/2}$,$(\alpha h\upsilon)^{1/3}$,$(\alpha h\upsilon)^{2/3}$,and $(\alpha h\upsilon)^{2}$ versus photon energy ($h\upsilon$ and selects the optimum linear part. It is found that the relation for r=1/2 yields linear dependence, which describes all films have allowed direct transition. The optical energy gap (E_g^{opt}) is then determined by the extrapolation of the portion at $(\alpha h\upsilon)^2$=0 as shown in Fig. (5) for different lasing energy. From the above fig. 5, we can observe that the increasing of lasing energy leads to decrease the optical band gap from 3.2 eV to 2.8 eV at RT. This result is consistent with previous researches[10]. the increasing lasing energy lead to decreased

levels of localized near valence band (decrease tails in the optical energy gap) as a result of crystallinety enhancement cause to decrease the optical band gap[10]. Table (3) shows the optical constants for pure SnO_2, In_2O_3 and their composite thin films on glass substrates with different ratio and annealing temperature at λ=500 nm and the energy gap values for these samples. In order to determine the performance of a solar cell device, as well as its electrical behavior, current-voltage (I-V) measurements was performed.

The I-V characteristics of SiC/p-Si solar cell prepared at different laser energy, under different illumination powers (30, 40, and 50) mW/cm^2 prepared at different laser energies (700, 800, 900 and 1000)mJ with the forward applied voltage were shown in Figs. (6). from this figure, it can be seen that the photocurrent density increases with increasing of the bias voltage, due decreasing the depletion region width with increasing of the applied forward voltage, which leads to increase the photocurrent density. Also the photocurrent density increases with increasing the used laser energy due to enhance the films crystallinety and reducing the grain boundaries which leads to the increase of the mobility and increase the photocurrent density. The solar cells parameters such as (V_o, I_{sc}, I_m, V_m, FF and the solar cell efficiency were calculated from these figures as shown in Table (4). This table illustrates that the optimum condition with highest efficiency.

CONCLUSION

we have successfully prepared SiC thin films by Pulse laser deposition technique using different lasing energy.All samples are showed in XRD image have amorphous structure goes to poly crystalline when annealing up to 400^{o}C. All sample have granular structure and higher surface area as shown in the AFM images. The grains sizes are decrease with increasing lasing energy. The I-V characteristics of Si/p-Si solar cell that the photocurrent density increases with increasing of the bias voltage and we can fabricate the higher efficiency solar cell.

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Table 1. Structural parameters: Inter-planar spacing, crystalline size of SiC thin films prepared at different laser energy.

Energy (mJ)	2θ (Deg.)	FWHM (Deg.)	d_{hkl} Exp.(Å)	G.S (nm)	d_{hkl} Std.(Å)	Phase	hkl	card No.
600	Amorphous							
	35.6100	0.970	2.5191	8.6	2.5082	Cub.	(111)	96-900-8857
700	41.4200	0.930	2.1782	9.1	2.1722	Cub.	(200)	96-900-8857
	60.1000	0.800	1.5383	11.5	1.5360	Cub.	(220)	96-900-8857
	35.6500	0.950	2.5164	8.8	2.5082	Cub.	(111)	96-900-8857
800	41.4800	0.910	2.1752	9.3	2.1722	Cub.	(200)	96-900-8857
	60.1500	0.690	1.5371	13.3	1.5360	Cub.	(220)	96-900-8857
	35.7000	0.890	2.5130	9.4	2.5082	Cub.	(111)	96-900-8857
900	41.5000	0.870	2.1742	9.8	2.1722	Cub.	(200)	96-900-8857
	60.1400	0.600	1.5373	15.3	1.5360	Cub.	(220)	96-900-8857

Table 2. AFM parameters (Average Diameter, RMS roughness and Roughness average) for SiC thin films prepared by PLD with different pulses and laser energies

Laser energy (mJ)	No. of pulse	Average Diameter (nm)	Roughness Ave.(nm)	RMS roughness (nm)
800	800	62.50	15.1	12.9
800	1500	84.41	4.56	3.91
1000	1500	73.48	4.36	3.56

Table 3. Optical constants at λ=500 nm and $E_g^{Opt.}$ for SiC thin films prepred at different energy

E (mJ)	T%	α (cm^{-1})	K	n	ε_r	ε_i	Eg (eV)
600	74.33	4944	0.020	1.971	3.886	0.078	3.20
700	35.97	17042	0.068	2.969	8.813	0.403	3.20
800	22.76	24673	0.098	3.240	10.487	0.636	3.10
900	10.34	37827	0.151	3.283	10.752	0.989	2.80

Table 4. The solar cells parameters

Laser energy (mJ)	Light intensity mW/cm²	I_{sc} (mA)	I_m (mA)	V_{oc} (V)	V_m(v)	F.F	η%
700	30.00	2.70	1.60	0.48	0.28	0.35	1.49
	40.00	4.00	2.30	0.54	0.34	0.36	1.96
	50.00	5.00	3.00	0.60	0.38	0.38	2.28
800	30.00	3.40	2.60	0.52	0.31	0.46	2.69
	40.00	5.20	3.60	0.57	0.36	0.44	3.24
	50.00	6.60	4.80	0.62	0.36	0.42	3.46
900	30.00	1.70	1.00	0.30	0.20	0.39	0.67
	40.00	2.70	1.40	0.40	0.25	0.32	0.88

	50.00	3.50	1.52	0.47	0.28	0.26	0.85
1000	30.00	0.80	0.50	0.20	0.10	0.31	0.17
	40.00	1.50	0.90	0.30	0.14	0.28	0.32
	50.00	2.00	1.10	0.38	0.22	0.32	0.48

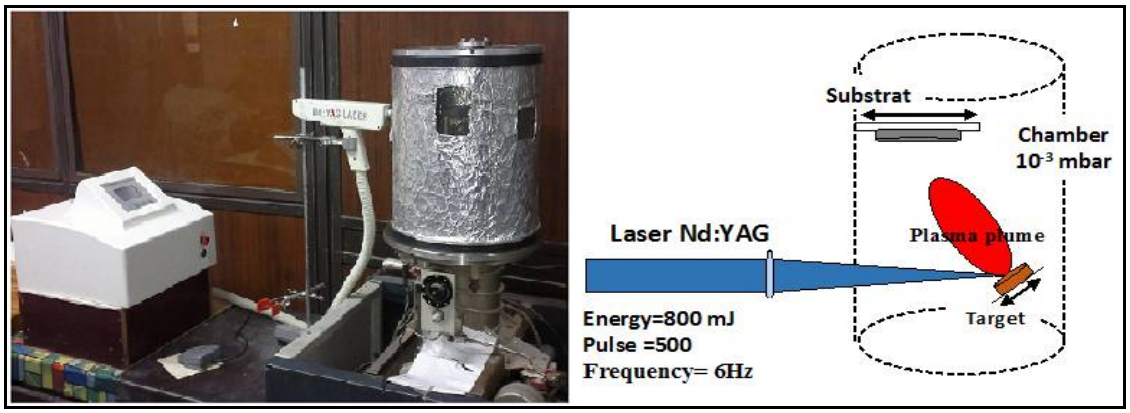

Figure 1. PLD using Nd:YAG .

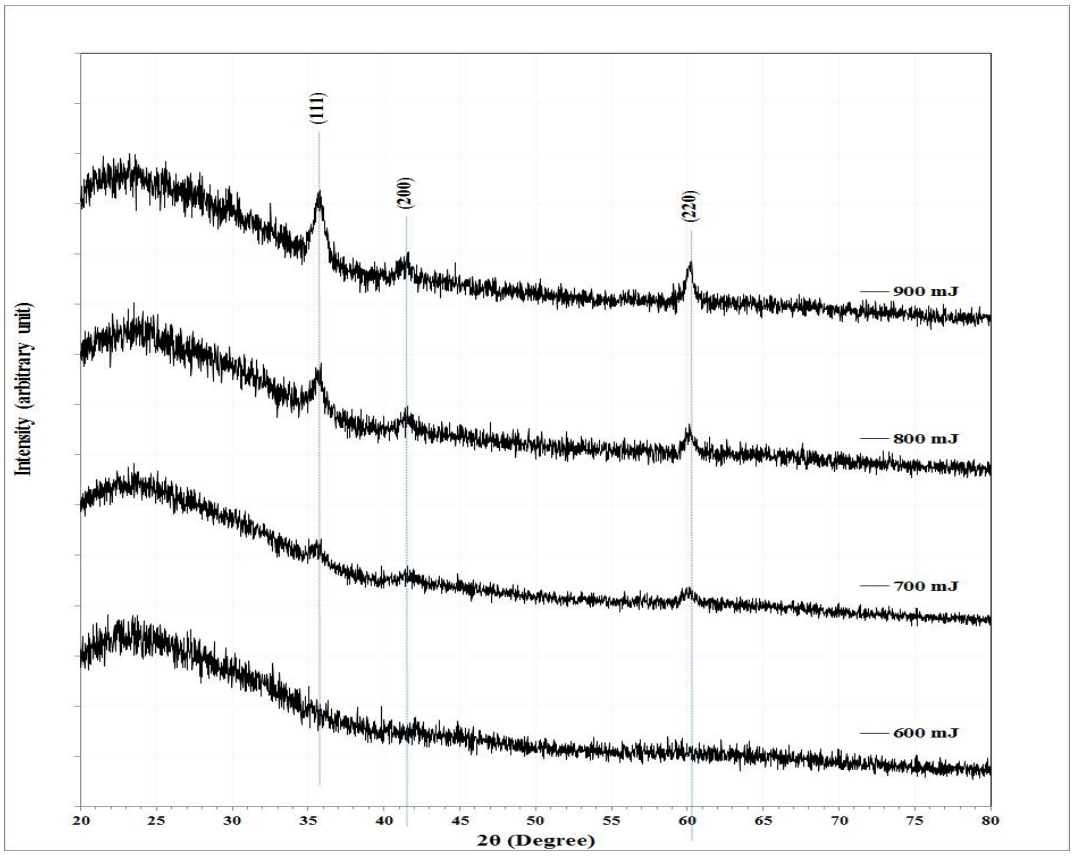

Figure 2. X-ray diffraction patterns of SiC thin films prepared at different laser energy.

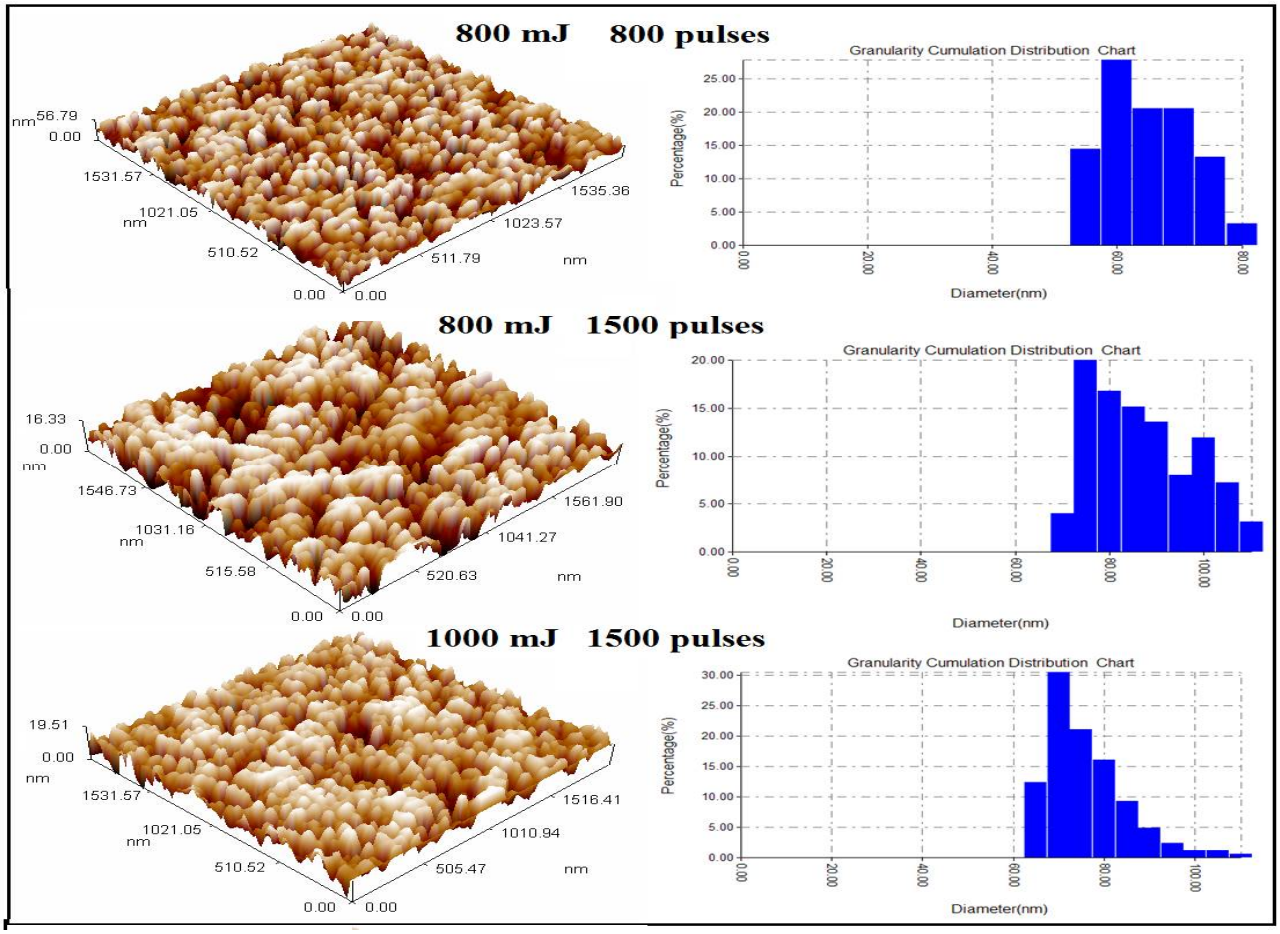

Figure. 3. 3D AFM image and their granularity accumulation distribution for SiC thin films prepared by PLD with different pulses and laser energy

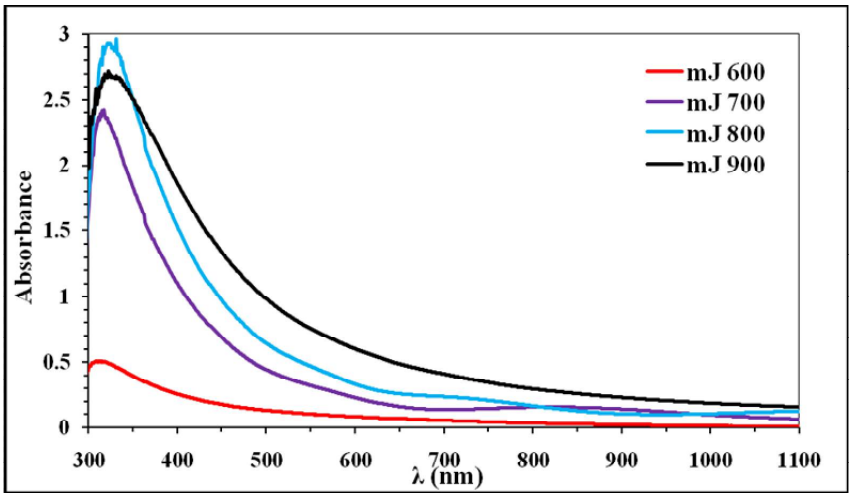

Figure 4. The variation of absorption with wavelength for SiC thin films prepared on glass slides by pulse laser with different energy

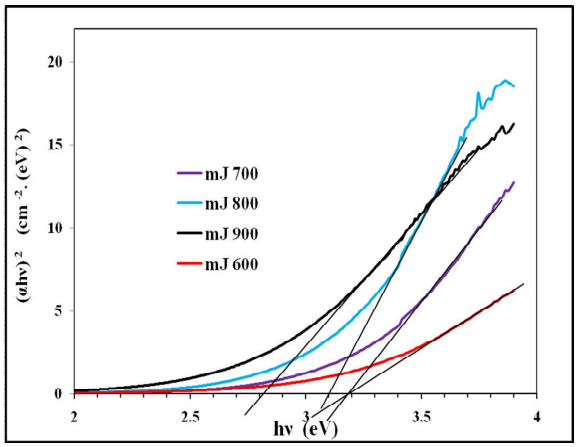

Figure 5. The variation of $(\alpha h\upsilon)^2$ versus photon energy $(h\upsilon)$ for SiC thin films prepared on glass slides by pulse laser with different energy

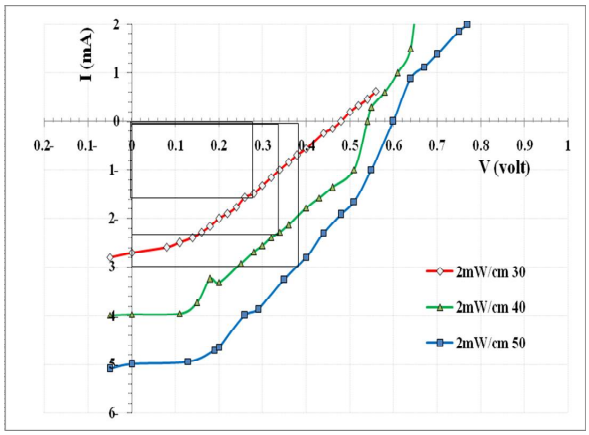

Figure 6. I-V characteristics for SiC/p-Si heterojunction Solar Cell prepared at 700 mJ laser energy at different falling intensity

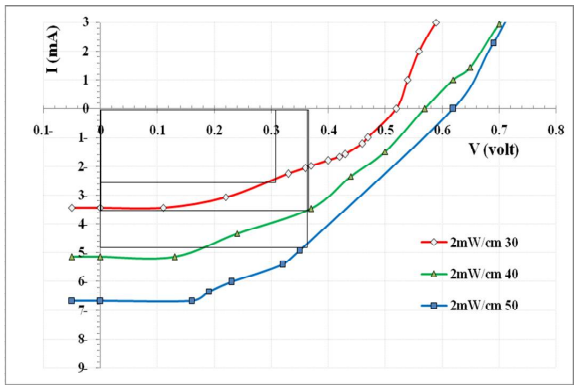

Figure 7. I-V characteristics for SiC/p-Si heterojunction Solar Cell prepared at 800 mJ laser energy at different falling intensity

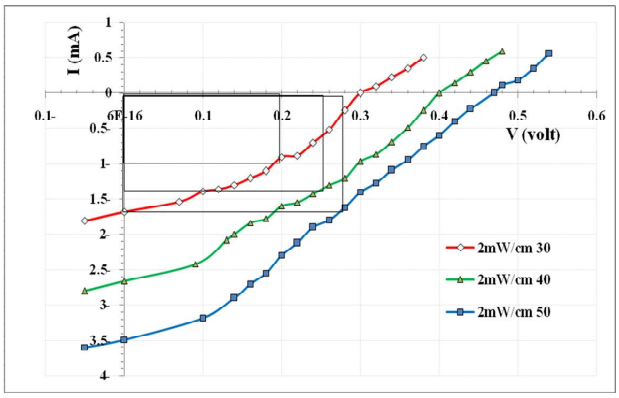

Figure 8: I-V characteristics for SiC/p-Si heterojunction Solar Cell prepared at 900 mJ laser energy at different falling intensity

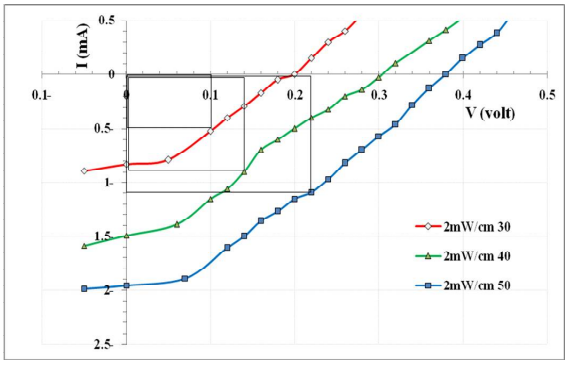

Figure 9. I-V characteristics for SiC/p-Si heterojunction Solar Cell prepared at 1000 mJ laser energy at different falling intensity

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RESEARCH ARTICLE

Gender Performativity in *How to Get Filthy Rich in Rising Asia*: Converging Literary and Linguistic Perspective

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ABSTRACT

The present research paper aims at the gender representation in Mohsin Hamid's novel *How to Get Filthy Rich in Rising Asia* with an effort to converge literary and linguistic perspective about gender. The study focuses on the stylistic analysis of the said novel by taking into account the category of adjectives at lexical level as suggested by Leech and Short (1981). The study of adjectives is supplemented with the notion of gender performativity by Butler (1990). The analysis of adjectives and their correlation with Butler's performativity delineates, differentiates and elaborates men and women to be sexually distinct as well as socially discrete genders entrenched in the cultural context of Asia. Men and women perform their specific gender roles through repetitive social acts, not by choice but as endorsed upon them by their being a part of a collective thought ruling over the individuals. Thus, the research at hand presents this performative aspect of gender through lexical choices of adjectives opted by the author forming a helpfulstudy for the researchers seeking to approachgender issues in literary texts via linguistic clues and evidences.

Keywords: Gender, adjectives, performativity.

INTRODUCTION

The debate about patriarchy, gender discrimination and oppression is spread far and wide, historically as well as geographically. The biological and social construction of gender identities and their rendering through discursive practices is a much lovedchoicefor inquiry for the social scientists and literary and linguistic researchers.Feminist literary theory is a diverse array of ideas on women oppression and its representation in literary texts. Such a profound existence of gender specific discourses have enabled myriads of inquiries in this domain with a claim that these writings have played a significant role in breaking stereotypes about gender and educating the recipients of

these texts towards gender esteem and equal rights. In fiction, representation of women by male and female writers has always innately presented the specific cultural demands from a female and a male in order to successfully fit into the social fabric where they are born and destined to live. Here, *female*is deliberately mentioned prior to *male*, of course breaking the normal discursive pattern, since it is a female who is often suppressed under the pressure of prescribed performative acts as compared to her male counterpart. The world of *Joseph Andrews* by Henry Fielding adores Fanny as a lovable character as she retains all the qualities, shy and submissive as she is. Elizabeth Bennett of Jane Austen is admired by the readers but her mother always has a special liking for the beauty of Jane Bennett and the cleverness of Lydia Bennett. Maggie Tulliver of George Eliot has to face criticism from her society because she did not act in accordance with the traditional tent around her. And there are many comparative instances of this kind in modern literature as well.

Hamid's novel guides us to the fact that a peculiar stylistic feature of his work is the choice of very specific lexical items for there presentation of men and women as sexually distinct and performing socially discrete gender roles. Adjectives used for the genders from the perspective of the narrator and that of characters minister to the readers and critics to form an idea of how specific gender roles are knitted in the very fabric of rising Asia. *How to Get Filthy Rich in Rising Asia*is a tale of an anonymous protagonist who is born in a rural setting in an unnamed country of rising Asia and then, with his migration to the city with his family, he steps into the economic struggle through his job as a CD seller and ends as a great businessman. His personal and business affairs are shown simultaneously in the novel. His relation with his mother, his sister, his beloved and his wife helps in understanding gender relations in the context of Eastern world. He dies like all, but his life is a wonderful tale of struggle which is the lot of so many if they want to become filthy rich in rising Asia.

Purpose of the Study

The purpose of the study is to stylistically analyse *How to Get Filthy Rich in Rising Asia* from gender perspective. In order to reinforce the idea of gender discrimination and culture specific gender roles, the text is analysed from both linguistic and literary perspectives. The analysis of the text is evident of the fact that gender representation is not only an important topic in fiction but more significant is the call for reading these texts in order to instruct the society to give space to men and women to perform their roles as both are intrinsic elements in turning the wheel of progress for any nation.

Significance of the Study

The interest in this study is retained by unifying linguistic analysis of adjectives with the critical analysis of gender roles in the light of ideas about sex and gender as presented by Judith Butler in her works, most importantly, the notion of performance elaborately addressed in her work *Gender Trouble*. In this way, effort is made to unite linguistic and literary perspective about gender and thus the gap between the two is bridged up through the research at hand. The research on Pakistani writers also helps to portray our values and norms to the rest of the world.

Population and Sample of the Study

The novel *How to Get Filthy Rich in Rising Asia* is selected for the study of adjectives that represent gender. The purposive sampling is used to select gender specific use of adjectives which are then discussed through the perform activity aspect.

Delimitation of the Study

The present study is limited in the following ways:

i. It takes into account only one novel by Mohsin Hamid, *How to Get Filthy Rich in Rising Asia.*
ii. The text is analysed on the basis of lexical category of adjectives only. The adjectives are also chosen on the basis of their use with male and female characters in the novel.
iii. The idea of gender performance by Judith Butler is only selected for data analysis and discussion in this research paper.

Research Questions

The present research seeks to answer the following research questions:

i. What kind of adjectives are used to represent men and women?
ii. Are these adjectives stereotypical or deviate from traditional gender percepts?
iii. How does Judith Butler's notion of performative acts of gender apply to the text?

Literature Review

The origin of Stylistics is attributed to two distinct and inter-related areas: linguistics and literary criticism. Stylistics can stand for both linguistic and literary criticism, which depends upon how one perceives and employs it (Short, 1997). Stylistics serves as the area where linguistics and critical exegesis overlap. Hence, Literary Stylistics is a podium where linguistic and literary interpretations may simultaneously be generated out of a text. Widdowson (1975) while considering Stylistics as a linking bridge between Literature and Linguistics studies literary discourse from a linguistic perspective. Widdowson (1975:4) has shown this relationship in the following figure 1 :

According to this relationship established between Linguistics and Literary Criticism through Stylistics, one can easily deduce the pedagogical implications of Stylistics providing grounds for the progression of a pupil from either language or literature towards either Literary Criticism or Linguistics. Style is a lexical and grammatical feature in a text that escorts the analyst to the meaning of the text. Fowler (1986) recognizes style as the standard feature of a language as well as its facet. An equivalent idea is maintained by Toolan (1998) where he interprets stylistics as the study of language in literature.The use and choice of certain lexical items and their repetition using specific syntactic structures and employing various literary devices are the interest areas of stylistic analyst to probe into the relation between author's choice of language and its function. These choices depend upon some influential factors like age, gender, education, social, cultural, regional background as well as idiosyncratic preferences etc. (Missikova, 2003). Fischer (2010) bases his idea of stylistic interpretation on the fact that meaning as a linguistic phenomenon gets decoded via linguistic analysis.

The study of fiction from gender perspective is not a novel topic. Many researchers have highlighted this issue in their works. Wollstonecraft was objectedwhen she boldly presented her ideas about women degradation and her strong perspective about the equal status and recognition of women along with men (Janes, 2017).Spivak (1988) addresses the theme of the west and the rest as superior and inferior respectively. The same idea is of great significance in studying the dominant position of men in relation to women which is vehemently presented in literature as well as through actual human actions in the real world. Beauvoir (1989) while evaluating the status of woman compares her with an object of beauty merely to please and entertain the rest. She is of the view that a woman is not born a woman but her society polishes her to assume this gender and her behavior is marked by the standards set by the powerful male strata of her society. Millet(2000)talks about the passive and ignorant view about women in literary writings and, in contrast, the powerful and dominating image of men.Kristeva, as cited in McAfee (2004), highlights the importance of language in creating beings who exhibit their cultural norms and identities with the use of spoken and written language.

Following the above idea about gender, the present research sets the stage for the application of Butler's notion of gender performance on the study of stereotypical and deviant roles of men and women as determined by their specific actsin the fictional setting structured by one of the influential Pakistani American writer, Mohsin Hamid.

MATERIALS AND METHODS

The text of the novel is subjected to close reading in order to locate adjectives used with male and female characters are extracted through purposive sampling. Their frequency of occurrence in the text is noted. The performative acts and discourse chunks are isolated and noted in order to discuss the gender specific stance in this paper. The selection of adjectives is based on the model of Leech and Short (1981)."A: Lexical categories 3. ADJECTIVES. Are the adjectives frequent? To what kinds of attributes do adjectives refer? physical? psychological? visual? auditory? color? referential? emotive? Evaluative?etc. Are adjectives restrictive or non-restrictive? Gradable or non-gradable? Attributive or predicative?" (p. 76).The adjectives in the text are carefully categorized and fit into the blocks suggested above by Leech and Short. The adjectives are further divided into categories of representation of stereotypes, social esteem, mutual relation between genders and antagonism. The data is then analysed in the light of gender performance by Butler (1990). She talks about heterosexual matrix which is a scale upon which culture plays its part to differentiate and define men as being different from women (Butler, 1990). The acts performed by men and women become discrete and naturalized through repetition and become the defining identity of each gender. Thus one is not born or becomes a man or a woman rather the repetitive speech acts and social performances measure masculine or feminine roles of individuals in society. The male and the female characters in the novels are studied in the light of these ideas about gender.

ANALYSIS AND DISCUSSION

Representation of Gender through Adjectives

In Hamid's novel, a peculiar stylistic feature is the choice of specific lexical items for differentiating men and women on the basis of their repetitive acts which rendering them sexually distinct and socially discrete genders in the cultural context of Asia. Adjectives used for the genders from the perspective of the narrator and that of characters minister to the readers and critics to form an idea of how specific gender roles are knitted in the very fabric of rising Asia. Following issues of gender roles are brought to the forefront after sorting out the category of adjectives used for men and women in the text.

Social Position and Esteem of Men and Women

A chain of Asian authors, especially Indian and Pakistani fiction writers in English, hold a special interest for the issue of gender and social esteem linked with men and women in their society. A general stance in Asian society is that the position of women is to undertake responsibility to protect and preserve what is called the home- an inner hub of the national culture and spiritual essence. Concomitantly, men are considered responsible to learn superior tactics of systematizing material life and rising socially for the protection of the same inner hub. Taking this primary notion in view, Hamid's choice of adjectives to differentiate men and women on the basis of social position and esteem in Asian context appears very apt and relevant. These choices perform the function of differentiating men and women in the very context of the fictional world crafted by the narrator. As an illustration of this differentiation in the social roles of genders, following extract from the novel can serve as evidence:

"She [mother] squats as your father is likely squatting, *handle-less* broom in her hand instead of a sickle, her *sweep-sweep* waddle approximating his own movements. Squatting is *energy efficient*, *better* for the back and hence *ergonomic*,

and it is not *painful*. But done for hours and days and weeks and years its *mild* discomfort echoes in the mind like the *muffled* screams from a *subterranean torture* chamber". (p.8, 9)

Here, it can be observed that gender identity is marked and determined by sustained social performances (Butler, 1990). The males and the females are judged on the basis of the efficiently performed roles which are naturalized and have got knitted in the cultural fabric of a society. Adjectives form an endearingly fine picture of the domestic duties of a woman and what feelings emerge out of delivering such responsibilities. In order to understand this point, a look at the table no. 1 deems significant where adjectives showing this difference have been fore grounded.

Through this tabular presentation of adjectives used with men and women in the novel, it becomes apparent that whereas men are presented with positive physical and mental traits, women are represented mostly on the basis of their emotional state of being. The character of the sister of protagonist is an instance to illustrate this point of view:

'Lately she alternates with alarming frequency between suppressed but globular tears and calm airs of smug superiority'. p.24

The adjectives representing women are emotive and psychological in nature more than physical ones. The reason is that woman as the softer sex is considered as the seat of emotions but, interestingly, it is shown that such emotions are adding strength to female characters rather than reducing them to weaker entities as compared with the male characters. Thus, we can also observe deviation from the stereotypical notions about gender.

'She [sister] does something you associate with women of girth and substance, not with slender slips of girls like your sister. She sings in a quiet and powerful voice'.p.26

Contextually, she is weak and miserable but adjective choices show that amidst hardships and shattered hopes and aspirations, the sister of protagonist still shines with the power of endurance and patience in odd circumstances. Same can be inferred from the representation of the characters of mother who fights courageously with cancer, wife of protagonist who remains successful as a well educated and self sufficient woman even after divorce and pretty girl who keeps whirling in the ocean of struggle to become famous till death puts an end to her life-long toil.

'She [mother] is perplexed by her wounded status, like a soldier who has been shot but as such sees no blood'. p.66

It can further be deduced from the study of the physical portrayal of women in the novel that Hamid deviates from the traditional norm of associating extraordinary beauty and exaggerating physical charms of women in his novel. Since the focal point of author in the novel is the position of individuals in a developing world of Asia so he deals most of his characters in the light of their goals, aspirations and actions rather than their physical charms and exotic appearances. Look at the portrayal of a major woman character in the novel, that is, pretty girl:

'*Her looks would not traditionally have been considered beautiful. No milky complexion, raven tresses, bountiful bosom, or soft, moon-like face for her. Her skin is darker than average, her hair and eyes lighter, making all three features a strikingly similar shade of brown'. p.38*

'I know. You [pretty girl] wanted to leave the neighbourhood and now you've done it. You're famous'. p.87

This female character is neither an archetypal nurturer nor a damsel in distress rather, she is a crusader and an independent woman who knows how to chalk out her own destiny, though how lucrative or filthy it may be. She can be contrasted with the mother and sister of the protagonist who are presented in somewhat traditional light. When she is exposed to the world of men she has 'jaunty' strut (p. 38) that gives a hint of the pleasure that she takes in vanity but when a man gawks at the sister of pretty girl, one can observe her 'altered' gaze (p. 28) exhibiting the shyness of a simple rural girl for whom this masculine stare is a matter of embarrassment and disrespect.

The adjective choices show that the central motif is neither beauty nor emotions but the labor to perk up from scratch to a sound social standing in rising Asia. Most of the characters deviate from the traditional gender polarization that defines categories of human beings into males and females while also devising their social roles, esteem and nature of socialization. Such polarization creates a barrier to attain an esteemed position in the socio-economic hub. But characters in this novel are unique in this regard because irrespective of their genders, they are all striving for a rise in their social and economic stipulation which is shown as a dream of most of the youngsters in a developing country like Pakistan.

Relationships within and across Genders

The study of adjectives shows that their function is multi dimensional when it comes to defining gender roles. Not only do they differentiate genders biologically and socially, but also the relationship within the same gender and across them is construed on the basis of adjectives. It means that study of adjectives may be very fascinating and exciting to wade through the nature of human relationships and social contacts. In this regard, the first evidence is the nature of relationship between two women, protagonist's mother and his paternal grandmother.

Your mother and grandmother play a waiting game. The older woman waits for the younger woman to age, the younger woman waits for the older woman to die. p. 9
In an all-female society your mother would likely rise to be queen, a bloody staff in her hand and crushed skulls beneath her feet. p.9, 10

The author's pick of adjectives for the portrayal of the identity constructions of a mother-in-law and daughter-in-law seems very relevant which can be illustrated with the help of the following table.

The adjectives mentioned in the table show a traditional association of negative attributes to a daughter-in-law by her mother-in-law. This implies that how adjectives create a pen picture of human relations.The relation between brother and sister is also constructed through specific lexical choices by the narrator which adds realism and honesty of portraying characters enwrapped in social and traditional cloaks.

'[…] your faces small ovals of the familiar in an otherwise unrecognizable world'. p.15
'[…] that she seeks you out not to comfort you, but rather for the comfort that you, her only recently recovered little brother, have in this moment of fragile vulnerability the capacity to offer her'. p.15

When gender identity and relationship is linked with the question of dominance and power, one can argue that it is the common sense of any community which gives a naturalized notion of gender roles in society converging at the point where no force or effort is needed to question that traditional role. That is why, despite being elder, the sister of the protagonist depends upon him for emotional support and strength in an unrecognizable world. Her character also reveals a desire of relatedness and belonging in a social world that is ghastly replacing genuine human relations with artificiality and materialism.The traditional viewpoint can be applied on other relationships presented in the novel. The mother and father of the protagonist typically represent the common perception of parents towards male and female children.

He says to your mother, "He's a strong child. This one" She says, "He's very strong". p.11
She demonstrated more enthusiasm for education in her few months in a classroom than your brother did in his several years… but your sister will not be sent there in his stead. p.28

The preference of strength and education given to a male child is very appropriately projected. The notion of a very young lady and her early marriage is embedded in the base of the cultural belt of countries like Pakistan and India in Asia. But we cannot call Hamid's choice of adjectives and projection of such a scenario as almost rigid and harsh.

Since the setting of the novel is rising Asia, hence, it remains an honest endeavor on the part of the author to capture the realistic image of norms, values and manners of the habitat of Asia. Lexical choices also construct the nature of the relationship between husband and wife through the character of the unnamed protagonist and his wife. It may be stated that Hamid has captured the spirit of this relationship by adding a third character and affixing the adjective 'other' to convey the real motif of her existence in the life of the protagonist. In order to form an idea of how two female characters are represented in the context, let us have a look at the following table showing differences of the use of adjectives for the difference of association of the protagonist with wife and pretty girl:

The adjectives differentiating wife and pretty girl in the first row show that the relationship enjoyed by the protagonist and pretty girl is romantically associated with the derivation of physical ecstasy promised in such contact. Whenever they meet, the description of the surroundings also reinforces the physical nature of their association. They used to live in *adjacent* streets and working in the *same* market (p. 41). The choice of the adjectives 'adjacent' and 'same' shows that the function of their use is to measure the probability of a relationship that can be established on common grounds. They had a number of *unspeaking* encounters (p. 53) in *dark* and dimly lit street and over the roof top and it is *starless* sky whenever they happen to see each other. This pattern of the use of adjectives suggests the physical aspect of this romantic contact where the bosom of love remains mainly entrapped into the bodily charms and pleasures. And by the time the protagonist gets married the pretty girl has assumed the status of an *archetypal* woman and a *living* memory in the life of the protagonist and her *languid* voice offers him some *wonderful* moments during which everything around him seems *less substantial*. The adjectives just used show the scale of their use by the narrator. In this instance, it can be observed that the growth of a relationship can be very emphatically presented through the use of adjectives. The pretty girl was an object of physical pleasure in the beginning but as the age and novel proceeds, the romantic version of love is modified into an eternal and immortal vision that remains with a person with or without the presence of the object of love.

The use of archetypal is adopted to show the feelings of a lover towards his beloved. Otherwise, the pretty girl fits only into the mould of the progressive vision of a woman but when one has to define the archetypal social esteem and associations of a woman, the character of the pretty girl hardly glues in that arrangement.A look at the adjectives used for the wife of the protagonist leads us to a number of other interpretations of their choice. From the above stated discussion on the nature of relation between the hero and his beloved, one cannot jump to the conclusion that Hamid has associated positive tone with that affair and marriage institution is blotted in his work. Marriage is esteemed to be a very sacred institution in the social world of Asia. It gives completion, security and strength to a pair joined in matrimonial bliss but it is assumed to offer more prerogatives to women than men. Keeping in view this primary notion, let us contrast the characters of the pretty girl and the wife of the protagonist on the basis of the lexical choices used distinctively for both of them as stated in the Table 3. The presence of the pretty girl in the life of the unnamed leading character affects the happiness tagged with married life. Because of her, he feels uninterested towards his own wife and their relationship remains flickering and it is a kind of burden borne by both him and his wife. Despite this, he calls his wife wondrous and she is presented with the positive attributes as shown in the Table 3. The positive adjectives like *indefatigable* [determination], *striking* [maturing beauty], *relative* [prosperity], *influential* [position], *pertinent* [advantage], *evident* [fearlessness], *self-sufficient* [fire], *disarming* [warmth] and *fortunate* are all used to given vivacity to the character of a wife. No adjective overshadows a negative trait embedded in her character. It can be generalized that a wife is held in high respect and esteem and she is considered a sacred being in traditional terms. On the other hand, a woman having an affair outside marital bond is seen with a doubtful eye and she can never enjoy the respect given to a wife in the sacred boundary of home. The pretty girl was called 'slut' by the mother of the hero. Her relationship with him might have been exciting 'breath-halting' feelings in the hero but at the same time he is shown to regret the possibility that could have made his married life successful had he not denied due share of love to his wife:

You shut your eyes, briefly seized by a strange regret, maybe for the delays to this project, or for the state of your marriage, or for becoming so late a father to your son, for being, in all likelihood, destined to overlap too limitedly with the span of his life. p.156

Though there is no sense of reproach for having a life-long relation with pretty girl, but a failed marriage is painful even in such a triangle. Such description reveals that though pretty girl enjoys the pleasure of being loved but she is denied the respect with which the society embellishes a wife. So we can say that tradition dominates in the portrayal of human relationships through adjectives.Another point of discussion emerges from the use of 'other [woman]' for the pretty girl. Though the debate on 'us' and 'them' or 'I' and 'other' is related with the postcolonial study of the developing and the developed nations but the concept can be borrowed for the present function in the context of this novel. 'Us' is a stronger body and occupies a position of dominance whereas 'other' pertains to a weaker entity in a subordinate position who lacks many privileges conferred upon the former one. That is why the weaker position of a woman is represented with lexical choices of 'them, other or subaltern'. But in the present case the distinction of us and other is to be made between two women, and not between men and women. The pretty girl leaves her home in order to get filthy famous in rising Asia but her foot as it crosses the threshold of her house closes door of any prospect of a promising domestic future and that is what the author shows through choice of adjective 'other' for her. She dominates the life of the anonymous hero but she could not entitle herself to any badge of honour and respect that is given to a wife.

Gender and Antagonistic Adjectives

Another aspect of the function of adjectives representing gender is their use to present antithetical ideas. The reason for the use of these antithetical adjectives is creating a sense of relativity of reality which is never absolute. Additionally, the choice of placing adjectives as opposites add a sense of obscurity resulting in enabling the readers to choose for themselves the possible meaning out of a situation projected in the novel. It may be noted that the author has used the contradictory adjectives while showing gender contact. The vague air of doubt engulfs the members of one gender when they try to establish an untraditional contact with a member of the other gender. In addition to this, the emotional state of a character, whether male or female, is represented via adjectives used in antithetical ways.Following is a list of adjectives used antithetically while describing characters and their feelings.

The pretty girl seems to the protagonist a woman who is familiar and unfamiliar at the same time, familiar as he enjoys an intimacy with her despite long pauses in between their occasional meetings and unfamiliar because that strange woman loves him but is not inclined to have a life-long matrimonial bond with him. That is why this obscurity of their relationship leaves them in a fix and this leads to meetings ending with state of happiness as well as fear and that of being surprised and unsurprised.The emotional state of characters is also embedded when the author uses antithetical adjectives. Students watch their teacher in horrified great fascination when he punishes the brightest student. The sister of the protagonist suppresses her tears when her fate is decided by her parents but these tears appear as globules. The hero meets his son after a couple of years and this warm reunion is both tearful and affectionate with a ferocious yet protective hug (p. 222). In this way the use of antithetical adjectives seems apt and relevant adding variety to the derivation of connotative meaning from the lexical choices at hand. Thus, Bulter's notion is successfully applied and inferred in the above stated discussion.

CONCLUSION

From the details discussed in the above section, it may be concluded that adjectives play an efficient role in describing the gender identity, differences and the specific roles assigned to each gender and their study can be very fascinating in order to form an idea of the style of an author's way of presenting gender in his work. The answers to the three research questions are sought in this paper. The first question was about the choice of adjectives for men and women characters in the novel. It is shown in the discussion section how a writer attributes gender roles to male and female characters by a distinct choice of adjectives for both. The second question revolved around the stereotypical or divergent gender specific roles of characters in the novel. It may be inferred that Hamid's novel gives a balanced view of performativity as we have observed how he sometimes deviates from the representation of

naturalized gender roles of women. The third question addressed Judith Butler's ideas about performative acts of gender. Here it may be concluded that both men and women are determined as two distinct social entities on the basis of their actions creating a heterosexual matrix in which goal, expectation and duties get distributed between the two according to the culturally and socially accepted norms which serve as a scale to gauge gender roles in the society.

The research is significant for the future researchers to study gender roles in the novels, and also for the different dimensions of research in literary stylistics. It is also an effort to blend and converge literary and linguistic perspective for the study of a literary text and the gap between two areas, that is, literature and linguistics for the study of fiction is bridged up and it may continue in the future endeavors that follow.

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Table 1. Adjectives representing Men and Women

Sr. No.	Men	Women
1.	Tall	Younger
2.	Good	Older
3.	Young	Jaunty (Strut)
4.	Strong	Fragile (Vulnerability)
5.	Lucky	Suppressed (tears)
6.	Surviving	Smug
7.	Important	Muffled
8.	Most loyal	Unmitigated (agony)
9.	Adorable	Baffled
10.	Voracious	Wondrous

Table 2. Adjectives showing the perspective of Mother-in-Law

Sr. No.	Adjectives
1.	Vain
2.	Arrogant
3.	Headstrong
4.	Unquenchable (disapproval)

Table 3. Relationship of Protagonist with Wife and Pretty Girl

Sr. No	Wife	Pretty Girl
1.	Unpleased, flickering, fraught (p.130)	Flushed, relaxed glibness (p.110)
2.	Wondrous, indefatigable (p.148)	Archetypal woman (p. 135)
3.	Charitable, religious (p.167)	Other woman (p. 147)

Table 4. Antithetical Adjectives

Sr. No.	Antithetical Adjectives	Characters
1.	Horrified, great fascination	Students
2.	Skinny and strong	Protagonist
3.	Familiar and unfamiliar	Pretty girl
4.	Happy and afraid	Protagonist/Pretty Girl
5.	Suppressed and globular tears	Sister
6.	Tearful and affectionate Reunion	Protagonist/Son
7.	Surprised and unsurprised	Protagonist/Pretty girl

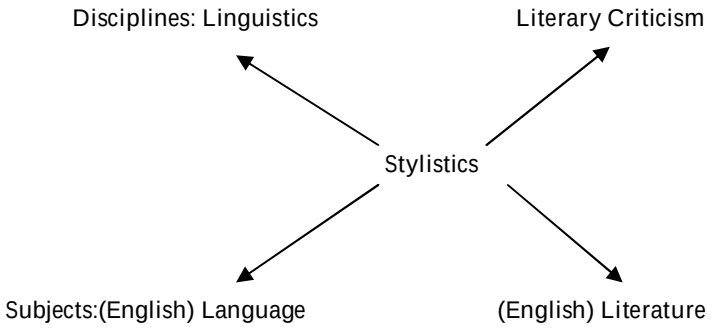

Figure 1.Widdowson's Perspective

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RESEARCH ARTICLE

Characterization of Copper Oxide Nanoparticles Prepared by Hydrothermal Method for Antibacterial Effect

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ABSTRACT

Copper oxide nanoparticles were synthesized by hydrothermal method, and characterized by x-ray diffraction, TEM, UV-Vis and FT-IR techniques. X-ray diffraction result confirmed the single-phase formation. The particles sizes were observed to be (11) nm. FT-IR spectra exhibited vibrational mode (500) cm^{-1} were assigned for cu-o stretching vibration. According to UV-Vis spectrum, the band were observed at (450) nm. Antibacterial activity of CuO nanoparticles were tested against bacteria strains (*Klebsiella*, *Staph.aureus*) using the gar diffusion –well method. Minimum inhibitory concentration (MIC) of these (*Klebsiella*, *Staph.aureus*) bacteria were found to be (123.02 and 144.5) μg/ml respectively.

Keywords: CuO nanoparticles, XRD, FTIR, TEM, UV-visible, Antibacterial activity.

INTRODUCTION

The properties of materials change as their size come close to the nanoscale [1]. CuO nanoparticles are prominent to their diverse applications in superconductors, optical [2], electrical [3] ,Nanofluids [4] , photocatalytic degradation [5], catalytic [6] ,gas sensors and in biosensors [7]. The properties of nanoparticles can easily be altered by reducing or changing their size, especially when the manipulations are done at the nanometer scale [8,9] .Nanoparticles with smaller particle size have been reported to show good antimicrobial activity [10]. Antimicrobial activity of nanoparticles has largely been studied with human pathogenic bacteria such as *Escherichia coil* [11] and *Staphylococcus aureus* [12] .Bacterial activity of such nanoparticles depends on size and concentration in the growth medium, the bacterial population growth can be inhibited by specific nanoparticles interaction [9].In general, bacterial cell size is in the micrometer range, while its outer cellular membranes has pores in the nanometer range. Since nanoparticles can be smaller in size than bacterial pores, they will have a unique ability of crossing the cell membrane. There lies a

strong challenge in preparing metal oxide (CuO) nanomaterial stable enough to restrict bacterial growth significantly while in nutrient medium.

EXPERIMENTAL WORK

Synthesis and characterization

Synthesis

The CuO nanoparticles were prepared by hydrothermal method using equimolar amount (0.1 M) of (CuCl2 .H2O) and hexamine (C6H12N4) dissolved in de-ionized water and transferred in to 50ml Teflon-lined stainless steel vessels. The vessels were tightly sealed and heated to 120◦c for 5 hours; they were, then, slowlycooled at room temperature. Precipitated powders were washed several times using de-ionized water and absolute ethanol .The precipitates were sonicated for 5 min prior to filtering, annealed at 200◦c,300◦c,400 ◦c for 1 hour, cooled to room temperature.

Characterization

The structure of the compounds was investigated using x-ray diffraction (XRD) with cu kα radiation (λ=1.54060 ◦A). Molecular analysis of the sample was performed by Fourier transform infrared (FT-IR) spectroscopy using (Affinty-1 CE) spectrometer recorded in the wave number range of 4000-400cm-1. UV-Vis spectroscopy was used to characterized the optical absorption properties of CuO. The absorption spectra of the sample were recorded in the wave length range of 190-1100nm.

Antibacterial activity of the CuO Nanopowder

Agar diffusion well

The antibacterial activity of the CuO Nanopowder was determined by agar well diffusion method [13] against both Gram-negative and Gram-positive microorganism. Once the medium was solidified, a suspension of each sample of the bacteria was diluted prior to 10^{-1}, $10^{-2}$and 10^{-3} (1ml of 108 cell/ml) and was spread on solid agar medium in petridishes. The wells were prepared by using sterile crockborer (6mm). Each well was filled concentration of Nano material. The plates were incubated at 37◦c for 24 hr. , the zone of inhibition was measured.

Minimum inhibitory concentration (MIC)

The lowest concentration of material that inhibits the growth of an of an organism [14] is defined as the minimum inhibitory concentration (MIC). MIC for metal oxide nanoparticles was determined by the agar diffusion method. A series of 4 test tubes were taken.

RESULTS AND DISCUSSION

XRD studies

XRD patterns of CuO nanoparticles are shown in figure (1). All diffraction peaks of sample correspond to the characteristics mono clinic structure of copper oxide. The peak positions of the samples which was confirmed from

the standard card (JCPDS, card No.05-0661). The average crystallite size (11.2) nm as calculated using Debye Sherrer's formula $D = 0.9\,\lambda/\beta\cos\theta$

FTIR

FTIR Spectroscopy is usefull in measuring the absorption of IR radiation by a sample and the results were shown by means of wavelength. The evaluation of the IR spectrum includes the correlation of the absorption bands (vibrational bands and the chemical compound in the sample [15].The FTIR spectra of CuO Nanopowder shown in figure (2). The CuO nanoparticles exhibited vibrational modes at 500 cm^{-1} were assigned for Cu-O stretching vibration.

UV-visible studies

The optical properties of CuO nanoparticles have been studied by UV-Visible spectrum , which shown in figure(3) UV-Visible spectroscopy is a most widely used technique to investigate the optical properties of the particles.The analysis was done in the range of (400-600) nm.

Transmission electron microscopy (TEM)

Spherical shape of CuO nanoparticles were observed from TEM Image , figure (4).The average size of copper oxide nanoparticles was found to be (11) nm.It was indicate that surfactant molecules form a film over the surface of CuO nanoparticles which prevented the agglomeration of nanoparticles .

Antibacterial activity of the CuO nanoparticles

Agar well diffusion method

The antibacterial activity of the CuO nanoparticles was determined by using agar well diffusion method against (*KlebsieII, Staph.aureus*) bacteria figure (5) shows the inhibition zone measurement with different temperature. Antibacterial activity results revealed that CuO NPs acts as excellent antibacterial agents against both Gram-negative and Gram-positive bacteria further more, previous studies have show that the smaller the CuO particle size, the greater the efficacy in inhibiting the growth of bacteria, however, nanoparticles of CuO were previously reported to act both as bactericidal a gents [16] and bacteriostatic [17] perhaps thereby limiting their biomedical use.

Minimum inhibitory concentration (MIC)

The results showed significant MIC values in table (1).*klebsiella* bacteria showed MIC at (123.02) μg/ml and *Staph.aureus* bacteria showed MIC at (144.5) μg/ml. Determine of the MIC is important in diagnostics laboratories because it helps in confirming resistance of a microorganism to an antimicrobial agents and it monitors the activity of new antibacterial agents.

CONCLUSION

CuO nanoparticles were synthesized by hydrothermal method. CuO nanoparticles with monoclinic structure. The crystallite size and particle size determined was ( ) nm by using XRD .TEM image indicate spherical shape of CuO nanoparticles of (11) nm in diameter. FTIR spectra confirm the presence of metal oxygen bond. Antibactrial characterization has been demonstrated against (*Klebsiella* and *Staph.aureus*)bacteria using the agar well diffusion

assay method. The well diffusion assay and MIC indicate the future potential of CuO nanoparticles for combating pathogenic microorganisms.

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Table 1. Show the MIC

Type of bacteria	**MIC μg/ml**
Klebsiella	123.02
Staph.aureus	144.5

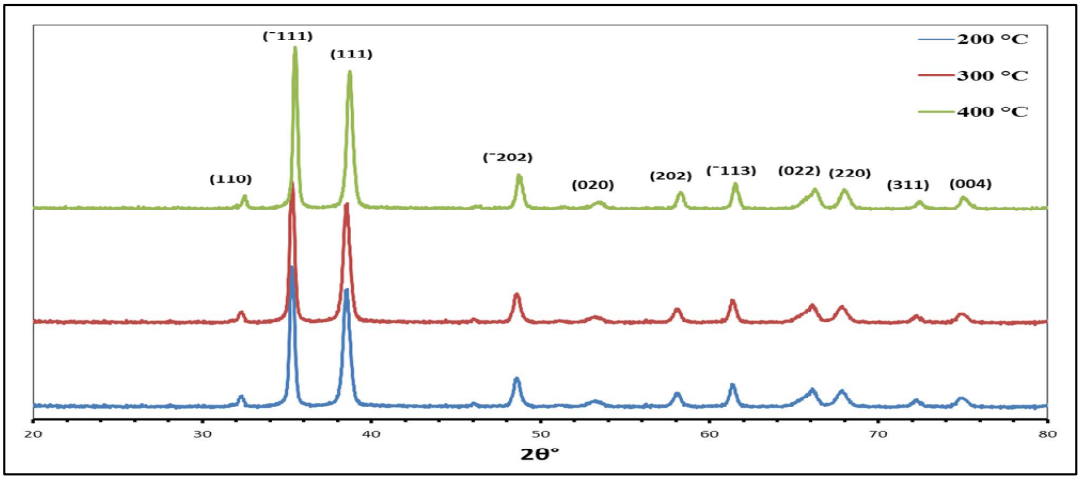


Figure 1. XRD pattern for CuO nanoparticles at different temperature (200,300, 400) °C

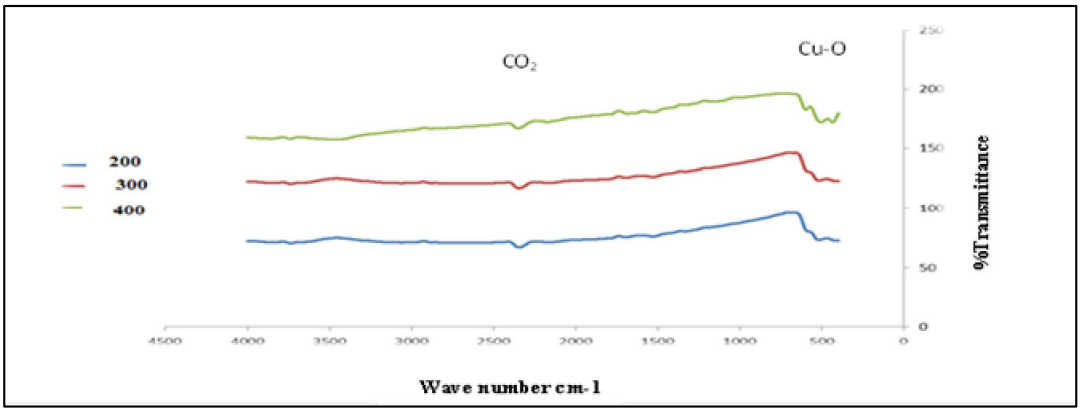


Figure 2. FTIR spectra of CuO NPs annealed at different temperature

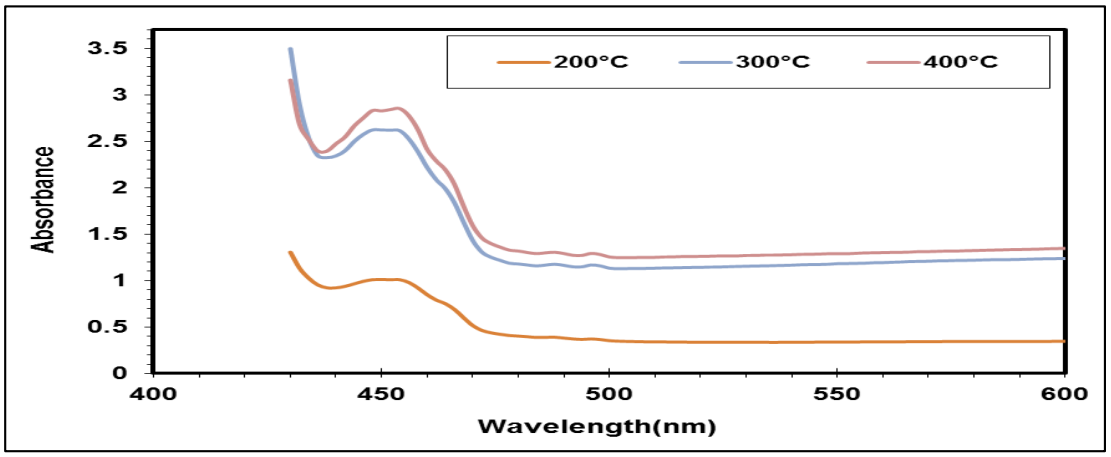


Figure 3. UV-Visible spectra of CuO NPs annealed at different temperature

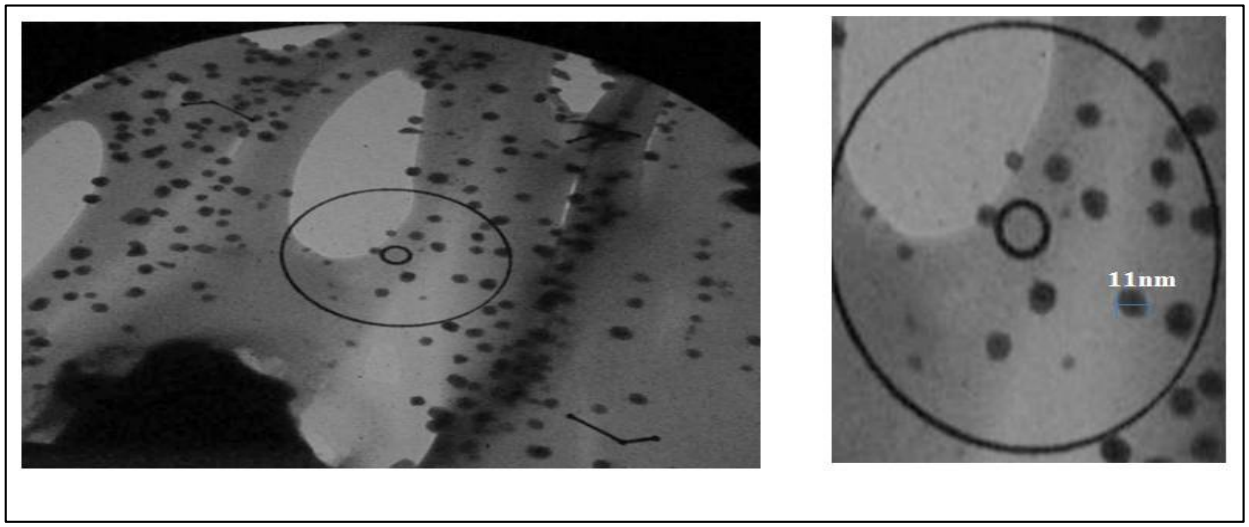
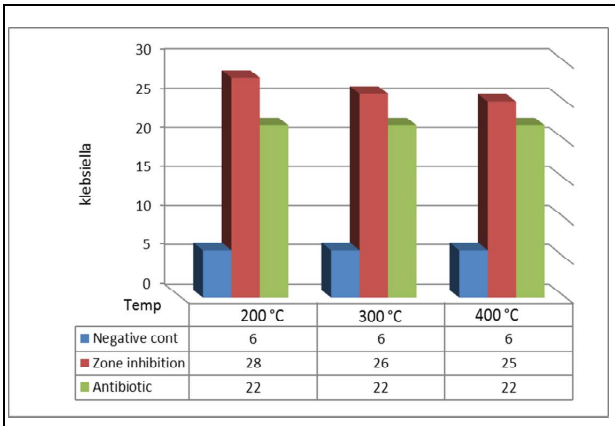
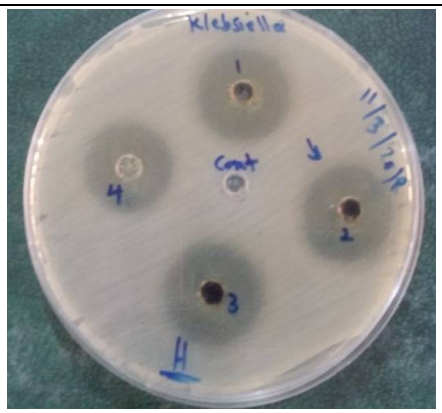
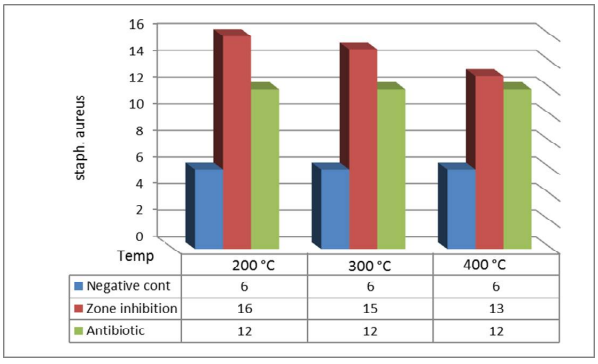


Figure 4. TEM image of CuO NPs







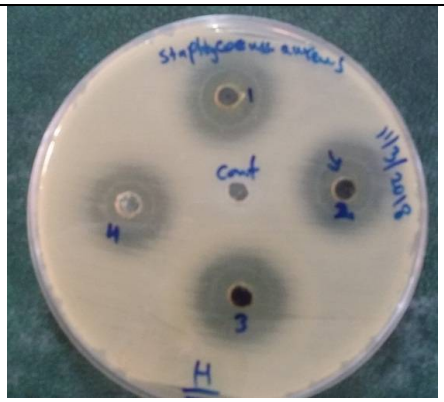


Figure 5.Inhibition zone ofCuO nanoparticles at different temperature (200, 300, 400)° Cagainst ((a) *Klebsiella* (b) *Staph.aureus*) bacteria. Numbers (1, 2, 3, 4, 5) represented the concentration at temperatures (200, 300, 400) ◦C and number (6) represented the tetracycline antibiotic

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RESEARCH ARTICLE

The Therapeutic Potential of *Nelumbo nucifera* Gaertn. Against Liver Toxicity Induced by Carbon Tetra Chloride in Rats

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ABSTRACT

Background: *Nelumbo Nucifera* Gaertn (Lotus) plant has been extensively used as an alternative medicine to treat various illnesses. Moreover, Lotus seeds contains noteworthy alkaloids and flavonoids that shows antioxidant and therapeutic properties.The object of the study is to determine effect of aqueous extract of Lotus seeds on hepatic toxicity induced by Carbon tetrachloride in rats. Methodology: Wistar Albino Animals (n=6) were divided in three group. Group I (control group), group II (received 0.8ml/kg of CCL_4 two times a week) and group III (received 0.8ml/kg of CCL_4two times a week and 200 mg/kg of aqueous extract of Lotus seedsorally for 21 days). The tissue and blood samples were collected from sacrificed animals for histology andLiver function tests. Results: Elevated levels ofALT, total bilirubin and AST in group II in contrast to group I shows successful toxicity induced by Carbon tetra Chloridewhereas, decreased levels of ALT, total bilirubin and AST were detected in group III in contrast to group II attributed protective effects of Lotus seeds. Furthermore, mild enlargement and lobular inflammation was observed in group II and group III. However, tissues were intact in group I.Conclusion: The study reveals that Lotus seeds exhibits potential therapeutic effects against hepatotoxicity.

Keywords: Lotus seed; Liver; Carbon tetrachloride; *Nelumbo nuicfera*; Hepatotoxicity.

INTRODUCTION

Nelumbo nucifera is a floating water plant comes under family Nelumbonacea. It has many other names such as water Lily, Sacred Lotus, Indian Lotus, Kanwal [1]. All parts of *N.nucifera* plant used for various purposes, rhizome used as vegetable, seeds show tremendous therapeutic properties. However, leaves and flowers are used for ornamental

purposes [2]. Many bioactive compounds are found in Lotus seeds such as flavonoids and alkaloids which are responsible for biological and pharmacological activities [3].The seeds contain three major active alkaloids; isoliensinine,liensinine, and neferine[4].The seeds contain rich content of proteins and essential minerals [5]. Lotus seeds exhibits anti-fertility, free radical scavenging and Hepatoprotective activities [6]. Additionally, customarily used to treat various illnesses such as Fever, indigestion, diarrhea, insomnia, tissue inflammation and cancer [7]. Moreover, Lotus has religious significance as it is considered divine and sacred [8].

The rise of ethno-medicines has change the horizon of treatment of diseases [8]. Herbal medicines including Traditional Chinese medicines, Unani medicines and Ayurveda medicines are widely used nowadays [9]. *Nelumbo nucifera* has been used as herbal medicine in different parts of world [8]. About 75-80% of population relies on herbal medicinesglobally [10]. Liver is a chief organ and play crucial role in many physiological functions. Due to its larger size and contribution in various functions, it is fragile and can easily be damaged. Mainly oxidative stress isa cause of injury to Liver [11].During oxidative stress, reactive oxygen species binds to biomacromolecules, alter enzymes and release cytokines [12]. Carbon tetrachloride (CCl_4) has beensuccessively used to produce Liver toxicity in rats[13]. CCl_4 cause activation of cytochrome P450 which leads to formation of trichloromethyl radical that binds to proteins and nucleic acids which in turn impairs cellular process. It also activates trichloromethyl peroxy radicals thatinduce chain reaction of lipid peroxidation [14]. The target of current study is to assess effect of aqueous extract of *Nelumbo nucifera*seeds on hepatic toxicity caused by CCl_4 in rats.

MATERIALS AND METHODS

Seeds collection

Lotus seeds were purchased from local herbal market, Karachi, Pakistan. The seeds were verified by Department of Botany, University of Karachi, Pakistan.

Extract Preparation

The aqueous extract of Lotus seed was prepared by crushing the seeds in a mortar to make 200gm of powder followed by soaking into 1000ml of distilled water. This mixture was placed in a shaking incubator for 24 hours and then filtered by whatmann no 1 filter paper. The filtrate so formed was stored at 4'C.

Experimental Animals

The Wistar Albino rats (n=6) weighing 150-200gm were obtained from DOW University of Health sciences. They were kept in glass cages in well aired area, provided with commercial rat chow and water *ad libitium.* Animals were handled with respect to Ethical guidelines of Institutional Review Board (IRB) and under standard Laboratory conditions.

Experimental Design

The rats were divided into three groups. Group I, control. Group II, given $CCL_4$0.8ml/kg subcutaneously for twice a week. Group II, given 0.8ml/kg of CCL_4subcutaneously for twice a week and 200mg/kg of aqueous Lotus seed extract orally through gavage daily for 21 days. On 22nd day, animals were sacrificed and tissue and blood samples were collected and stored till further investigation.

Detection of AST, ALT and Total Bilirubin

The separated plasma from centrifugation of blood at 3000rmp for 10 minutes used for biochemical tests such as detection of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)[15] and total bilirubin[16] levels by commercially prepared Randox kits.

Tissue Examination

Liver tissues were placed in Formalin solution. The tissues were cleared, embedded, sliced and stained with H & E stain. The morphology of tissues was observed under electron microscopy.

Statistical Evaluation

The date values were depicted as Mean ± Standard Deviation. Evaluated by One way analysis of Variance (ANOVA) with Duncan's Multiple Range test. $P< 0.05$ indicates statistical significance.

RESULTS

Impact of Carbon tetra chloride and Lotus seeds on Liver Enzymes and Total Bilirubin Levels in Experimental Groups

The result shows AST levels ($p>0.05$) were increased in CCl_4 treated group against control group. However, AST levels ($p>0.05$) decreased inCCl_4 + Lotus seed extract treated group compared to CCl_4 treated group (Figure3). Additionally, ALT levels and total bilirubin levels ($p< 0.05$) were increased CCl_4 treated group in contrast to control group. However, ALT levels and total bilirubin levels ($p< 0.05$) showed significant curtailment in CCl_4 + Lotus seed extract treated group contrary to CCl_4 treated group (Figure 2 and Figure4).

Impact of Carbon tetra chloride and Lotus seeds on Body weight and Liver weight in Experimental Groups

In recent study, changes in initial and Final body weight ($p< 0.05$) were observed in all groups. Body weight in CCl_4 treated group was reduced whereas, it increased in control rat group and CCl_4 + Lotus seed treated group by the end of study. (Figure 5 and Figure6). Additionally, elevated Liver weight was observed ($p< 0.05$) in CCl_4 treated group contrary to control group with dark colored, swollen Liver indicating Liver injury. However, reduced Liver weight ($p<0.05$) was observed in CCl_4 + Lotus seed extract treated group in contrast to CCl_4 treated group attributed to hepatoprotective effect of Lotus seed extract. (Figure7).

Impact of Carbon Tetra Chloride and Lotus seeds on Histopathology of Liver

Enlargement and Lobular inflammation was observed in CCl_4treated group along with CCl_4 + Lotus seed extract treated group. However, hemorrhage was found in CCl_4 treated group signifying hepatic damage. Moreover, no pathological evidence was found in control group. (Figure8).

DISCUSSION

Liver plays a significant role in metabolism and removal of xenobiotics that makes it susceptible to injury by exposed chemicals. Chemicals usually convert biomolecules into free radicals that ultimately damage Liver structure or its

functions [17]. CCl_4 is a renowned solvent to induce Liver toxicity [13]. It induces acute Liver injury and used as a hepatotoxicity model to investigate protective effect of natural drugs on the Liver [18]. CCl_4 metabolized by cytochrome P450 which form trichloromethyl radical that leads to lipid peroxidation [19].Moreover, CCl_4 activates transforming growth factors (TGF)-α and -ß tumor necrosis factor (TNF) α, and nitric oxide (NO) that cause self-destruction of cells [14]. Lotus seed has profound free radical scavenging activity due to presence of flavonoids, alkaloids and glycoproteins [20,7]. Additionally, Lotus Plumules contained in the seeds have enormous alkaloids that are responsible to prevent lipid peroxidation and inhibition of TNFα and promotes IκB-alpha (nuclear factor of kappa- B-cells inhibitor, alpha) [11]. Parameters such as, level of enzymes located in hepatocytes, total bilirubin levels, body weight and histopathology have been used to assess extent of Liver injury.

Serum level of transaminases increases in various Liver diseases [21] as indicated in present study, ALT ($P<0.05$), AST ($p> 0.05$) levels increases in CCl_4 treated group in contrast to control group. (Figure 2 and Figure 3). Earlier studies revealed that ALT, AST enzymes moves out into blood stream once the Liver is intoxicated with CCl_4[22]. Membrane permeability of Hepatocytes increases when treated with CCl_4 which allows transaminases to release into blood and are the indication of Liver inflammation [23]. Liver cells have function of regulating bilirubin [24]. CCl_4 damage Hepatocytes which flows bilirubin back to blood and Hence Serum total bilirubin is increases [25] which shows in our present study, total bilirubin level increases in CCl_4 treated group against control group whereas, it decreased in CCl_4 + Lotus seed treated group contrary to CCl_4 treated group. (Figure4). The current results coincide to a study by [11]. Evaluation of organ weight and body weight in a toxicological study is important to determine potential effect of chemical and drug [26]. The final body weight decreases in CCL_4 treated group as compared to control group and CCL_4 + Lotus seed treated group that is evident of toxic injury and physiological changes in rodents [27] (Figure6). Moreover, the Liver weight increased in CCL_4 treated group as compared to control group and CCL_4 + Lotus seed treated group. (Figure7). Liver damage follows disappearance of fenestrae in Liver cell, incapacitate hepatic stellate cells and Kupffer cells, increases collagen that leads to accumulation of Fibers in Liver and resulting changes in Liver weight [28].Our biochemical analysis and estimated body and organ weight correlates with histopathological features.(Figure8)

CONCLUSION

The findings shows *N.nucifera* seeds improve enzyme levels and protect Liver cells against toxicity caused by carbon tetrachloride.The study supports notable use of *Nelumbo nucifera* Gaertn seeds in Traditional medicines.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

Declares none

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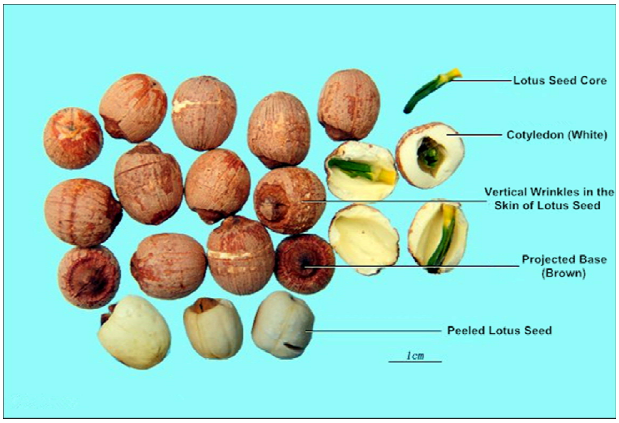

Figure 1. Structure of Lotus seeds [7]

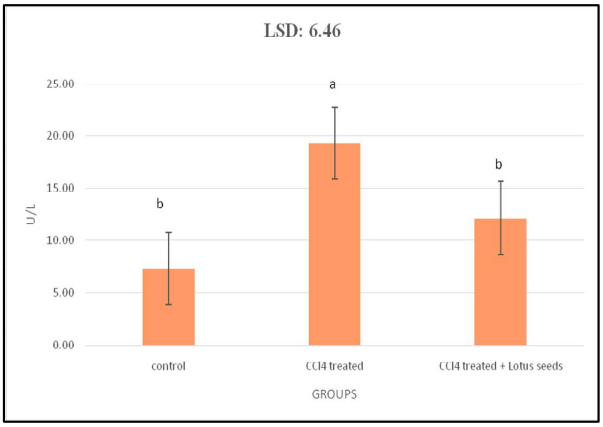

Figure 2. Alanine aminotransferase (ALT) levels in experimental groups. Data evaluated by One way ANOVA followed by Duncan's Multiple Range Test. Similar alphabets are non-significant. Significant figure at (p<0.05).

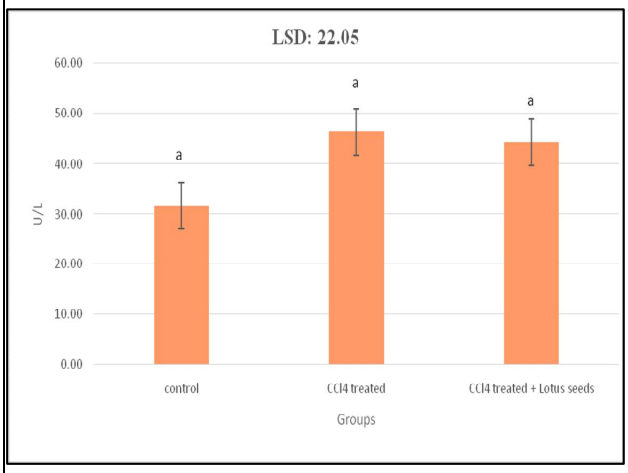

Figure 3. Aspartate aminotransferase (AST) levels in in experimental groups. Data evaluated by One way ANOVA followed by Duncan's Multiple Range Test. Similar alphabets are non-significant. Significant figure at (p<0.05).

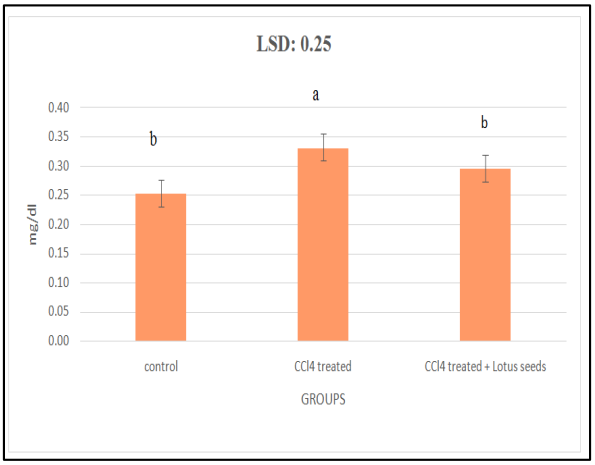

Figure 4. Total bilirubin levels in in experimental groups. Data evaluated by One way ANOVA followed by Duncan's Multiple Range Test. Similar alphabets are non-significant. Significant figure at (p<0.05).

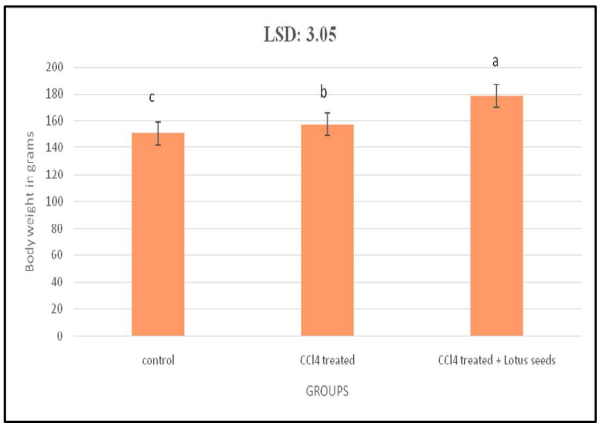

Figure 5. Initial body weight in in experimental groups. Data evaluated by One way ANOVA followed by Duncan's Multiple Range Test. Similar alphabets are non-significant. Significant figure at (p<0.05).

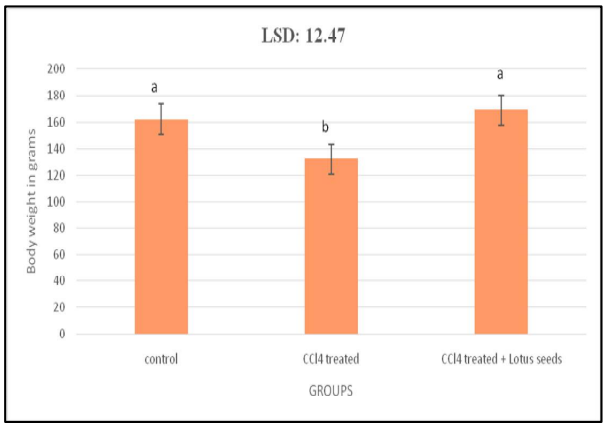

Figure6: Final body weight in in experimental groups. Data evaluated by One way ANOVA followed by Duncan's Multiple Range Test. Similar alphabets are non-significant. Significant figure at (p<0.05).

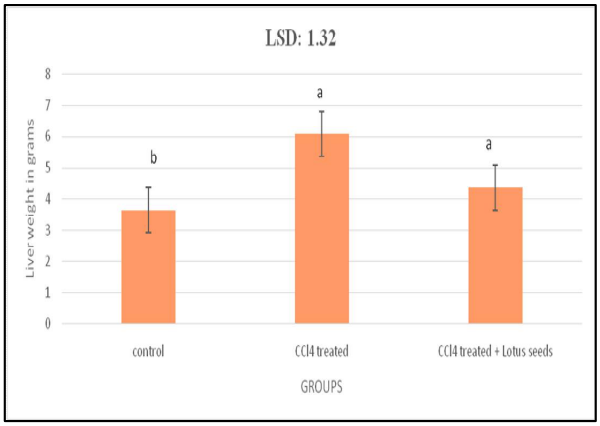

Figure 7. Liver weight changes in in experimental groups. Data evaluated by One way ANOVA followed by Duncan's Multiple Range Test. Similar alphabets are non-significant. Significant figure at (p<0.05).

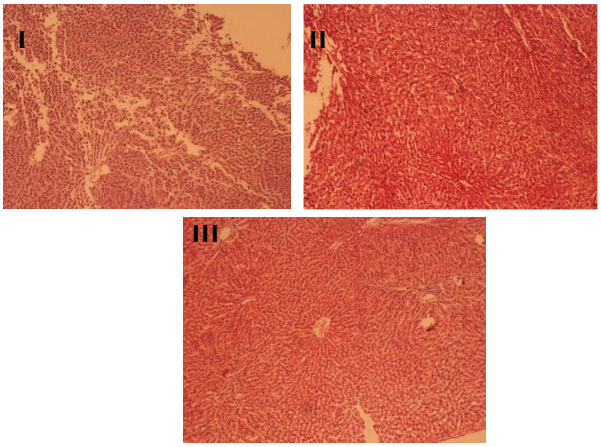

Figure 8. Microscopic examinations of Liver tissue at magnification 4X. I (Control) II: Treated with CCl_4, III: Treated with CCl_4 and Lotus seed extract

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RESEARCH ARTICLE

Isolation and Identification of Extended Spectrum Beta Lactamase (ESBL) *Escherichia coli* and *Klebsiella pneumonia* in Urine Samples of UTI Patients

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ABSTRACT

This study was undertaken to observe antimicrobial resistance pattern amongst extended spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumonia* clinical isolates.Eight hundred and fortyfive (845) urine specimens were processed in Microbiology department of United Laboratory Dabgari Garden, Peshawar, during June 2012 to April 2013. Isolates were screened out for ESBL production against ceftazidime, ceftriaxone, cefuroxime, cefepime in combination with an amoxicillin-clavulanic acid. Antimicrobial susceptibility testing was performed by disc diffusion method. Among the processed urine samples, 96 (11.3%) were reported as ESBL producing *E. coli* and 67(7.9%) *K. pneumonia*while the rest of them were reported as ESBL negative. High resistance rate was recorded for ESBL producing *E. coli* against aztreonam (100%), cefixime (100%), sulphamethoxazole (94%), trimethoprim (97%) and ciprofloxacin (85%). Increased resistance pattern of ESBL producing *K. pneumonia* was observed against

aztreonam (100%), ceftriaxone (100%), co-amixoclave (100%), trimethoprim (94%) and ciprofloxacin (80%).ESBL-producing *E. coli* and *K. pneumonia* had an excellent susceptibility to meropenem, piperacillin-tazobactam, nitrofurantoin, and amikacin. Emerging antimicrobial resistance in bacteria is imposing serious complications in the treatment while ESBL producing bacteria have further limited the use of broad spectrum cephalosporin in clinical settings.

Keywords: *Escherichia coli*; Extended Spectrum Beta Lactamase; *Klebsiella pneumonia*; UTI

INTRODUCTION

Extended Spectrum Beta-Lactamases (ESBL) are plasmid mediated enzymes that hydrolyze a large number of β lactams, including third generation cephalosporins, aztreonam andpenicillins, but showing susceptible to carbapenems or the cephamycins [1,2]. ESBLs were first identified in 1980 in Enterobacteriaceae [3]. Presently, it is more common in enteric bacilli other than *Escherichia coli* or *Klebsiella* spp. (e.g. *E. cloacae, Enterobacter aerogenes*, *Serratia marcescens, Providentia, Morganella morganii*, *C. koserii* and *Citrobacter freundii*), additionally in non-enteric bacilli (e.g. *Pseudomonas aeruginosa*) [4]. Bacterial drug resistance is becoming a worldwide issue and it is endangering the efficacy of antibiotics, which are providing safe guards to millions of human lives [5]. As recently, a new strain of *E.Coli* with gene MCR-1 indicated that a gene that can confer resistance to the last-resort antibiotic colistin has spread widely in clinical settings in China [6]. Since the first human treated with antibiotics and mankind won the war against many infectious agents, have again become a big threat to human lives. By 2050, drug-resistant infections could kill more people than cancer, according to the new report [7]. While on other hand there is no new discovery of any antibiotics since three decades, had made situation worst. After a 30 years wait, a new antibiotics is reported from human nasal secretion [8]. The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications [9].

Worldwide data manifest that there is an increasing rate of resistance among urinals tracts infections (UTIs) pathogens to conventional drugs. ESBL enzyme producing strains have varying susceptibility rates for fluoroquinolones, aminoglycosides, and fourth-generation cephalosporins [10]. This issue is more evident in uropathogens predominantly in*E. coli* causing difficulties in treatment. Community strains of *E. coli* are exhibiting increased resistance against commonly used drugs like co-trimoxazole (10-30%) and ampicillin/ amoxicillin (60%) [11]. *K. pneumonia*is considered second to *E. coli* as a urinary tract pathogen that is the leading cause of resistance to oxyimino- cephalosporins and aztreonam [12]. The clinical microbiological tests are used for detecting ESBLs using clavulanic acid (β-lactamase inhibitor) in combination with the Third Generation Cephalosporins such as ceftriaxone, ceftazidime or cefotaxime[13]. The current study was aimed to isolate and identify ESBL producing *E. coli* and *K. pneumonia* in urine samples of UTI patients in district Peshawar, Pakistan.

MATERIALS AND METHODS

Bacterial isolates

A total of 845 urine specimens were processed for the isolation and identification of Extended Spectrum Beta Lactamase (ESBL) *E. coli* and *K. pneumonia* from urine samples of UTI Patients [14].

Laboratory Methods

Urine samples were streaked on CLED agar (Oxoid Ltd., Basingstoke, UK) and were further screened for the presence of ESBL *E. coli* and *K. pneumonia*. The isolates were identified by biochemical tests such as indole, urease,

and triple sugar iron (TSI). After these tests ,theywere subjected to antimicrobial susceptibility testing by disc diffusion method.

ESBL Detection by NCCLS Phenotypic Method

ESBL phenotypic confirmatory test was performed with ceftazidime (30 μg) and amoxicillin plus clavulanic acid (20 μg+10 μg) against *E. coli* and *K. pneumonia* on Muller-Hinton agar plate [15].

Antimicrobial Susceptibility Test

Sensitivity of the isolates to numerous antibiotics was checked following Kirby Bauer disc diffusion method against selected antibiotics (Oxoid Ltd., Basingstoke, UK), namely aztreonam (30 μg), ceftazidime (30 μg), cefoxitin (30 μg), cefixime (5 μg), amikacin (30 μg), sulphamethoxazole (25 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), cefuroxime (30 μg), co-amoxiclave (30 μg), nitrofurantoin (100 μg), fosfomycin (50 μg), gentamycin (10 μg), cefepime (30 μg), imipenum (10 μg), ertrapenum (10 μg), meropenem (10 μg), piperacillin-tazobactum (110 μg), trimethoprim (5 μg) and sulzone (105 μg) (Bauer et al., 1966). The results were interpreted as per National Committee for clinical laboratory standards (NCCLS) recommendations [16].

RESULTS

For 845 urine samples, 163 isolates were screened as ESBL producers,out of these, 96 were positive for *E. coli* and 67 for *K. pneumonia* by NCCLS phenotypic method (Fig. 1). Isolates were separated as sensitive, intermediate and resistant according to NCCLS guidelines (Table 1, 2). Out of all 96 samples the ESBL producing *E. coli* were susceptible to meropenum (MEM) 97.91%, imipenum (IPM) 97.91%, followed by piperacillin-tazobactum (TZP) 96.87%, nitrofurantoin (F) 95.83%, gentamicin (CN) 96.87%, Fosfomycin (FOS)91.66%, amikacin (AK) 89.58% and Sulzone (SCF) 83.33%. Aztreonam (ATM), ceftazidime (CAZ), cefixime (CFM), co-amoxiclave(AMC), sulphamethoxazole (SXT), ceftriaxone (CRO), cefuroxime (CXM) and trimethoprim (W) were confirmed to be more resistant antibiotics in the study. Out of 67 samples of ESBL producing *K. pneumonia* showed susceptibility to piperacillin-tazobactum (TZP) 92.53%, Nitrofurantoin (F) 92.53%, sulzone (SCF) 91%, gentamicin (CN)89.55%, fosfomycin (FOS) 88%, Meropenem (MEM) 85%, imipenum (IPM) 83.58% amikacin (AK) 82%. Resistance pattern of *K. pneumonia* isolates toward broad spectrum antibiotics was also recorded as high (Table 2). In the case of aztreonam (ATM), ceftazidime (CAZ), ceftriaxone (CRO), cefixime (CFM), co-amoxiclave (AMC) and cefuroxime (CXM), 100 % resistance was observed. The resistance was decreased in a stepwise pattern towards trimethoprim (W), sulphamethoxazole (SXT) and ciprofloxacin (CIP) respectively.

DISCUSSION

The study was aimed to screen out ESBL producing *E. coli* and *K. pneumonia* in clinical specimens of UTI patients. The members of Enterobacteriaceae possess different mechanisms of resistance to β-lactam antibiotics, although, β-lactamases are the most common and clinically significant mechanism of resistance to β-lactam antibiotics among this bacterial group[17]. In this study ESBL producing *E. coli* were *96* (11.3%) and *K. pneumonia* were 67 (7.9%). Isolates were screen out in urine specimens of UTI patients as reported by Men et al. (2012) [17].Our results are relative to previous studies about ESBL prevalence. Another similar study reported that ESBL producing *E. coli* (9.6%), *Klebsiella pneumonia* (11.3%) and Enterobacter species (10.14%) were present in urine specimens of UTI patients[18].Amoxicillin and clavulanic acid were used in combination with other cephalosporins to detect the presence of ESBL. Isolates of *E. coli* and *K. pneumonia*were diagnosed as ESBL producers by phenotypic confirmatory test (Fig. 1). A combination of a clavulanic acid with third generation cephalosporins was used to detect ESBL production by clinical microbiological tests in gram-negative bacilli [13]. Antibiotic susceptibility or resistance to commonly used antibiotics (Table 1)

revealed that *E. coli* isolates were highly susceptible to most of antibiotics like meropenem (97.91) and imipenum (96%), piperacillin-tazobactum (96.87%), nitrofurantoin (95.83%), gentamicin (96.87%) and amikacin (89.58%). Resistance was shown to aztreonam (ATM), ceftazidime (CAZ), cefixime (CFM), co-amoxiclave (AMC), sulphamathoxazole (SXT), ceftriaxone (CRO), cefuroxime (CXM), ciprofloxacin (CIP) and trimethoprim (W) by isolates of ESBL *E. coli* (Table 1). Rhee et al., stated that aztreonam was resistant drug against *K. pneumonia* in diabetes and chronic renal patients[19]. A study conducted by Ali et al., (2012), had shown that 90-93% of *E. coli* isolates were ciprofloxacin resistant[20]. According to Maina et al.,(2013), antibiotic resistance pattern was maximum in ESBL *E. coli* towards ceftazidime (90.6%), sulfamethoxazole/trimethoprim (90.6%) and cefuroxime (99.4%) [21]. In our study, meropenum (MEM) 97.91%and piperacillin-tazobactum (TZP) 96.87% had shown maximum sensitivity. ESBL-producing *K. pneumonia* had developed resistance to majority of antibiotics but remained susceptible to pipracillin-tazobactum[22], nitrofurantoin,[23] gentamicin and sulzone[24]. The resistance pattern of *K. pneumonia* towards Aztreonam, ceftazidime and cefuroxime were 10%. Sikka et al., (2004),also observed 100% resistance towards aztreonam in surgical patients[25]. Similarly, Maina et al., (2013), reported 90.6% and 99.4% of resistance to ceftazidime and cefuroxime[21]. Resistance was gradually decreased towards sulfamethoxazole and ciprofloxacin i.e. 2.5% and 8.5% respectively, also80% resistance towards ciprofloxacin but only 40% towards sulphamethoxazole was reported. Increased resistance to ciprofloxacin may be due to overuse of this antibiotic in this area. In current study, it was observed that ESBL producing *E. coli* and *K. pneumonia* isolates were highly prevalent in UTI patients. Resistance pattern of these isolates was also high towards commonly used antibiotics in clinical practice. ESBL production should be screened out and antibiotic susceptibility should be performed on all isolates showing decreased sensitivity to common antibiotics[15].

CONCLUSION

Our study revealed that *K. pneumonia* showed resistance to half of availble antibiotics as compared to *E.Coli*. Pattern of drug resisatnce in peshwar is almost similar to other parts of country. Our study confirmed that antibiotics drug resistance is increasing in Peshwar district. We recommend governmnet should adopt some policies and guidelines toward use of antibiotics in provicne. As Peshwar is provinical capital of Khber Pakhtoon Khwa (KPK) province and rout to Afghnistan and centeral asian states, So this drug resistance can spread to neighbouring regions.

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Table 1. Sensitivity profile of selected antibiotics against *E. coli* isolated from Urine samples

S.No.	Antibiotics	Sensitive	Resistant	Intermediate
1	Aztreonam (ATM)	0	96	0
2	Ceftazidime (CAZ)	0	96	0
3	Cefixime(CFM)	0	96	0
4	Sulzone (SCF)	80	12	4
5	Cefoxitin (FOX)	58	35	3
6	Amikacin (AK)	86	8	2
7	Co-amoxiclave (AMC)	0	96	0
8	Sulphamathroxazole (SXT)	5	90	1
9	Gentamicin (CN)	93	2	1
10	Ciprofloxacin (CIP)	12	82	2
11	Ceftriaxone (CRO)	0	96	0
12	Cefuroxime (CXM)	0	96	0
13	Nitrofurantoin (F)	92	2	2
14	Fosfomycin (FOS),	88	3	5
15	Cefipime (FEP)	2	93	1
16	Imipenum (IPM)	92	2	0
17	Ertrapenum (ETP)	92	3	1
18	Meropenem (MEM)	94	1	1
19	Pipracillin-tazobactum (TZP)	93	2	1
20	Trimethoprim (W)	2	93	1

Table 2. Sensitivity profile of selected antibiotics against *K. pneumonia* isolated from Urine samples

S.No.	Antibiotics	Sensitive	Resistant	Intermediate
1	Aztreonam (ATM)	0	67	0
2	Ceftazidime (CAZ)	0	67	0
3	Ceftriaxone (CRO)	0	67	0
4	Cefixime (CFM)	0	67	0
5	Co-amoxiclave (AMC)	0	67	0
6	Cefipime (FEP)	1	65	1
7	Cefuroxime (CXM)	0	67	0
8	Trimethoprim (W)	2	63	2
9	Sulphamathroxazole (SXT)	3	62	2
10	Gentamicin (CN)	60	2	5
11	Ciprofloxacin (CIP)	12	54	1
12	Amikacin (AK)	55	11	1
13	Nitrofurantoin (F)	62	3	2
14	Fosfomycin (FOS)	59	5	3
15	Cefoxitin (FOX)	40	26	1
16	Imipenum (IPM)	56	09	2
17	Ertrapenum (ETP)	52	14	1
18	Meropenem (MEM)	57	10	0
19	Pipracillin-tazobactum (TZP)	62	2	3
20	Sulzone (SCF)	61	5	1

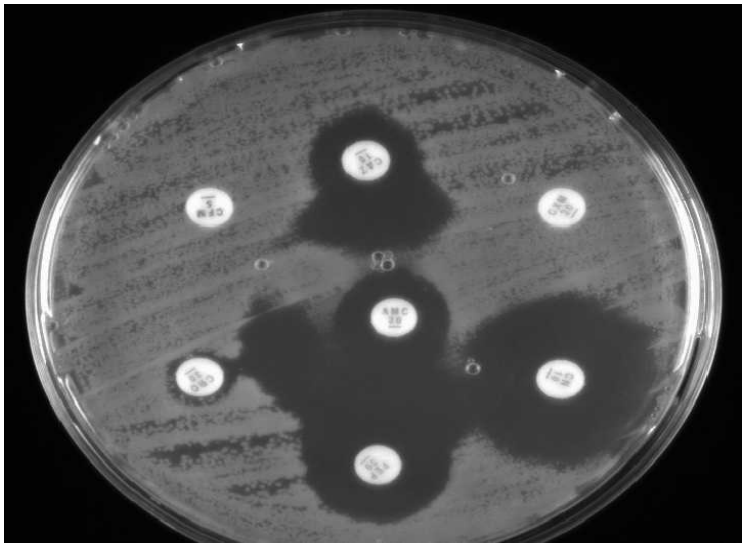

Fig. 1. A positive test for ESBL detection by phenotypic confirmatory method

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RESEARCH ARTICLE

Protective Effects of *Prunus dulcis* Against Dyslipidemia Induced By Letrozole In Polycystic Ovarian Rat Model

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ABSTRACT

The current study was conducted to unravel the antihyperlipidemic effects of *Prunus dulcis* (almond oil) against the letrozole induced polycystic ovarian syndrome (PCOS) in female rats. Almond oil is extensively used for different purposes but it can also be used as a medicinal essential oil because it contains some valuable components. Monounsaturated fatty acids (MUFA's) and Zinc is present in abundant quantity which are helpful in reproductive health and lowers the hyperlipidemic conditions duringPCOS. Letrozolebeing an aromatase inhibitor has been used widely to make the PCO model in rats as it produces more reliable characteristics. After purchasing the rats they were divided into three groups (n=6) based upon their age and size. Group A was considered as a control group whereas group B and C were induced with letrozole (1mg/body weight (b.w) in 0.9% NaCl) for 21 consecutive days. In addition group C received almond oil (1ml/kg b.w) after 7 days of experiment. Body weights were recorded and vaginal smears were prepared on daily basis to deduce the phases of estrus cycle. After 21 days the rats were dissected and their blood plasma was stored to further biochemical assessments. Estrus cycle comprises of four phases: estrus, diestrus, metestrus and proestrus. After letrozole was induced in group B the cycle was blocked at diestrus phase and group C was even given almond oil which showed positive results as the estrus cycle reverted from diestrus phase.Our biochemical test exhibited positive results as there was decrease in the total cholesterol ($p < 0.05$) and low density lipoprotein levels ($p < 0.05$). It is therefore concluded that *Prunus dulcis* (almond oil's) prolonged usage may exert some beneficial effects in PCOS.

Key words : Polycystic ovarian syndrome, Letrozole, Prunus dulcis, estrus cycle.

INTRODUCTION

A perplexing and most persistent endocrine disorder was defined by Stein and Leventhal in 1935 and since then polycystic ovarian syndrome (PCOS) is affecting around 6% - 10% of females of reproductive age(Stein, 1935). There are distinct reproductive, metabolic and psychological aspects which arecorrelated to PCOS(Boomsma et al., 2006). Based on these aspects, PCOS is manifested by menstrual irregularities, anovulation, amenorrhea, hirsutism, acne and the most common infertility(Boomsma et al., 2006). Women suffering from PCOS display an increased incidence of glucose intolerance and insulin sensitivity, along with hyperandrogenism and genetic abnormalities. Hyperinsulinemia and hyperandrogenism are both associated with abnormal lipoproteins pattern in the body as there are high levels of triglycerides and cholesterol whereas HDL are decreased(Frayn, 1993). The root cause of PCOS remains uncertain but it is presumed that insulin resistance and hyperandrogenism can be responsible for the onset of it(Franks et al., 1997). Besides the fact that there are multiple methods to make animal models of PCOS, but not most of them fulfill the pathologic conditions as seen in humans, but letrozole induced PCO model is said to be more reliable as they have more features as in humans(Mahajan, 1988). In letrozole induced PCOS model, acyclicity was seen and the conversion of estrogen from androgen was blocked which causes hyperandrogenism(Kafali, Iriadam, Ozardalı, & Demir, 2004).

One of the most economic and well known nut oil*Prunus dulcis*(almond) was used in this study.**Almond oil** was used as a therapeutic agent to treat the symptoms and causes of PCOS. Almonds are known for its cholesterol free nature and contains many nutrients like zinc and L-arginine which can be beneficial for reproductive health(fertility, april 2016). Essential oils have a long history of serving as alternative medicine and are economically viable too as compared to other medicines. Our purpose of this investigation was to observe the changes in PCOS symptoms in letrozole induced PCO rat model after almond oil treatment.

MATERIALS AND METHODS

Eight weeks old female Wistar rats were purchased from the Dow University of Health Sciencesweighed around 170-190 g.They became acclimatized to the environment of the animal house where they were kept in a 12hr light/dark cycleand they could easily excess to diet and water.

Ethical Guidelines

The experiment was performed under the Institutional Ethical Review Board's ethical guidelines which are internationally acknowledged ethical practices for animal care (Health Research Extension Act of 1985).

Preparation of Oil

Bitter Almonds were purchased from a local market in Karachi. Their shells were removed and were kept in water to further get rid of their covering. They were coarsely blended in a grinder to make a paste. Three phase partitioning was done to extract the oil from almonds by adding ammonium sulphate and t-butanol (Dennison & Lovrien, 1997).

Study Design

Animals were divided into three groups corresponding to their weights and each group (n=6). Group A served as a control group while group B and C were treated with letrozole (in 0.9% NaCl, 1mg/b.w) for 21 days. Group C was treated with almond oil (1ml/kg b.w) starting from day 7 of the experiment. After 21st day the animals were sacrificed and blood was taken out in the heparinized syringes and was centrifuged later on. Plasma was extracted from the sample and stored at -80□.

Vaginal Smear Slide Preparation

Vaginal smears were prepared every day for 21 days to determine the phase of the estrus cycle. At first, rats had normal 4 day estrus cycle but when letrozole (in 0.9% NaCl,1mg/b.w) was induced acyclicity could be seen. A cotton swab was submerged in NaCl and moved inside the vaginal wall to take up a few cells. The swab was moved on the slide and it was stained with 1% crystal violet solution. The slide was dried first and then observed under the high resolution microscope.

Assessment of Lipid Profiles

Lipid profile consists of total cholesterol(Lieberman), high density lipoprotein(HDL)(Bairaktari, Seferiadis, & Elisaf, 2005), triglycerides(Fossati & Prencipe, 1982), LDL(Fukuyama et al., 2007)evaluated by the commercially prepared Global kit.

Statistical analysis

The results were exhibited as mean± standard deviation (SD). Stastical analysis was completed bySPSS version 16. The data was statistically analyzed by sample independent T- test and one way analysis of variance (ANOVA) along with least significance difference (LSD).ϱ values which are lesser than 0.05 were reported as significant.

RESULTS

Determination of estrous cycle through different phases

Estrus cycle is determined by vaginal smear in which different cells can be seen in order to judge the phase of estrous cycle. Majorly there are three types of cells round and nucleated are the epithelial cells, irregular and not nucleated are cornified cells and lastly the round and small ones are leukocytes. Epithelial cells are seen in the Proestrus (P) phase, cornified cells are seen in the Estrus (E) phase, abundant leukocytes are seen in Diestrus (D) phase and in Metestrus (M) phase all the cells are present in equal amount (Table 01-04).

Body Weights

Body weights are differing in different stages of the experiment, in the control group the weights have increased gradually while in letrozole treated group there are no major differences in the weights. Lastly in the letrozole + almond oil treated group weights have increased after the treatment (Fig. 01).

Biochemical Assessment

Effect of Almond Oil on Cholesterol, Hdl and Ldl Levels in Control, Letrozole Treated and Letrozole + Almond Oil Treated

Cholesterol levels have decreased in letrozole + almond oil treated group while it certainly did increase in letrozole treated group which gives a positive result in the study as the levels have decreased (Fig.02-04).

DISCUSSION

The main purpose to carry out this study was that to investigate the beneficial effects of almond oil on letrozole induced PCOS in rats. It would eventually lead us to see that if there is a chance to treat women suffering from PCOS efficiently. Polycystic ovarian syndrome comes along with different causes like insulin resistance, hyperandrogenism

and other genetic abnormalities(Azziz, 2018).In this present study the female rats had normal 4 day estrous cycle, but after the administration of letrozole the cycle had changed(Kumar, Woods, Bartolucci, & Azziz, 2005).Letrozole being an aromatase inhibitor, would inhibit the cycle by blocking the conversion of estrogen from androgens and this would lead to hyperandrogenism which is a main factor which influences PCOS manifestations(Diamanti-Kandarakis & Piperi, 2005).There are previous studies which show that letrozole model by far is the most reliable to make PCO rat model as it depicts more human characteristics (Kafali et al., 2004). In this study it is seen that the untreated control group A had normal four day estrus cycle while the Group B and C switched to diestrus phase where their cycle was blocked which was an indication that the PCO model is being formed. After the induction of Almond oil from the 7th day of experiment there were slight changes in the cycle of group C and once the cycle even reverted back to the estrus phase but as our study was for concise days further changes could not be seen.

As almond oil has many beneficial effects on the body one of the major benefits is that it has a cholesterol free nature which would surely decrease the cholesterol level which is increased during PCOS causing Dyslipidemia and along with that there is an impact on the low density lipoprotein(LDL) and high density lipoprotein(HDL). This present study shows some positive results which can be associated with other studies too(Croston, Milan, Marschke, Reichman, & Briggs, 1997). PCOS induced group showed significant increase in total cholesterol (TC) ($p < 0.001$), LDL ($p < 0.001$) and decrease in HDL ($p < 0.05$) levels too. Almond oil displayed its antihyperlipidemic action by considerably decreasing serum TC, LDL while slightly decreasing the HDL levels.

CONCLUSION

In conclusion, the results based on the biochemical assessment, histological findings and vaginal smears in this study demonstrates that prolonged use of almond oil can have beneficial effects in PCOS phenotypes by producing an antiandrogenic effect.

ACKNOWLEDGEMENTS

I would like to acknowledge my sincere gratitude towards my mentor Dr Lubna Naz, who provided with her sheer guidance in my research work. I am even thankful to my teammate Miss Zoha who assisted in this research work.

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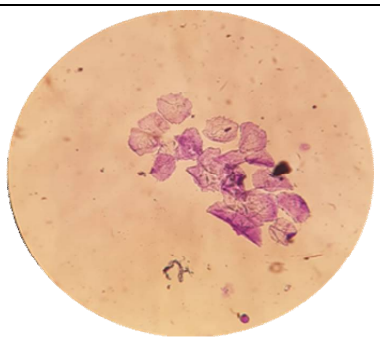
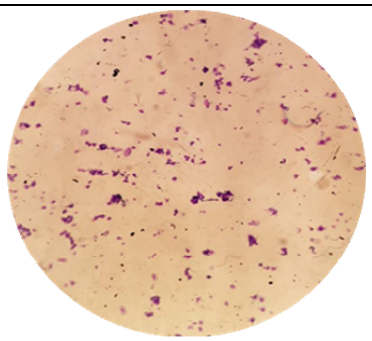
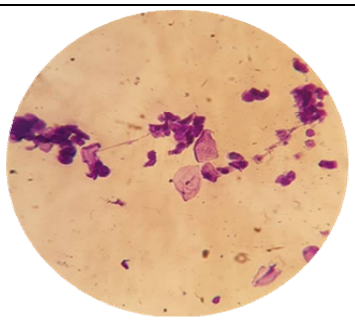
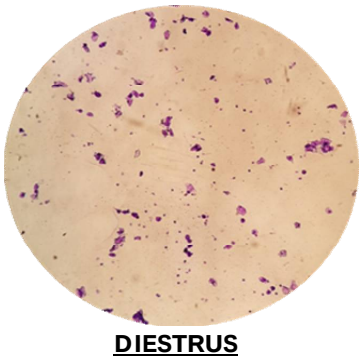
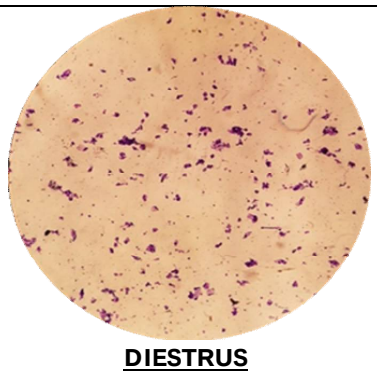
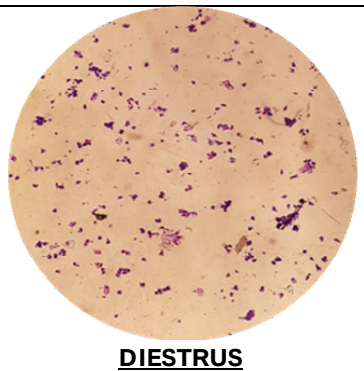
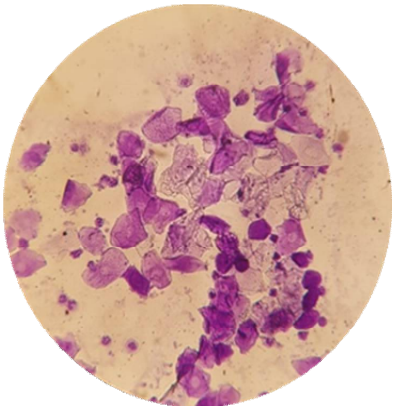
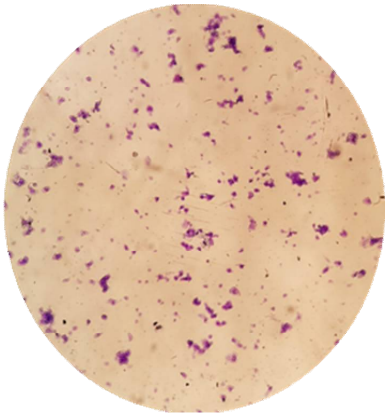
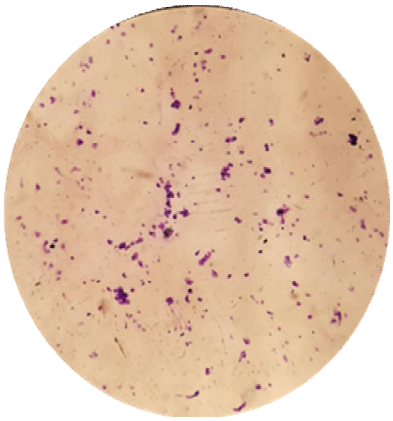
Table 01-04: The vaginal smear of rats with various stages of estrus cycle in theuntreated control group, letrozole treated and letrozole + almond treated at magnification of 40X.

DAY 1		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE + ALMOND TREATED**
 **ESTRUS**	 **DIESTRUS**	 **PROESTRUS**

DAY 6		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE + ALMOND TREATED**
 **DIESTRUS**	 **DIESTRUS**	 **DIESTRUS**

DAY 15		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE + ALMOND TREATED**
 **DIESTRUS**	 **DIESTRUS**	 **DIESTRUS**

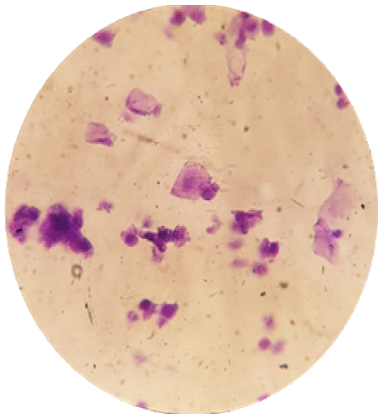
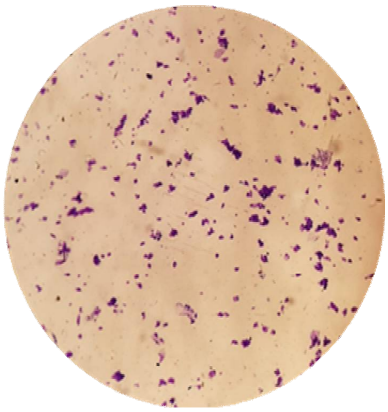
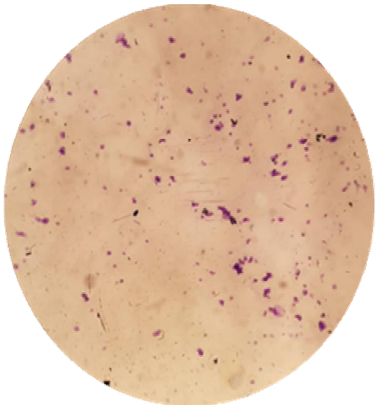
DAY 21		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE + ALMOND TREATED**
 **METESTRUS**	 **DIESTRUS**	 **DIESTRUS**

Table 01-04. The major cells in four phases are: estrus phase has cornified cells, diestrus phase has mainly the leukocytes and the proestrus phase mainly the epithelial cells. All the cells are in equal amounts in the metestrus phase.

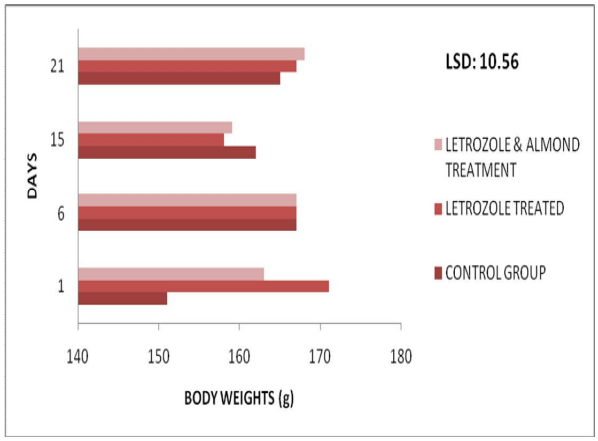


Fig. 01. Body weights taken on different days of control group, letrozole treated group and letrozole + almond oil treated group. LSD within the groups is 10.56.

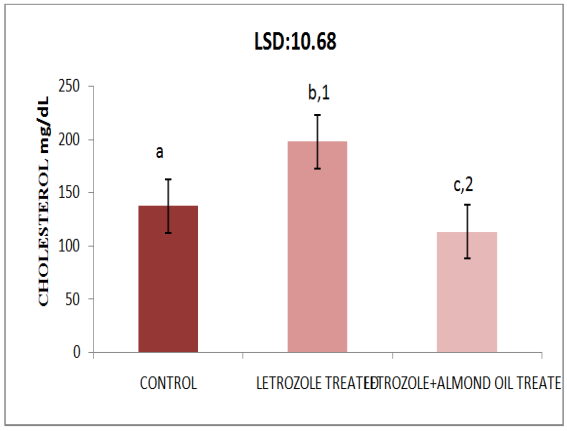


Fig. 02. Cholesterol levels in the control group, letrozole treated group and letrozole + almond oil group. Values are demonstrated as mean ± SD and ONE way ANOVA and Duncan Multiple range test where unique alphabets are significant and similar alphabets are non-significant.($p<0.05$) 1: control group compared with the letrozole treated.2: letrozole treated group compared with letrozole + almond oil treated group.

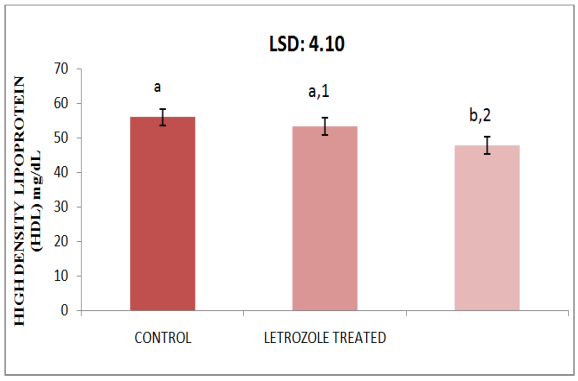


Fig. 03. High density lipoprotein (HDL) levels in the control group, letrozole treated group and letrozole + almond oil group. Values are demonstrated as mean ± SD and ONE way ANOVA and Duncan Multiple range test where unique alphabets are significant and similar alphabets are non-significant.($p<0.05$)
1: control group compared with the letrozole treated.
2: letrozole treated group compared with letrozole + almond oil treated group

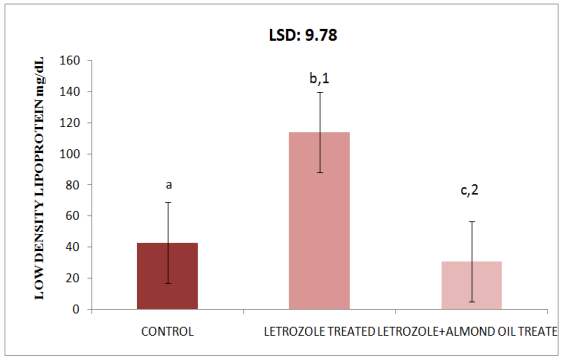


Fig. 04. Low density lipoprotein (LDL) levels in the control group, letrozole treated group and letrozole + almond oil group. Values are demonstrated as mean ± SD and ONE way ANOVA and Duncan Multiple range test where unique alphabets are significant and similar alphabets are non-significant.($p<0.05$)
1: control group compared with the letrozole treated.
2: letrozole treated group compared with letrozole + almond oil treated group

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RESEARCH ARTICLE

Hepatoprotective Effects of Taurine against Carbon Tetra Chloride Induced Hepatotoxicity in Rats

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ABSTRACT

This study was aimed to investigate the protective effects of Taurine on antioxidant enzyme activity and lipid peroxidation in CCl_4 induced hepatotoxic rats. Taurine is a sufonic acid widely distributed in animal tissues. It has many fundamental functions including antioxidation, osmoregulation and membrane stabilization. Study included four groups (n=6). Group I remained healthy control rats, group II , received CCl_4 (0.8 ml/Kg b.w, s.c, for 8 weeks, twice a week), group III received CCl_4 (0.8 ml/Kg b.w, s.c, for 8 weeks, twice a week) together with taurine (1% w/v with drinking water daily for 8 weeks); Group IV received taurine (1% w/v with drinking water daily for 8 weeks). Biochemical analysis included total bilirubin, liver enzymes, antioxidant enzymes & MDA. CCl4 created hepatotoxicity as indicated by elevated bilirubin, liver enzymes and MDA levels and reduced antioxidant enzyme activity. Taurine supplementation markedly reduced hepatic damage and restored the antioxidant enzymes and MDA activity. The histologic findings indicated portal and periportal inflammation and fibrosis with degeneration of hepatocytes in CCl_4 treated rats whereas slight periportal fibrosis with apparently no degenerative hepatocytes were seen in rats treated with taurine along with CCl4. The results indicate that taurine successively mitigates the CCl_4 induced hepatotoxicity.

Key words: Antioxidant, CCl_4, Cirrhosis, Hepatotoxicity, Taurine.

INTRODUCTION

Taurine is a derivative of cysteine, and a sulfur-containing amino acid occurs especially in seafood and meat. It is also found in tissues such as CNS, ocular tissues, epididymis, perirenal white adipose tissues, and brown adipose tissue (Tappaz*et al.* 1998, Ide *et al.* 2002) but synthesis of taurine is a primary responsibility of liver and CNS. Taurine is involved in cytoprotection, cell development including cell's nutrition, survival, control of growth and differentiation (Ripps&Shen, 2012). It enhances expression and activities of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase (Jang *et al.* 2009). It may also affect oxidative damage by limiting the availability of lipids for lipid peroxidation and research studies suggest that taurine supplementation prevents oxidative stress induced by toxic substance, alcohol, exercise ischemic reperfusion and mechanical injury (Murakami*et al.* 2010, Silva *et al.* 2011). The protective effects of taurine against toxins, oxidative stress and carcinogenesis have been demonstrated by various studies. (Sinha*et al.*2009, Erman*et al.* 2004). It is a potent antioxidant especially in liver that effectively inhibits lipid peroxidation either by directly scavenging free O2 radicals or controlling membrane permeability induced by oxidative stress (Koch *et al.* 2004). Researchers have found that its derivatives such as taurolidine and Taurochloramin display anti-neoplastic effect both in vitro and in vivo; through suppressing cell proliferation, enhancement of tumor cell apoptosis (Duffy*et al.* 2006).

Liver Cirrhosis is the final worst stage of chronic liver damage associated with great morbidity and mortality. In cirrhosis substitution of normal liver tissue by fibrosis, scar tissue, regenerative nodules occur that eventually leads to liver failure. Liver functions as detoxification center where metabolism of toxins and destructive agents occur for their subsequent excretion. Toxins can be converted to intermediate metabolites and reactive radicals that can cause damage to liver parenchyma resulting in degenerative, necrotic and atrophic hepatocytes thereby creating a state of hepatotoxicity. The mechanism of liver cirrhosis has been investigated by researchers through several animal models like carbon tetrachloride treatment, bile duct ligation, alcoholism and thioacetamide administration. $CCl_4$is one of the chlorinated hydrocarbons that produces harmful effects on different body systems. It is widely used to induce liver damage, and to establish experimental models. CCl_4 induced liver injury is characterized by histological, biochemical and molecular alterations CCl_4 Exposure to high concentrations of carbon tetrachloride including vapor can affect the central nervous system and degenerate the liver and kidneys and after prolonged exposure may result in coma and even death (Raja *et al.* 2007). The aim of this study was to investigate the protective effects of taurine against hepatotoxicity induced by CCl4 in rats.

MATERIALS AND METHODS

24 male Albino wistar rats weighing 190-250g were purchased from the animal jouse of ICCBS (International center for chemical and biological sciences, Karachi, Pakistan) for the study. Animals were acclimatized to the laboratory conditions prior the initiation of the experiment and caged in a quiet temperature controlled animal room (23±4ºC). Rats had free access to water and standard rat diet.

Ethical guidelines

The experiments were conducted with ethical guidelines of institutional ERB (Ethical Review Board) and internationally accepted principles for laboratory use and care in animal research (Health Research Extension Act of 1985)

Study design

The rats were randomly divided in to four experimental groups, each of six rats. The experimental phase lasted for 60 days. Taurine was purchased from Avon chemicals whereas CCl_4 and other chemicals used in present study were purchased from BDH laboratory supplies, Fisher Scientific UK limited and Fluka AG.

Group I : Untreated control
Group II : CCl4 treated
Group III : CCl4 + taurine treated
Group IV :Taurine treated

Group I served as control and received only 2 mL/kg normal saline solution for 60 days. Group II (CCl4 treated) & III(CCl_4+Taurine treated) received CCl_4, 0.8 ml/kg of body weight, subcutaneously, twice a week for 60 days. Group III(CCl4 + taurine treated) & IV(Taurinetreated) received daily 1% taurine with drinking water for 60 days. At 60^{th} day rats of all groups were decapitated. The blood was collected from the neck wound in the lithium heparin coated tubes and centrifuged to collect plasma. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at -70º C until analysis.

Assessment of AST, ALT, ALP &Bilirubin

Plasma ALT (Reitman & Frankel,1957), AST(Reitman & Frankel, 1957), ALP (Rec, 1972) and total and direct bilirubin (Jendrassek&Grof, 1938) were analyzed using commercially prepared reagent kits from Randox.

Preparation of post mitochondrial supernatant

Liver homogenate was prepared by taking 1gm of tissue in 10 ml of 5 mM potassium phosphate buffer (pH 7.8) by using a homogenizer. The homogenates then centrifuged at 800 g for 5minutes at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4°C to get post mitochondrial supernatant which was used to assay SOD, Catalase, MDA & glutathione reductase activity.

Estimation of thiobarbituric acid substances

Themalondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in in the form of thiobarbituric acid reacting substances (TBARS) by the lipid peroxidation method (Ohkawa*et al.*1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10% (w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Estimation of catalase

Catalase activity was assayed by the method of Sinha (Sinha,1972). Briefly the assay mixture consisted of 1.96 ml phosphate buffer (0.01M, pH 7.0), 1.0 ml hydrogen peroxide (0.2M) and 0.04 ml PMS (10% w/v) in a final volume of

3.0 ml. Then 2 ml dichromate acetic acid reagent was added in 1 ml reaction mixture, boiled for 10 minutes & cooled. Changes in absorbance was recorded at 570nm.

Estimation of superoxide dismutase

Superoxide dismutase levels in the cell free supernatant were measured by the method of (Kono,1978). Briefly 1.3 ml of solution A (0.1 ml EDTA containing 50 mM Na2CO3, pH 10.0), 0.5 ml of solution B (90 µm NBT nitro blue tetra zoliumdye) and 0.1 ml of solution C (0.6% Triton X-100 in solution A), 0.1 ml of solution D (20 mMHydroxlamine hydrochloride, pH6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 ml of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) require in one minute.

Estimation of glutathione reductase

GSH activity was determined by continuous spectrophotometric rate determination (Calberg&Mannervik, 1985). In a clean glass test tube, 0.3 ml of 10% BSA, 1.5 ml of 50 mM potassium phosphate buffer(pH 7.6), 0.35 ml of 0.8 mM βNADPH and 0.1 ml of 30 mM oxidized glutathione was taken and finally added 0.1 ml of homogenate, mixed well by inversion. Absorbance was recorded at 340 nm at 25°C for 5 minutes on kinetic spectrophotometer PRIM 500 (Germany) with automatic aspiration and thermostat. The activity was calculated using the molar coefficient for NADPH of 6.22 µmol $^{-1}$ x cm $^{-1}$ and expressed in unit/gram tissue.

Histopathological examination

Liver tissue samples were fixed with formalin and embedded in paraffin blocks, tissue then sectioned at 4µm and stained with eosine and hematotoxylin for microscopic examination. The degree of hepatic injury was evaluated from the histologic sections via gradings and numerical scores according to French *et al.* 2000.

Score 0 = no visible damage
Score 1 = focal hepatocyte damage on less than 25% of the tissue
Score 2 = focal hepatocyte damage on 25-50% of the tissue
Score 3 = extensive, but focal hepatocyte lesion
Score 4 = global hepatocyte necrosis

Statistical analysis

Results are presented as mean± standard deviation. Statistical significance and difference from control and test values were evaluated by Student's t-test. Statistical probability of a= P<0.05, b= P<0.01, c= P<0.001 were considered to be significant.

RESULTS

Effects of CCl4 and Taurine treatment on body & liver weight in control and treated rats

CCl4 administration has significantly decreased body weight of rats as compared with control (P<0.0001). Taurine administration has shown to increase body weight significantly in CCl4+Taurine treated group as compared with CCl4 treated group (P<0.01) (Table I). Body weight were almost similar in taurine treated group and control. Body weight loss was observed in CCl4 treated and CCl4+taurine treated whereas weight gain was noted in control and

taurine treated rats. There is no significant difference between liver weight and relative liver weight in taurine treated rats and control (P>0.05). Liver weight and relative liver weight were significantly higher in CCl4 treated group as compared with control (P<0.01) whereas lower in CCl4 + taurine treated rats as compared with CCl4 treated rats (P<0.01) (Table I).

Effects of CCl4 and Taurine treatment on liver enzymes (ALT, AST, ALP) & Bilirubin in control and treated rats

Table-II shows that CCl4 treatment has significantly increased the levels of hepatic enzymes AST (P<0.001), ALT(P<0.01)& ALP(P<0.01)in rats as compared with control . Taurine treatment has significantly reduced hepatic enzymes AST(P<0.001)& ALT(P<0.0001) in CCl4 + taurine treated group as compared with CCl4 treated group but no significant change in ALP(P>0.05) was observed in CCl4 + taurine treated group as compared with CCl4 treated group. Serum hepatic enzymelevels were nearly similar in control group and taurine treated group (P>0.05). CCl4 treatment has significantly raised bilirubin level in rats as compared with control (P<0.001). Bilirubin level was reduced significantly in CCl4 + taurine treated group as compared with CCl4 treated group (P<0.001). There was no significant change in rats treated solely with taurine as compared with control (P>0.05).

Effects of CCl4 and Taurine treatment on hepatic concentration of catalase in control and treated rats

Table-III shows significantly lower activities of Catalase in CCl4 treated rats as compared with control (P<0.0001). Taurine intake along with CCl4 in taurine+CCl4 group has been shown to increase significantly (P<0.01) the reduced catalase activity. Significantly increased activity of catalase was observed in rats treated with taurine alone as compared to control (P<0.0001).

Effects of CCl4 and Taurine treatment on hepatic concentration of SOD in control and treated rats

Activity of SOD was significantly reduced in CCl4 treated rats as compared with control(P<0.05)(Table III).Taurine treatment in taurine+CCl4 has been shown to increase significantly SOD activity(P<0.0001). Activity of SOD was more or less identical in taurine treated group and control group(P>0.05). (Table 3).

Effects of CCl4 and Taurine treatment on hepatic concentration of GSH in control and treated rats

Table III shows that activity of Glutathione reductase was reduced significantly in CCl4 treated rats as compared with control(P<0.0001).Taurine intake in taurine+CCl4 has been shown to increase significantly the reduced GSH (P<0.05) as compared to CCl4 treated group. Significantly reduced activity of glutathione reductasewas observed in taurine treated rats as compare to control (P<0.05).

Effects of CCl4 and Taurine treatment on hepatic concentration of MDA in control and treated rats

Levels of MDA were raised significantly in CCl4 treated group as compared with control (P<0.05). Taurine treatment along with CCl4 in taurine+CCl4 group has been shown to decrease MDA level non-significantly as compared with CCl4 treated group(P>0.05). MDA activity wasalmostsimilar intaurine treated group as compared with control (P>0.05)(Table III).

Histopathological findings

Liver morphological changes were scored, described and summarized in Control, CCl4 treated, CCl4 +Taurine treated &taurine treated groups (Table IV. No evident histological changes were observed in liver tissues of control

group (Figure 1). Severe histopathological changes in CCl4 treated rat liver tissues were noted. The prominent changes include portal and periportal inflammation, portal and periportal fibrosis, focal lobulitis and intracellular pigment deposition. Degeneration of hepatocytes was also noted and degenerative hepatocytes constitute for 30-40% (Figure 2). Our experimental results showed that taurine treatment along with CCl4 has partly improved and reversed the liver histological changes induced by CCl4. Histological sections indicated slight periportal fibrosis, slight bile ductular proliferation and marked sinusoidal expansion with apparently no degenerative hepatoytes Figure 3). However enlargement, paleness, hydropic degeneration & dysplasia were absent in rat liver lobules of both CCl4 treated &CCl4 +taurine treated groups. Hepatic architecture in taurine treatment group is similar to control group with no necrosis &degeneration. Only slight periportal fibrosis was noted in taurine treated rat liver tissues (Figure 4)

DISCUSSION

In the present study the protective effects of taurine against CCl4 induced hepatotoxicity and oxidative stress was investigated. Administration of CCl4 produced hepatotoxicity and cirrhotic changes in rats, which is exhibited via increased levels of hepatic enzymes; ALP, AST, ALT and bilirubin in CCl4 treated group(Table II). Hepatic functions were resumed by taurine treatment in CCl4+taurine treated group and a significant decrease in hepatic enzymes activity and bilirubin was noted (Table II). Activities of Liver function enzymes, AST, ALT and ALP in plasma are generally tested as indicators for liver functions and levels are proportional to hepatic injury, because CCl_4 causes peroxidation of membrane lipids of hepatocytes. This membrane disintegration of hepatocytes results in subsequent release of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and g-glutamyltransferase (g-GT) (Singh *et al.* 2008).CCl4 is selected in this study to induce hepatic damage as it is widely used to establish experimental models. Carbon tetrachloride (CCl_4) is a well-known hepatotoxin used in diverse experimental models. Its exposure to high concentrations can affect the central nervous system and degenerate the liver. CCl_4 metabolism takes place by cytochrome P450 and generates agents that lead to initiating of a chain of lipid peroxidation and thereby causing liver fibrosis and inflammation (Fang *et al.* 2008). CCl4 administration is believed to decrease hepatic taurine content and is shown by various studies. Therefore it is reasonable to think that taurine supplementation is not only effective against taurine depletion caused by CCl_4 but also protects against hepatotoxicity induced by CCl4. In present study, CCl4 treated rats showed a reduction in antioxidant enzyme catalase, SOD & GSH activity and an increase in MDA activity (Table III).

This is because CCl_4 not only initiates lipid peroxidation but also reduces tissue CAT and SOD activities, and this depletion may result from oxidative modification of these proteins. CCl_4 is not only metabolized by hepatocytes, but also by endogenous macrophage-like Kupffer cells that results in severe hepatic necrosis (Augustyniak*et al.* 2005). In this study taurine supplementation in CCl4+taurine group raised the activity of antioxidant enzymes catalase, SOD and GSH whereas slightly reduced MDA(Table III) which is a crucial indicator of lipid peroxidation, thus, taurine treatment prevented oxidative stress possibly via scavenging ROS generated by CCl4 thereby reducing oxidative stress that otherwise can cause potential hepatotoxicity. Several studies also have shown that taurine may modulate the hepatotoxicity of CCl_4 and the effectiveness of taurineagainst CCl4-induced liver disease in rats has been reported widely (Erman*et al.*2004, Miyazaki *et al.* 2005). Sun also indicated anti-oxidant and anti-inflammatory functions of taurine (Sun *et al.* 2012). In sime in vivo studies taurine has been shown to protect hepatic tissues and hepatocytes against hepatotoxicities, oxidative stress and hepatocarcinogenesis (Miyazaki *et al.* 2005). The histological findings reported in this study are in accordance with the biochemical results. Hepatotoxicity induced by CCl4 is confirmed by histological changes in liver tissues which indicate portal and periportal inflammation and fibrosis with intracellular pigment deposition and hepatocyte degeneration. Similar changes in liver tissues were noted by Akram*et al.*2012. Our experimental results showed that taurine intake along with CCl4 protected against hepatotoxicity and only slight periportal fibrosis, bile ductular proliferation and marked sinusoidal expansion were observed with no apparent hepatocyte degeneration(Table IV) thereby suggesting a preventive role of taurine against

hepatotoxicity. Supplementation of taurine to CCl4 induced hepatotoxic rats resulted in improvements in body weight(Table I), reduction of liver damage markers ALP. ALT , AST& bilirubin and elevation of antioxidant enzymes catalase, SOD & GSH activity as well as reduction in lipid peroxidation marker MDA. This suggests that taurine is a potent anti-injury agent against hepatotoxicity induced by CCl4. Thus it is reasonable to think that taurine mediates regeneration of hepatocytes with subsequent tissue repair which is the basis of survival from liver injury. Therefore it is concluded that taurine, a very important sulfonic acid can be used successfully to suppress hepatotoxicity and will be helpful in providing awareness and defining the protective effects of taurine against pathological conditions especially liver cirrhosis.

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Table I. Comparison of body weight, liver weight and relative liver weight in Control, CCl4 treated, CCl4 + Taurine treated &Taurine treated groups

	CONTROL (n=6)	CCl41 (n=6)	CCL4 + T[1,2] (n=6)	TAURINE[1,2,3] (n=6)
Initial body weight	214.66±4.50925	212.67±2.5166	209.33±1.1547	204±3.6056
Final body weight	237.33±11.1505	121.667±7.63[d]	183.3±2.88 [b,c]	219.33±9.0185 [a,n,b]
Weight Gain	22.67±6.6412			15.33±5.4129
Weight Loss		91.003±5.1204	26±1.7321	
Liver Weight	4.85±0.6403	7.42±1.4056[a]	4.725±0.2872 [n,a]	4.525±0.4113 [n,a,n]
Relative liver weight (g liver/100 g body)	2.119±0.2444	6.486±1.411 [b]	2.6201±0.2017[a,b]	2.0843±0.2404[n,b,a]

The data is expressed as mean ± standard deviation.

1= As compared with control, 2= As compared with CCl4, 3= As compared with CCl4+Taurine, a= P<0.05, b= P<0.01, c= P<0.001, n= P>0.05(Non-significant)

Table II. Comparison of Serum enzymes & Bilirubin levels in Control, CCl4 treated, CCl4 +Taurine treated &Taurine treated groups

	CONTROL (n=6)	CCl4[1] (n=6)	CCL4 + T[1,2] (n=6)	TAURINE[1,2,3] (n=6)
AST (U/l)	8.5±0.212	25.16±5.48[c]	10.5±0.96[a,c]	7.17±4.49[n,c,n]
ALT (U/l)	17.5±3.27	24.62±3.68[b]	2.98±2.03[d,d]	3.48±0.44[c,d,n]
ALP (U/l)	290.70±111.43	683.56±219.52[b]	546.6±74.07[n,n]	452.09±309.43[n,n,n]
BILIRUBIN (mg/dl)	0.4±0.0967	2.03±0.53[c]	0.566±0.04[b,c]	0.45±0.208[n,c,n]

The data is expressed as mean ± standard deviation.

1= As compared with control, 2= As compared with CCl4, 3= As compared with CCl4+Taurine, a= P<0.05, b= P<0.01, c= P<0.001, n= P>0.05(Non-significant)

Table III. Comparison of Liver antioxidant enzymes and MDA activity in Control, CCl4 treated, CCl4 +Taurine treated &Taurine treated groups

	CONTROL (n=6)	CCl4[1] (n=6)	CCL4 + T[1,2] (n=6)	TAURINE[1,2,3] (n=6)
Catalase (µmol/g tissue)	17.312±2.183	4.831±0.685[d]	14.63±4.76[n,b]	1.28±0.17[d,a,b]
SOD (Unit/g tissue)	1.436±0.638	0.422±0.2511[a]	1.7±0.25[n,d]	6.36±1.43[n,c,a]
GSH (Unit/g tissue)	0.811±0.08	0.0214±0.0015[d]	0.03±0.003[a,a]	0.08±0[a,b,a]
MDA (µmol/g tissue)	0.906±0.327	1.710±0.7166[a]	1.51±0.61[a,n]	0.806±0.205[n,a,a]

The data is expressed as mean ± standard deviation.

1= As compared with control, 2= As compared with CCl4, 3= As compared with CCl4+Taurine, a= P<0.05, b= P<0.01, c= P<0.001, n= P>0.05(Non-significant)

Table IV.Histopathological features in Control, CCl4 treated, CCl4 +Taurine treated &Taurine treated groups

Histopathological findings	Control	CCl4	CCl4 + Taurine	Taurine
Enlargement	0	0	0	0
Paleness	0	0	0	0
Fatty change	0	0	0	0
Hydropic degeneration	0	0	0	0
Portal inflammation	0	2	0	0
Periportal inflammation	0	2	0	0
portal fibrosis	0	2	0	0
Periportal fibrosis	0	2	1	1
Focal lobulitis	0	2	0	0
Total Score	0	10	01	01
Intracellular pigment deposition	absent	present	absent	Absent
Sinusoidal expansion	absent	absent	Marked	mild
Degenerative hepatocytes	absent	30-40-%	absent	Absent
Ballooning degeneration	absent	absent	absent	Absent
Bile duct proliferation	absent	absent	slight	absent

Degree of hepatic injury is expressed as scores observed via light microscopy. Score 0 = no visible damage; Score 1 = focal hepatocyte damage on less than 25% of the tissue; Score 2 = focal hepatocyte damage on 25-50% of the tissue; Score 3 = extensive, but focal hepatocyte lesion; Score 4 = global hepatocyte necrosis

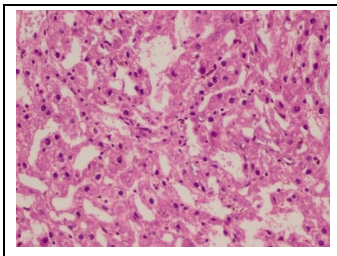

Figure 1. Control

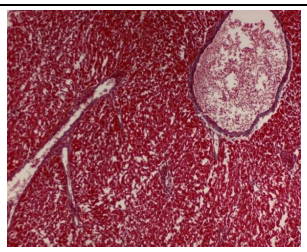

Figure 2. CCl4 treated

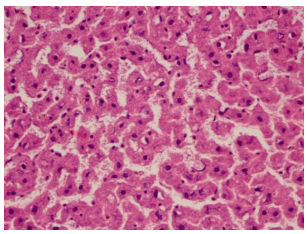

Figure 3. CCl4 + Taurine treated

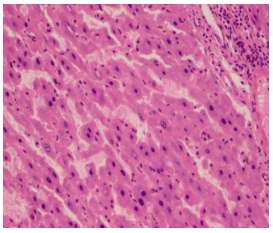

Figure 4. Taurine treated

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RESEARCH ARTICLE

The Anti-lipidemic Effect of *Juglan regia* Linn. in Polycystic Ovarian Syndrome Rats Treated with Letrozole

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ABSTRACT

Polycystic ovary syndrome (PCOS) remains a disorder of unknown etiology, characterized by metabolic features which if left untreated can lead to serious medical conditions. A cardiovascular disease including dyslipidemia is one of the consequences of PCOS. *Juglan regia* L. (walnut) is a source of monounsaturated fats (MUFAs) and polyunsaturated fats (PUFAs) and evidently lowers cholesterol by reducing low-density lipoprotein concentration. Unsaturated fatty acidsameliorate the cholesterol profile inPCOS women.This research was conducted to determine the effect of *Juglans regia L.* on cholesterol profile of letrozole induced PCOS rats. Aged matched Wistar rats were divided into three groups: Control which remained untreated letrozole treated and letrozole + walnut treated.PCOS model was developed by administering a dose of 1mg/kg body weight of letrozole per day for 21 days. Group III rats were given cotreatment of walnut oil for 15 days. To evaluate the estrus cycle vaginal smears were taken daily at 10 am.Their body weight was observed daily. On 22th day they were sacrificed for biochemical assessments. As indicated by the vaginal smear PCOS ratsremained in diestrus phase throughout the experiment furthermore Letrozole treated rats showed high values of cholesterol (p=0.001) and low-density lipoprotein (LDL) (p=0.0001) as compared to that of control group while Walnut +Letrozole treated PCOS rats demonstrated significant reduction in cholesterol levels (p=0.004) with a slight increase in high-density lipoprotein (p=0.02) and significant decrease in LDL (p=0.0001). Body weights remained constant after an initial decrease. Our data supported the hypothesis that walnut can lower the cholesterol profile which is important for women with PCOS in lowering the risk of dyslipidemia.

Key Words: Walnut, Letrozole, Cholesterol, Dyslipidemia, Obesity

INTRODUCTION

Poly Cystic Ovary Syndrome (PCOS) is an idiopathic disorder affecting 6% to 21 % females(1). PCOS is presented with reproductive, psychological and metabolic manifestations, that cause ovarian dysfunction, hormonal disruption including abnormal gonadotrophin, insulin and androgenconcentrations(2). It is a heterogeneous condition and the phenotype varies widely depending upon age, ethnicity, genotype and environmental factors including physical activity and bodyweight(3). Untreated PCOS complicates fertility leading to Gestational diabetes pre-eclampsia, miscarriage or premature births. It also significantly increases the risk of cardiovascular disease. PCOS sufferer have difficulty handling energy balance and have high tendency to gain weight (4)(5) leading to an increase in body mass index BMI. Which also causes dyslipidemia of varying patterns in PCOS patients(6), (7). There are also many reported types and extent of lipid aberrations seenassociated with PCOS (8)(9) including evidence of decreased HDL cholesterol, enhanced triglyceride levels similar lipid profile is seen associated with insulin resistance (7) and very-low-density-lipoprotein levels (8). PCOS women have elevated waist/hip ratio (i.e. centrally distributed fat), making them more vulnerable and prone to dyslipidemia since this distribution exerts an adverse effect on blood lipids (10). Visceral obesity predispose to CVD and diabetes, insulin resistance, hypertension and dyslipidemia (11). These risk factors promote increased risk of endothelial dysfunction, coronary artery calcification and increased carotid artery intima media thickness in PCOS (12). Followingstudies provesthat women with features of PCOS have increased risk of coronary artery disease (13)(14). Because CVD is characterized by a long incubation period, metabolic abnormalities observed in the teens and twenties among PCOS women might translate into measurable vascular abnormalities by middle age long-standing exposure at an early age to an adverse cardiovascular profile, as observed among women with PCOS, leads to premature atherosclerotic changes (15).

Juglan regia (walnut) is a nut with high nutritional value and isenrichedwith omega 3 and 6 poly unsaturated fatty acids (PUFAs) of which ω -6 PUFAs is thought to be associated with an increased proinflammatory vascular response. Researchers have concluded that consuming ω-3 and ω-6 PUFAs of walnuts (30-100 g/day) regularly lowers CVD risk factors in non-hyperlipidemic individuals (16). Aortic endothelin 1 (ET-1) concentration and other CVD risk markers in hamsters fed with high fatty acids diets were measured by Davis and colleagues with the inclusion of walnuts, and concluded that ET-1 regulator levels reduced with increased Walnut consumption (17). We conducted our research to demonstrate the lipid lowering effects of walnut oilin rats to decrease the risk of developing dyslipidemia which is associated with PCOS.

MATERIALS AND METHODS

This research followed health research extension act of 1985 and Ethical Guidelines of Institutional ERB. Standards measures of animal care were insured. Rats were purchased from (DUHS) Dow University of Health and Sciences, Ojha Campus. Controlled condition of 12-h light/12-h dark cycle at 24-26 C temperature was maintained and rats were allowed free access to standard diet and water.

Walnut Oil Hydraulic Press Extraction

Walnuts (dried) were purchased from local Sadar Bazar Karachi and then grounded into small size between 1-3 mm. Pilot plant hydraulic press was used for oil extraction. A pressure of 60 kg cm–2 was applied on 1 kg nuts foratleast 10 minutes. Extracted oil was then centrifuged to eliminate remaining solid particle.

Study Procedure

The rats were divided into three groups (n=6). Group I (control group) was given no treatment. Group II (letrozole treated) was treated with letrozole approx. 1 mg/body weight daily for 21 days and Group III (letrozole + walnut oil

treated group) was administered with letrozole + Walnut oil in concentrations 1 mg/kg and 1 ml/kg body weight daily for 21 and 15 days respectively. letrozole was given every morning for 21 days, to induces acyclicity in their estrus cycle and develop a PCOS model(18), (19) and (20). Group II and III were administered with letrozole stock solution (10mg in 50 ml 0.9% NaCl) Enterally via gavage.PCOS model was confirmed once the rats were in diestrus phase. Cotreatment of walnut oil was given to Group III PCOS rats for 15 days.

Vaginal Smear Preparation

Vaginal cytology of rats was observed for 21 days to determine their estrus phase microscopically. Vaginal smears were obtained using cotton swab, dipped in normal saline (0.9%). The swab wasswiped against vaginal wall at an angle of about 45° to obtain cells. Slides were prepared by heat fixation and then stained with crystal violet. Slides were examined 10x and 40x magnification.Estrous phase was determinedby identifying the type of cells present (cornified epithelial, leukocytes, and nucleated epithelial cells)(21).

Biochemical Assessments

Control, Letrozole treated and Letrozole + Walnut treated rats were assessed for Total cholesterol (22), LDL and High-density lipoprotein by(23).

Statistical analysis

Our results are shown by using mean ± standard error of mean. SPSS (Statistical Package for the Social Sciences) Version-16 was used for statistical analysis. (ANOVA) one-way analysis of variance followed by the (Least Significance Difference Post hoc multiple comparison test) called as LSD test was done for Statistical significance and comparison between different groups, Statistical significance was tested minimally at $p<0.05$.

RESULTS AND DISCUSSION

Identification Tool for Estrus cycle

The identification of each phase depends on the proportion cells prese in the vaginal smear:

- Estrus phase:contain cornified cells which are large,irregularly shaped and non-nucleated.
- Pro-estrus phase:has epithelial cells which are mostly nucleated and have a granular appearance.
- Metestrus phase: identified by epithelial cells which are non-nucleated and less granular.
- Diestrus:has Leucocytes which are very small and round (24)

Dyslipidemia is seen associated with Obesity and Hyperandrogenemia which are also very common in PCOS however, the pathologic mechanisms involved in their association is still unknown. Body weight of rats changed throughout the experiment.There was an initial increase in weight of Walnut + Letrozole treated rats and then it remained constant as compared to Letrozole treated rats which showed an initial increase, an abrupt fall and then a rise in weights (Graph=1). Dyslipidemia is also linked with the effects of testosterone on adipocytes of PCOS women (25) and Insulin Resistance (IR). 81% of IR PCOS patients have lipid abnormalities, compared with 65% of those that don't have insulin sensitivity (6). Several studies have indicated the lipid-lowering effect of walnut. (26) because of the presence of unsaturated fatty acids. Total cholesterol profile of Walnut + Letrozole treated rats significantly decreased with the p-value ($p<0.004$) (Graph 2) as compared with only Letrozole treated rats (=0.001) which showed a high cholesterol level, confirming the state of dyslipidemia in PCOS.

Walnut is especially rich in linoleic= 57%-76% and linolenic =2%-16 % acids). (27) showed that linolenic acid can reduce triglyceride levels and decreases the risk of coronary artery disease (26), these properties of Walnut makes it an efficient herb for reducing cholesterol and lipid levels in PCOS patients. High-density lipoprotein levels of rats who were administered with walnut oil showed a significant decrease with a p-value of HDL lied on the significant side being $p<0.022$ (Graph 3).

Our data supports the studies conducted by (27) and (26) that proved that dietary α-linolenic acid, oleic acid and linoleic acid helps lower blood cholesterol levels. The biochemical assessments showed significant reduction in LDL levels ($p<0.0001$) of Walnut + Letrozole treated rats when compared with letrozole treated group (Graph 4).

CONCLUSION

In conclusion walnut oil can be considered as the best alternative to modern medicine. It is beneficial to health and when taken in right doses it provides protection against many CVD including dyslipidemia which is a questionable manifestation of PCOS.

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LIST OF ABBREVIATIONS

PCOS = Polycystic Ovary Syndrome.
MUFAs = Mono-unsaturated Fatty Acids.
PUFAs = Poly-unsaturated Fatty Acids.
HDL = High-Density Lipoprotein.
LDL = Low-Density Lipoprotein.
CVD = Cardio-Vascular Diseases.
BMI = Body Mass Index.
DUHS = Dow University of Health and Sciences.
ET-1 =Endothelin 1
LSD = Least Significance Difference Post hoc multiple comparison test.

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Table. 1. Images demonstrates the vaginal smear of rats on the 1st day of experiment. Control and letrozole + Walnut treated group show cornified cells, Letrozole slide shows leukocytes

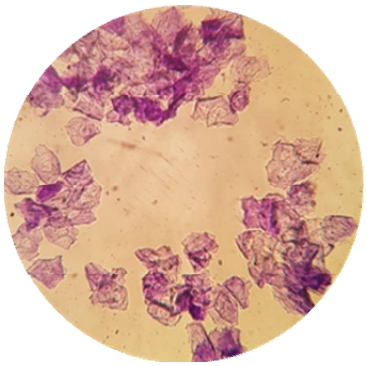
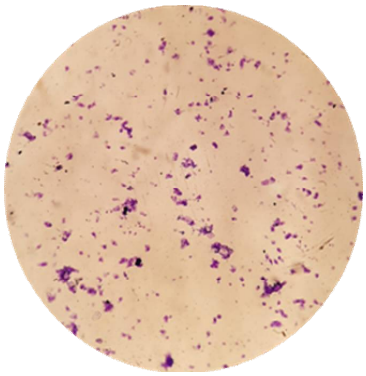
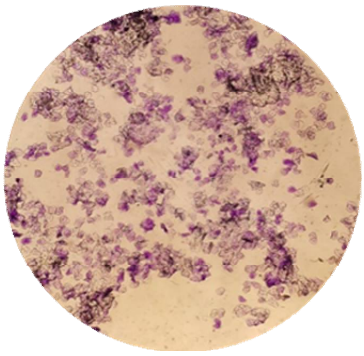
DAY: 1		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE+WALNUT TREATED**
		
ESTRUS	**DIESTRUS**	**ESTRUS**

Table. 2. Table of vaginal smear taken on 6th day.Diestrus phase indicates PCOS in rats. All the slides show leukocytes confirming diestrus phase.

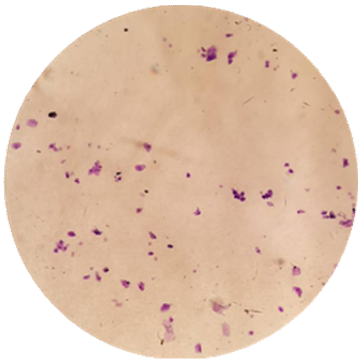
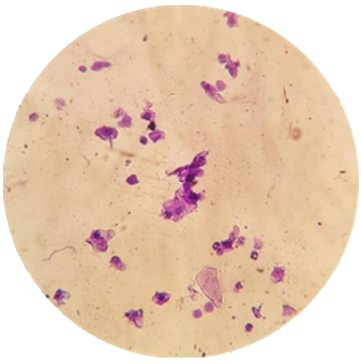
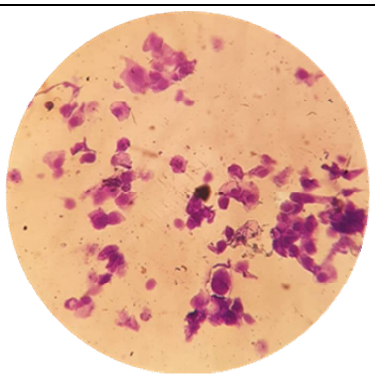
DAY: 6		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE+WALNUT TREATED**
		
DIESTRUS	**DIESTRUS**	**DIESTRUS**

Table.3. Table of vaginal smear taken on 15th day.Diestrus phase and Metestrus phase

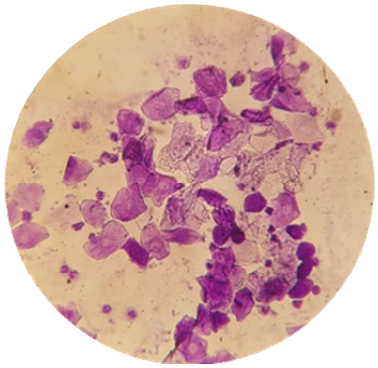
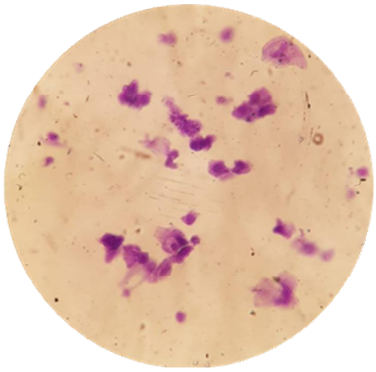
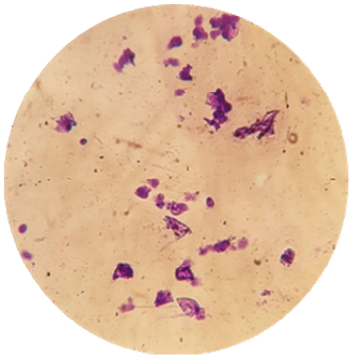
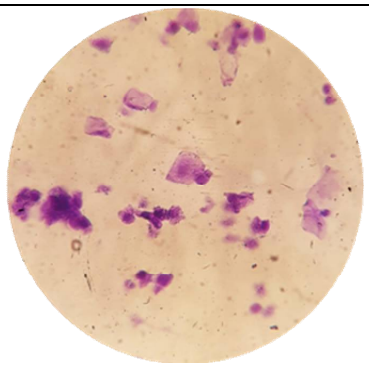
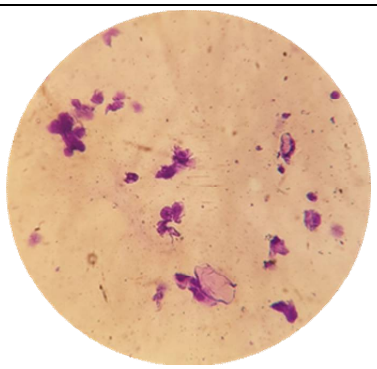
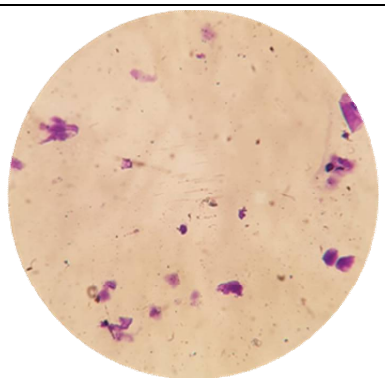
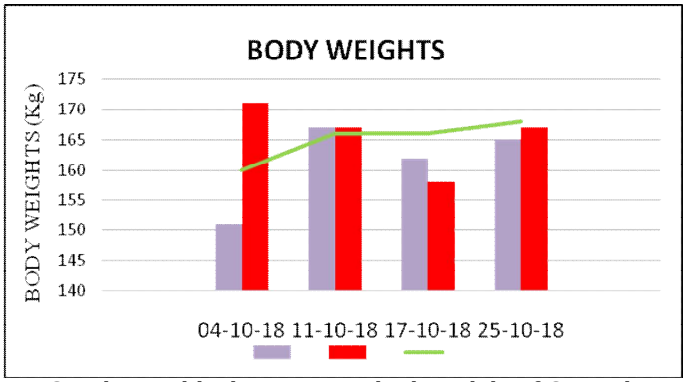
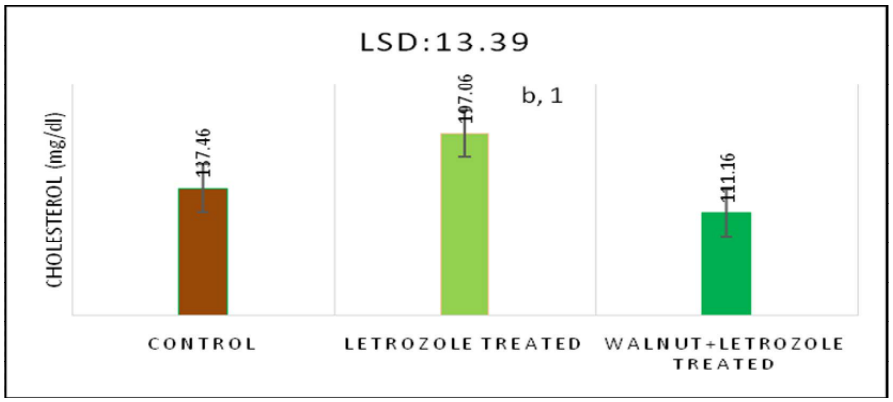
DAY: 15		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE+WALNUT TREATED**
		
METESTRUS	**DIESTRUS**	**DIESTRUS**

Table. 4. No change in the vaginal smear was observed on the last day. All the slides show leukocyte cells

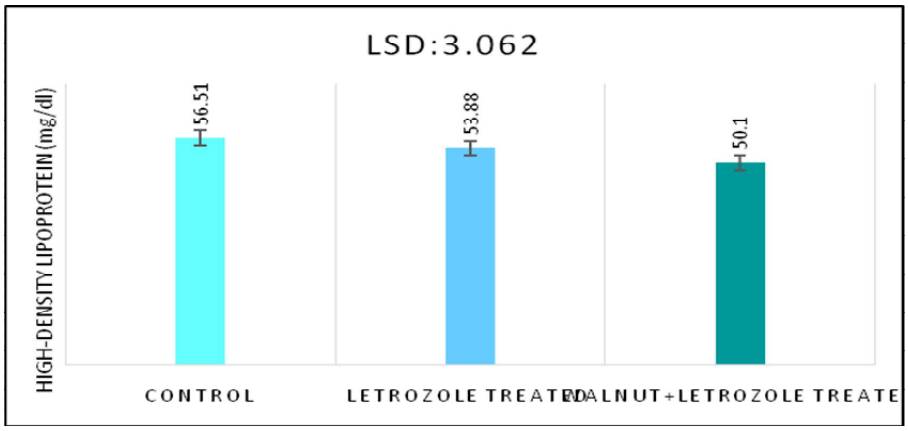
DAY: 21		
CONTROL GROUP	**LETROZOLE TREATED**	**LETROZOLE+WALNUT TREATED**
		
DIESTRUS	**DIESTRUS**	**DIESTRUS**



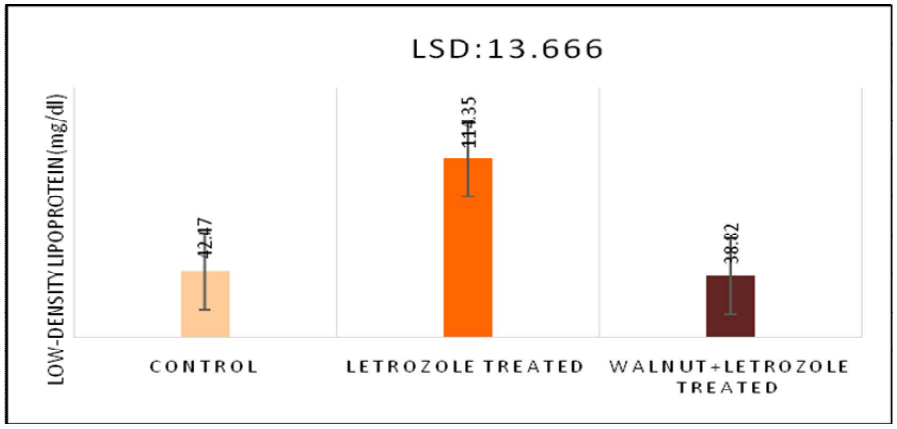
Graph. 1. Table demonstrates body weight of Control (C), Letrozole treated (LT) and Letrozole + Walnut treated (LWT) rats.



Graph. 2. Biochemical Assessment of Cholesterol of Control, Letrozole treated, Walnut + Letrozole treated. 1= when compared with Control, 2= when compared with Letrozole Treated. Where, Similar alphabets = non-significant & Unique Alphabets = significant.



Graph. 3. Biochemical Assessment of High-density Lipoprotein of Control, Letrozole treated, Walnut + Letrozole treated.



Graph. 4. Biochemical Assessment of Low-density Lipoprotein of Control, Letrozole treated, Walnut Letrozole treated.

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RESEARCH ARTICLE

Smart Home Prototype Controller using Human Hand Locations within Head and Shoulders Level

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ABSTRACT

Human hand detection has significant role in computer vision application. In this study, an effective smart home prototype designed of low cost andcontrolled switchON/OFFeight LEDs using human hand. The hand is detected in real time video by train computer to detect human hand using Matlab software to obtain an XML-file of hand classification model. The computer is trained based on Viola-Jones method using two image datasets which are positive (hand) and negative (non-hand).Where the location and size of hand of all positive images is reguired. In this paper, a new idea is used to determine location and size of positive images automatically using skin color detection.Eight hand cases of different location within head and shoulders level are detected using XML- training file. The eight hand cases used to control ON/OFF eight LEDsin prototype. The delay time between showing hand case in front of webcam and LEDs controlling is 0.3 second.

Keywords: smart home, Viola-Jones method, hand detection, Arduino.

INTRODUCTION

Computer vision field used to train computer to see and recognize different objects. Computer vision development has great advancements to create a natural interaction between human and computer.The basic goal of Human Computer Interaction,such as hand, head, facial expression, voice and touch,is making the computer more receptive to user needs[1].Intelligent human computer interaction in smart home environments is getting a lotof attentions from researchers in many fields such as architectural, electrical, computer, and biomedical engineering. This kind of human-computer interfaces would allow a human user to control remotely wide variety of devices like hand which consideredvery simple and natural body language.This kind of intereaction helps disabled and elderly people to

communicate to the world around them by using their gesture. In particular, a hand gesture-based human and machine interface is a more intuitive, natural, and intelligent way thanthe traditional interface methodologies [2, 3]. The problem in hand detection is how to make computer understands hand movement. Hand detection achived using both non-visionand vision approaches [3]. In general vision based approaches are more natural as they require no devices instead it depends on web camera to extracts the video frame while non-vision approaches required sensors (optical or mechanical) which hinders the ease and naturalness of the user interaction with surrounding[1]. Many researches developed hand detection systems such as Qing Chen *et.al.*[4] to recognize hand gestures in real time with a single web camera as an input device.It achived in two steps, the first is real-time recognition for hand postures using Haar-like features and the AdaBoost learning algorithms.

The second, the hand gesture recognition implemented using the syntactic analysis that based on a stochastic context-free grammar. Van-Toi NGUYEN*et.al.*[5] proposed a framework of hand detection method based on Viola-Jones face detector. This method compolsury focused on the inside hand region only to extract features. While 2015 Ruchi M. Gurav, and Premanand K. Kadbe detects hand gesture using AdaBoost based hand-pose detectors.Theexperiment is developed on open source library for computer vision application called Open ComputerVision Library (OpenCV).They detect the hand of live images but only for two gestures[1]. Aashni Haria *et.al.* designed hand gesture recognition system using Haar cascade classifier and then translate detected hand gesture into softwarecommands such as opening websites and launching applications like VLC Player and PowerPoint[3]. In this work,a prototype system has been designed to be used as a smart home controller.This system consist of acomputer, Arduino card,and abreadboardwith eight LEDs. The location and size of region of interest (human hand) determined automatically based on skin color detection algorithm.Matlab software is used to generate XML-file to detect hand based on hand features that saved in XML-file.A suggested algorithm designed to detect human hand in different location within head and shoulders levels.Ultimately, eight hand cases studied used to control LEDs switching in smart home prototype.

METHODS AND ALGORITHMS

Human hand can be detected depends onset of features by training hand dataset basedViola Jones method for object detection [6].Viola-Jones used in their method many stages: integral image, Haar features, Adaboost algorithm, and cascade classifier. The first step in Viola Jones algorithm is convert input image into integral image to reduce computation time of Haar features.Haar-like features (so called because they are computed similarly to the coefficients of Haar wavelet transforms) were used combined with the Adaboost algorithm to extract the features characteristics of the hands [7]. Each Haar-like feature $J(x)$ consists of two, three, or four connected “black” and “white” rectangles. The value of a Haar-like feature is the difference between the sums of the pixel values in the black and white rectangle, i.e.[1],

$$f(x) = \sum_{x=black} I(x) - \sum_{x=white} I(x) \qquad (1)$$

where $I(x)$ is input image pixel value.

Adaboost technique algorithm based on the learningmachine idea that combine hand features in all directions of Haar feature[10, 11].A cascade of classifiers is a degenerated decision tree consist of stages of increasing complexity.Thefirst stage of classifier trained to detect almost all objects of interest (hand). This triggers the evaluation of classifier of the second stage which has also adjustedto achieve very high detection rate. A positive resultfrom the second stage triggers a third stage, and soon[13].

Experimental details

Human hand detected based on Viola-Jones algorithm using Matlab cascade object detector. Cascade training includes processing steps for training the system to detect human hand in a complex background.Matlab code *train Cascade Object Detector* used to get hand classification model configured as XML-file whichused to detect hand in iuput image. In this paper, XML- file is used to discriminatehand within head and shoulder level where theimage plane divided into nine parts as explained in (Algorithm to Detect hand location) section.

Environment Setup and Tools

The experiment was done using the available environment conditions and it can be changed by keeping the same circumstances. The room dimensions were 7m length, 3.7m width, and 3m height. The distance between webcam and hands was 100 cm with appropriate lighting condition for detection of hand. Environment lightening with two LED strip light of 15watt powerused to get 70 Lux that measured in Luxmeter device. Figure (1) shows the diagram of the environment experiment.The camera which has been used for capturing the positive dataset of hand is the built-in Webcam of a computer with a spatial resolution of 1280x720 pixel and a temporal resolution 30 fps. The camera also used to test real time detection after trainging. Tools andhardware of the electronic system thatshown in Fig. (2) are eight LEDs which turns ON/OFF when it receives commands, Arduino-Uno card provide voltage of (5 volts) to LEDs, Jumper wire to connect LEDs into Arduino, Breadboard to connect LEDs and jumper wire on it, and USB cable to connect Arduino to computer.

Electronic System Setting

Electronic system tools assymbled using eight LEDs fixedon breadboardwhich wired to the Arduino that gives 5 volts as an output. The negative end of each LED connected to the ground GND of Arduino, and the positive end connectedto the digital output pins.The connection of the prototype system is illustrated in Fig. (3) with real and schematic diagram.

Algorithm steps to detect hand

Cascade Training Algorithm

Cascade trainingis used to train computer to detect region of intereset in complex background using negative and positive dataset. In this paper, thefirst set includes 12000 negative images that does not contain the Region of Interest (ROI) which is in this study a hand, whereas, the second set includes 2900 positive images of ROI. Positive images set contain ROI ofpalm posture captured using the built-in webcam of a computer with a resolution of 1280x720 pixelwith a temporal resolution of 30 fps. These images captured with different lighting conditions with different distance from the camera. The dataset is collected usingdifferent size, color, tilt, and orientation of hands.Figure (4) shows example of positive and negative images that used in the training.

Positive target labelingis a process in Matlab software to label ROI using *Image Labeler* application. This process gives two column table or two-field structure known as positive samples.The first table column or structure field contains image file names, specified as character vectors.The second table column or structure field contains an *M*-by-4 matrix of *M* bounding boxes enclosed ROI. Each bounding box has the format [*x, y, width, height*] to specify an object location in the corresponding image.This process is time consumption because the labeling must be implemented manually for each positive image. Therefore, this study introduced a new idea to determine the label of ROI automatically depending on skin detection method. This technique gives the same details as image labeler application by means of x, y coordinate of upper left corner of rectangle enclosed positive target, with width and height of rectangle. Figure

(5) shows skin detection of positive image and the enclosed rectangle of detected skin of positive image. For cascade training two sets of database are required positive samples database and negative images.The databases used to train computer for hand detection depending on *train Cascade Object Detector* Matlab function.The training based on cascade of boosted classifier of Haar-like features.An XML-file of hand detection is resulted to be used in any computer which contain hand features. Figure (6) shows block diagram of training process.

Algorithm to Detect hand location

Different locations of hand are detected within head and shoulders level using algorithm steps showed in Fig. (7). In real- time detection,one image framethat selected from the real-time video is divided into nineregions (blocks).Figure (8) shows the divided image frame into nine blocks.The reason behind this is to determine the location of hand within shoulder and head level.These nine blocks scanned by XML-file in order to detect hand and determine its location.As a results, blocks B2 and B8 represent head level while blocks B3 and B9 demonstrate shoulders level.The remaining blocks are not checked in this study because the hand in these blocks is not commonly appeared. However, it is easy to study these blocks in case to extend the project. The probability of the hand cases locations is shown in table (1).For one raised hand case, a specific decimal code numbers *CN* suggested and equal to 4, 8, 1, and 2 for the blocks B2, B3, B8, and B9, respectively.In case two raised hand, the *CN* became 5, 6, 9, and 10 according to hand case appearance. The output index (*id*) is used to arrange the cases ascendingly in this study only. The LED ON/OFF status is controlled using hand case of specific *id* number which sent to Arduino as acommand.

RESULTS

In this work, a new prototype system presented to employ right and left human hand to control switch LEDs ON/OFF. Human hand is detected by training computer based on Viola-Jones method using Haar-like features. Skin detection method adopted to develop the hand detection techniques. The adopted method helps to make the computer training more accurate and faster. Eight hand cases studied to control set of LEDs in a smart home prototype. These cases are choosen to show the realiablity of the suggested prototype to be extended to other applications. The studied cases depends on the position of human hands compared with head and shoulders. Depending on the hand position, a cetain command can be send to the prototype. The first step, showing hand in front of the webcam to control LEDs in prototype in real-time. Then the image frame is segmented into nine regions to determine hand location using an XML-file of training. Second step, determine hand position regarded to head and shoulders level depending on the suggested algorithm showed in Fig. (7).

After that, the suggested method organized the hand position cases into index table to send command to the Arduino to control specific LEDs. Input hand case is used to turn LED ON and it also used to turn the same LED OFF in case it appears again. The lighting condition is vital to detect hand in different location, snd the luxemeter reading was 70 lux which gives appropriate results. All the cases are not shown within this paper because it is beyond the paper limit. Figure (9) shows some hand cases that used to control threeLEDs ON/OFF. Hand cases illustrated in table (1) used to turn 8 LEDs ON/OFF.For instance, the hand case of id=1 used to turn ON/OFF LED 1. The response time of algorithm to detect hand cases and determine output index is of 0.4 secand the delay time between showing hand in front of camera and LED turned ON/OFF is of 0.5 sec. The response time and delay time is determined of suggested algorithm that implemented using Matlab R2018a runs on Lenovo ideapad 520 laptop of Windows 10, 8th Gen Intel® Core™ i7 processing of 16 GB RAM. The response time and delay time depend on computer processor and Matlab version.

CONCLUSIONS

The suggested setup and algorithm gives a reliable performance of using Arduino board that can be applied in smart home prototype. The introduced assembled systemis low cost and used only eight LEDs and can be extended more. This system designed to detect hand and turn ON/OFF LEDs using eight cases of detected hand.From the results, the response time of LEDs to switch ON/OFF is 0.5 second. While, the hand cases detected using software part is of 0.4 second. The eight hand cases detected with accuracy of 100%.

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Table 1. Input and output of hand cases.

Input hand case		Code number (*CN*)	Output index (*id*)	LED No.	Input hand case		Code number (*CN*)	Output index (*id*)	LED No.
Right hand	Left hand				Right hand	Left hand			
		1	1	LED 1			5	5	LED 5
		2	2	LED 2			10	6	LED 6
		4	3	LED 3			9	7	LED 7
		8	4	LED 4			6	8	LED 8

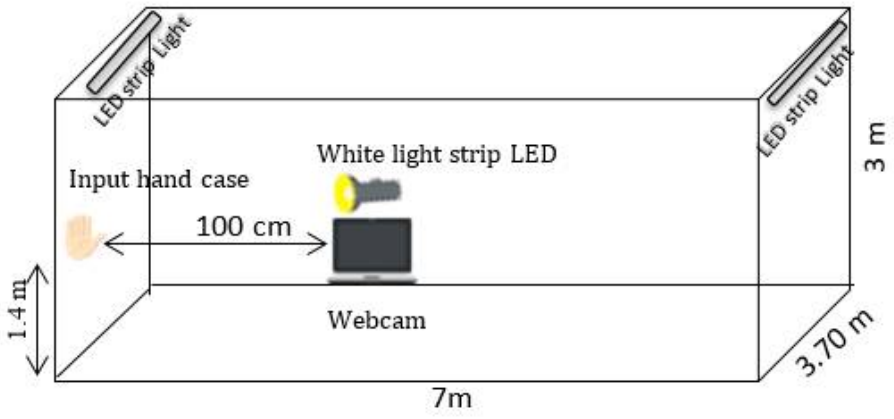


Figure 1. Sketch of the Setup System Enviroment.

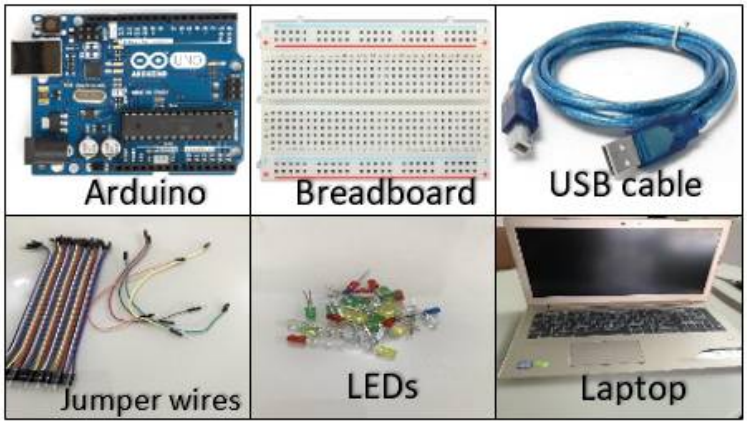


Figure 2. Hardware components of the suggested system.

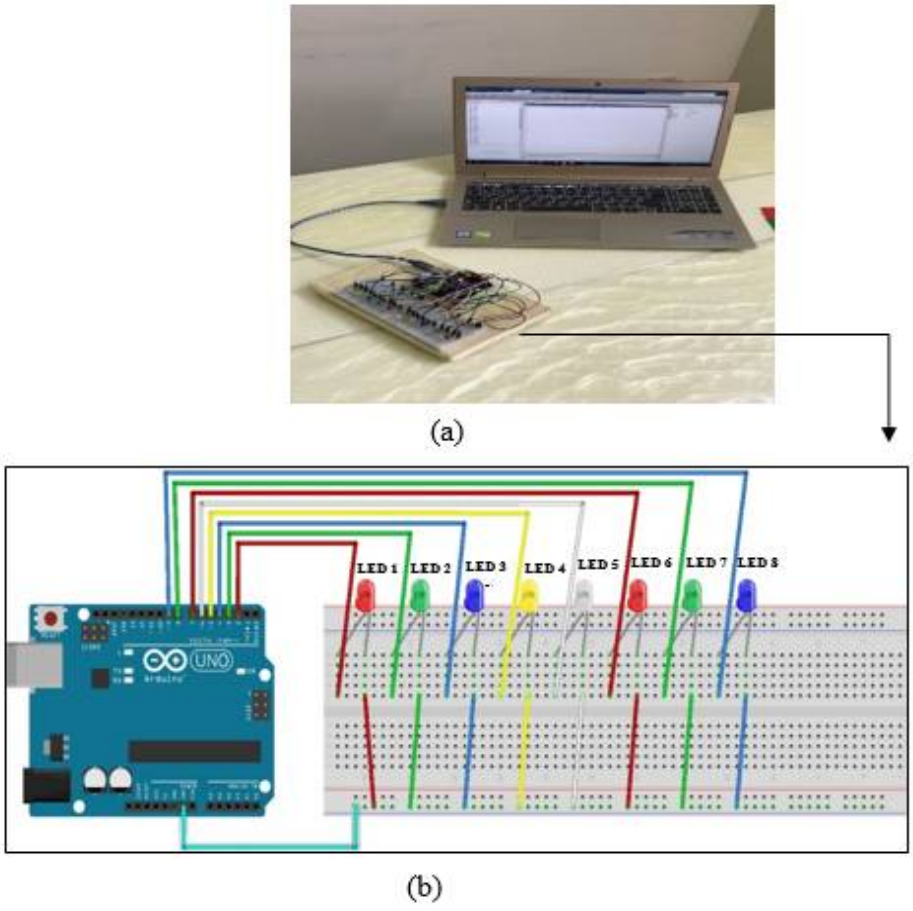


Figure 3. (a) The suggestedprototype system, (b) Schematic of the introduced system.

Figure 4. the training images (a) positive and (b) negative.

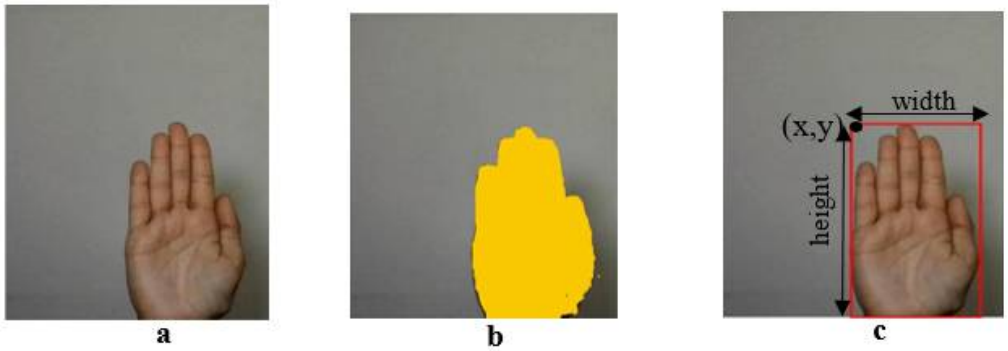


Figure .5. (a) positive image, (b) detected skin of positive image, (c) rectangle information

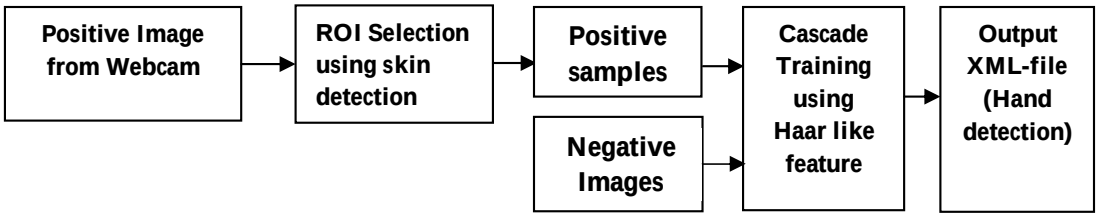


Figure 6. Block diagram of training using cascade hand detector.

Input:

- image frame (*I*) extracted from input real-time video.
- XML- training file.
- Arduino object definition as (*a*).

Output:

- Indices *id*s of hand case.
- LEDs controlling.

Step 1: Divide image frame (*I*) into nine regions as in Fig. (8)using:

$rr = \frac{r}{3}$ and $cc = \frac{c}{3}$ where *r* and *c* represent length and width of (*I*), *rr* and *cc* are the dimension of one reigon.

Step 2: using XML file to check the subdivided regions.

Step 3: Determine code number *CN* and index number*id*.

Step 4: Send the index *id* to *a* to control specific LED.

Figure 7. Hand location detection Algorithm.

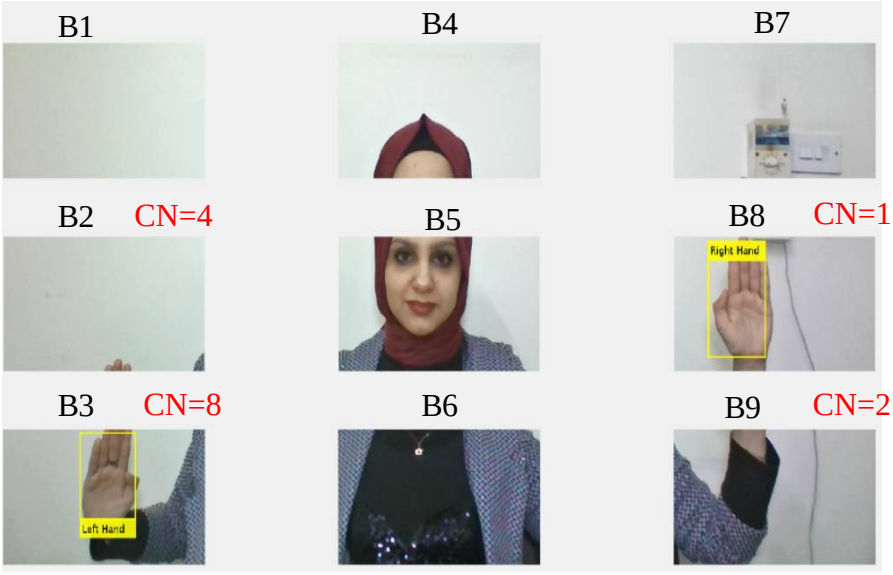
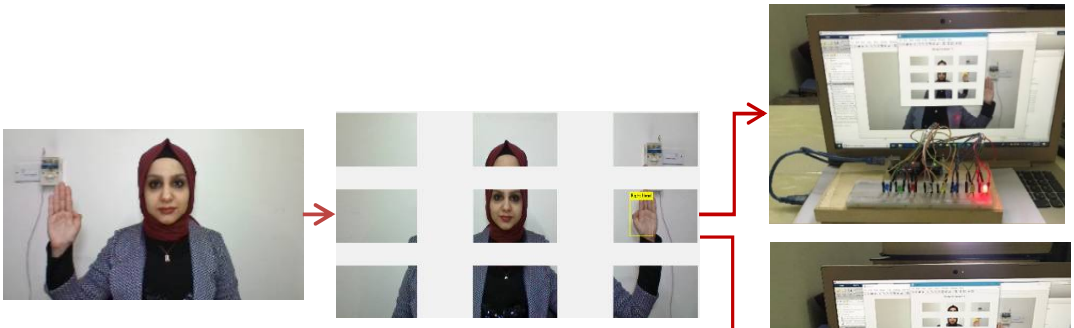
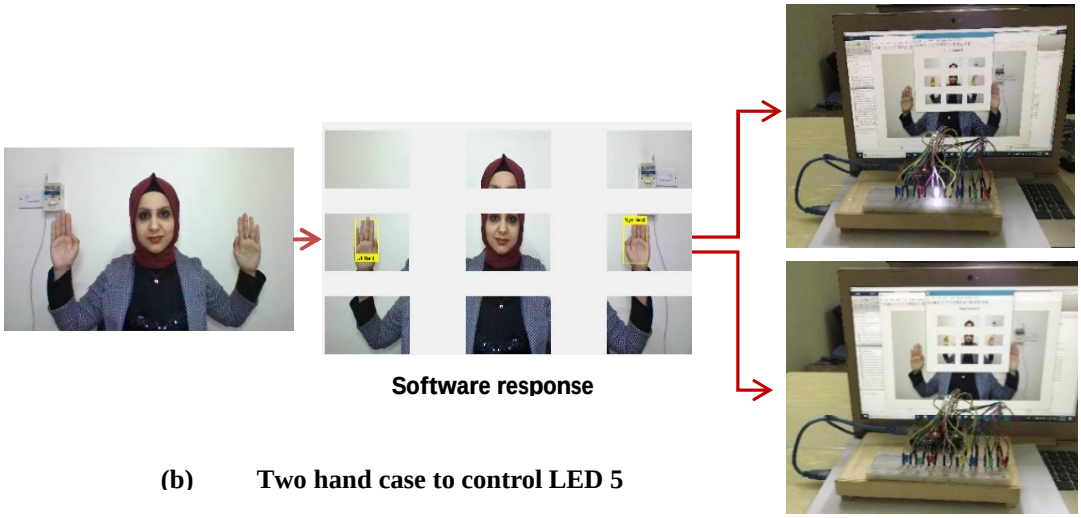


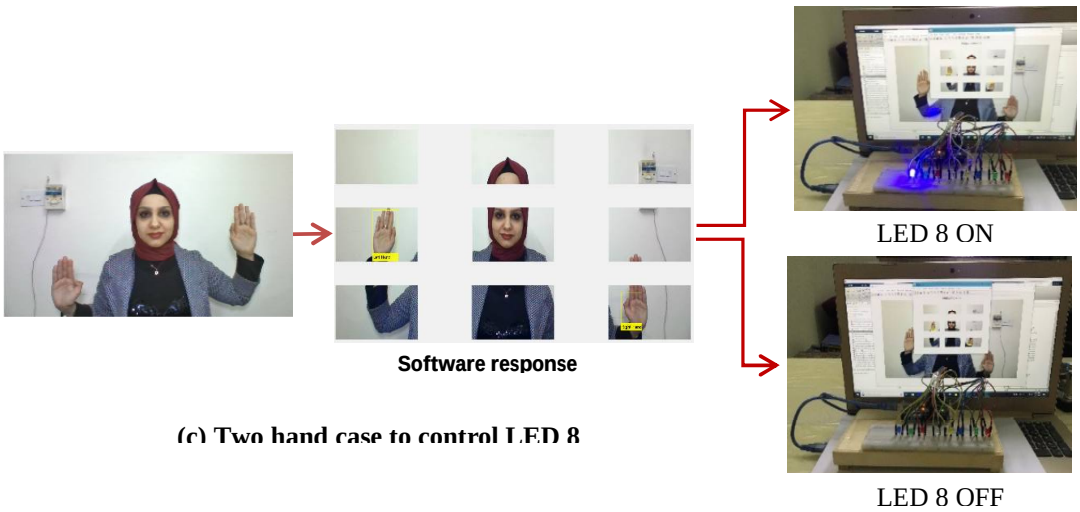
Figure 8. Frame input image divided into nine regions with its region label.



(a) One hand case to turn ON/OFFLED 1



(b) Two hand case to control LED 5



(c) Two hand case to control LED 8

Figure 9. Input image cases and output actions

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RESEARCH ARTICLE

Is AHSG Gene Polymorphisms (rs4918) Association with T2DM in Iraqi Population?

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ABSTRACT

The relationship of AHSG gene polymorphisms (rs4918) with the incidence of Iraqi diabetic patients was evaluated in this study. Two hundred subjects about 100 of them are with type two diabetes mellitus and 100 apparently healthy individuals were used in this study. Blood samples were obtained for DNA analysis and biochemical parameters measurements. Genotyping of the AHSG gene was carried out by using Taq man Polymerase chain reaction assay, non-Significant differences were observed in allele and genotype frequencies of AHSG gene polymorphisms (rs4918) among studied groups. However, age, BMI, HbA1c levels, fasting blood sugar and lipid profile were observed in T2DM patient carriers of CT genotype. There was significantly elevation of C allele in T2DM Compared with control (11% versus 9%) and increase risk of T2DM among them whom compared with healthy (OR 1. 24, χ^2 0.22). The GC and GG genotypes were significantly differences in patients compared with healthy individual, the risk of T2DM was increased tow times for the GC phenotype (OR 2.27, χ^2 8.11) than in GG phenotype (OR 0.4, χ^2 9.74).

Keywords: AHSG, gene, diabetic, Genotype, sugar, Polymerase, biochemical, analysis .

INTRODUCTION

People with type 2 diabetes mellitus (T2DM) have a higher cardiovascular morbidity and mortality risk, and are disproportionately affected by cardiovascular disease (CVD) compared with non-diabetic subjects (1). Fetuin -A- is a negative acute phase glycoprotein that synthesis and secreted from the liver (2). It have effect on insulin receptor, where fetuin -A- binds reversibly to the insulin receptor tyrosine kinase in peripheral tissues and inhibit the insulin induced intracellular signal cascade (3). The changes in the fetuin -A- level may influences the body defense mechanisms such as inflammatory regulation. The reduction of fetuin -A- level are linked to many disease such as

T2DM (4), fatty liver (5), adipocyte dysfunction (6), obesity (2008), and metabolic syndrome (8). Fetuin -A- is encoded by α2- Heremans-Schmid glycoprotein (AHSG) gene, which located on chromosome 3q27 (9). The most common SPN is thr256>ser in exon7, which was examined in this study as it thought to have a role in the development of DM. Type 2 diabetes mellitus is the most common type of diabetes (90% of cases) (10). It is effected by the genetic factor and/or environmental factor, some of the environmental factor are obesity, no or less physical activity, old ages, family history and some time the race (11), those factor may affect the pancreatic β-cell which lead to effect the production or secretion of the insulin which lead to insulin resistance which is the main causes of the T2DM (12).

Kotronen and Yki-Jarvinen (2008) found that fetuin-A levels were negatively correlated with HDL-C (13). While Khalil and Kuobaili (2013) reported that elevated serum fetuin-A levels in T2DM patients were significantly associated with atherogenic dyslipidemia (14), thus indicating that fetuin-A- may be one of the contributing factors to the increased incidence of coronary heart disease (CHD) in T2DM patients. Ix *et al*, (2012) reported that higher level of fetuin-A was associated with higher triglycerides, and LDL-C (15). Because of AHSG gene polymorphisms (rs4918) are involved in the pathogenesis of T2DM, and activated insulin resistance. So, we analyzed the distribution of AHSG gene polymorphisms at 766 C/G substitution of chromosome 3q27 by taqman polymerase chain reaction and studied the association of these variants with clinical and laboratory findings for the T2DM in Iraqi population.

MATERIALS AND METHODS

Patients and methods

A case-control study was designed to include 100 Iraqi patients with T2DM, and 100 apparently healthy as a control group. The samples was collected between the periods of the 1st of March 2018 till the 30th of September 2018. The patients with T2DM were diagnosed previously by Al-Imam Al-Hassan center for endocrine and diabetes in Al-Imam Al-Hussein medical city in holy Kerbala province. Permission was taken from all subjects of T2DM and the control groups after they were told about the aim and advantages of this study. The collected blood samples was divided into two parts, first part was collected in an EDTA anticoagulant tubes for the DNA extraction and to measure the HbA1c, and the second part was collected in a gel tube to measure the lipid profile and fasting blood glucose (FBG) by colorimetric method (16,17). For all participants BMI, the atherogenic index, and non-HDL-C was calculated according to Nada 2017 (18).

DNA Extraction and RT-PCR assay

The DNA was extracted from whole blood samples using the genomic DNA extraction kit (Promega, USA). The protocol was carried out as recommended in the instruction of the kit. DNA concentration and purity were measured with the use of BioDrop, UK. Genotyping of AHSG thr256>ser polymorphism was performed by real-time polymerase chain reaction technique (RT-PCR) with the use of thermocycler (Roto-Gene/ Germany). A proper nucleotide primer and probe were designed at the location of SNP, the DNA fragments were amplified using 2 primers selected as the forward: 5′-CTGGGAGGAGGAAGCAAACT-3′ (0.6 μM); and the reverse: 5′-TAACACATGGGAGTCTGGGG-3′ (0.6 μM), and two probe as FAM 5′-TGAGCTCACAGCCCCAAC-3′ (0.2 μM), and VIC: 5′-ACCTCACAGCCCCAACCAG -3′ (0.1 μM). The amplification was performed in a total volume of 20 μl consisted of 10 μl Go TaqMan master mix (Promega, USA), a 0.5 mixture of the forward and reverse primer with the FAM and VIC probe and 4 μl genomic DNA as a template and finally 5.5 nuclease free water. The PCR reaction program protocol was contain two hold, the first hold was in 50°C for 15 min and the second hold was in 95^{O}C for 15 min, then was followed by 45 cycles divided into two cycling stages, the first cycling was of 95 °C for 5s, 60 °C for 20 s, 72 °C for 15 s for 5 cycles only and the second cycling was the same temperature and time but for 40 cycles and on the step of annealing was added the acquiring Green and Yellow fluorescence's (FAM and VIC).

Statistical analysis

Student *t*-test and ANOVA test were used to compare numerical data of the T2DM and control groups using SPSS v. 22.0 software (SPSS Inc., Chicago, IL). Genotype distribution and allele frequency for the T2DM patients and control groups were tested by multinomial logistic regression analysis. Polymorphisms were tested for Hardy-Weinberg's equilibrium using online software (www.oege.org).

RESULTS AND DISCUSSION

AHSG gene polymorphisms (rs4918) were not significantly deviated from the Hardy–Weinberg's equilibrium either in T2DM patients or in control individual. The genotype and allele frequencies in patients and healthy are shown in Table 1. As indicated in this table, there were a significant differences were revealed for AHSG gene polymorphisms (rs4918) genotype frequencies between T2DM patients and the control group (X^2= 9.74, P = 0.631), and (X^2= 8.11,P = 0.004) for GG and GC genotyping, respectively, with an odds ratio (OR) were calculated as 0.4 95% confidence interval (CI) = 0.22 - 0.71 for GG genotype and 2.27 95% confidence interval (CI) = 1.28 - 4.03 for GC genotype. As mentioned table (1) the percentage of patients group with wild GG genotype carriers was highly significant decreased (p < 0.0001) in T2DM (35%) as compared with healthy control (57%). Also, the present study demonstrated that AHSG gene polymorphism may be linked with the danger of DM two times than in healthy control, these results were disagreement with some studies like (Khalil and Kuobaili 2013) who was observed that CC genotype in DM patient have 50%, CG genotype have 42.85%, and GG genotype have 7.14% (19). Otherwise, Maréchal, *et al*., found the genotype of CC, CG, GG were recorded as 47.65%, 42.23%, 10.10 % respectively, but it is in agreement with the HWE which have (0.78) and p value 0.17 (20).

The mutation of AHSG (rs4918, thr256) on chromosome 3q27 is a missense mutation (21) which is a change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by the gene, which is the A**G**C serine changing into A**C**C threonine in the 256 position, and this change have been linked to low level of fetuin -A- (22, 23), thus, it well loss its function in inhibition of the insulin receptor tyrosine kinase (3) and have low effect on the prevalence of DM, but it has been reported the low level of fetuin A is associated with increased risk of CVD. On the other hand, other study have been reported that the increase level of fetuin A is associated with DM, thus why we did not have increase the prevalence of G allele. The current results are consistent with those of (Siddiq *et al.,* 2005) who showed that the C allele of rs4918 SNP is associated with DM (24). Also the current results found that AHSG (rs4918) associate with T2DM with dyslipidemia and it's in agreements with the study of metabolic traits in the white Danish subjects (25). On the other hand, the present study is inconsistent with those of (El-Batch *et al.,* 2015) which report that the G allele is not significant between DM and control group (23).

Table 2 shows the characteristics of the studied groups. There are a significant difference concerning the fasting blood glucose (FBG), total cholesterol (TC), high density lipoprotein (HDL-C), Glycosylated hemoglobin (HbA1c), non HDL-C and LDL-C/HDL-C as atherogenic index (P value > 0.05). Otherwise, there were no significant difference concerning the body mass index (BMI), age, triglyceride (TG), very low density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C), between diabetic patients and control group (P > 0.05). The result of this study was observed an elevation in total cholesterol, and HbA1c with a highly significant differences (P<0.05). These results show the abnormal total cholesterol, and HbA1c metabolism remains as a strong risk factors for DM, and also observed an elevation in FBS (P<0.001), and decrease HDL-C (P<0.001) in patient with T2DM, body mass index (BMI), triglyceride (TG), very low density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C), levels were increased not significantly in T2DM when compared with healthy control group (P > 0.05). It has been revealed that fetuin-A directly or indirectly leads to these changes through its inhibitory effects on the insulin receptor tyrosine kinase, which lead to increases lipolysis and efflux of free fatty acids from adipose tissue (5). This may lead to increased production of apolipoprotein B - containing very low density lipoprotein (VLDL-c). Furthermore, the

hypertriglyceridemia may lead to a decrease in the cholesterol content of HDL-C enhancing HDL-C clearance from the circulation, thereby potentially leading to the atherogenic lipid profile as such observed in table (2) (14). Also Jensen *et al,* 2013 have been reported that rs4918 was not appear to be related to metabolic markers including insulin levels, lipids, BMI, and fasting glucose, as it in agreement with our result (26). It has been reported the elevation of non-HDL above 160 mg/dl (27), or the atherogenic index (LDL/HDL) above 0.24 were increases the risk for CVD (28).

CONCLUSION

Our study was found an association between the AHSG (rs4918) gene polymorphism associated with DM. where the C allele frequency of thr256>ser (rs4918) was more frequent as two times in diabetic patients than the control group. Also the atherogenic index was highly significant increased, and there level was not associated with AHSG (rs4918) gene polymorphism. Also a marked difference in the non-HDL levels between the T2DM and control group, thus the non-HDL could be consider a predictor marker for CVD.

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Table 1. allelic and genotype frequency for AHSG (rs4918) polymorphism

AHSG	Group							
	Control n=100		DM n=100		X^2	P value	OR	CI 95%
Genotype	H W O.F.	H W E.F.	H W O.F.	H W E.F.				
GG	57 57%	55 55%	35 35%	39 39%	9.74	0.001*	0.4	0.22 - 0.71
GC	34 34%	38 38%	54 54%	47 47%	8.11	0.004*	2.27	1.28 - 4.03
CC	9 9%	6 6%	11 11%	14 14%	0.22	0.639	1.24	0.49- 3.16
Total	100	100	100	100				
X^2	1.35		2.13					
P-value HWE	0.245 NS		0.144 NS					
Allele frequency								
G	0.74		0.62					
C	0.26		0.38					

Where the H W O.F. = Hardy Weinberg observed frequency, H W E.F. Hardy Weinberg expected frequency, X^2= chi-sequear, p= probability, OD= Odd ratio.

Table 2. Clinical and biochemical characteristics of studied subjects

Parameter	DM (n=100) (mean ± SD)	Control Group (n=100) (mean ± SD)	P value
Number (M/F)	50 / 50	52 / 48	----
BMI (Kg/m²)	26.04 ± 3.08	26.78 ± 2.03	0.215
Age (y)	56.57 ± 5.98	55.84 ± 5.58	0.053
FBS (mg/dl)	241.09 ± 51.81	95.88 ± 17.11	< 0.001**
TC (mg/dl)	167.17 ± 39.02	171.26 ± 31.39	< 0.001**
TG (mg/dl)	239.53 ± 54.48	170.25 ± 45.07	0.045
VLDL-C (mg/dl)	47.91 ± 18.49	34.05 ± 17.01	0.454
LDL-C (mg/dl)	112.65 ± 27.41	93.82 ± 34.14	0.021
HDL-C (mg/dl)	37.29 ± 7.74	47.59 ± 5.99	< 0.001**
HbA1c%	9.4 ± 1.8	4.48 ± 0.87	< 0.001**
Non HDL-C	130.6 ± 36.85	123.43 ± 33.33	0.001 **
LDL-C/HDL-C	3.22 ± 0.926	2.02 ± 0.882	0.001**

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RESEARCH ARTICLE

Exploration of Professional Development Barriers Confronted by In-Service O level English & Urdu Language Teachers

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ABSTRACT

The current study is about identification of professional development (PD) barriers confronted by O level language teachers. The study was descriptive in nature. The objectives of the study were to identify professional development barriers faced by language teachers and to find out the remedial measures (solutions) of O level teachers' professional development. The population of the study was comprised of 846 English and Urdu language teachers of 149 registered O level schools in the province of Punjab. A sample of 394 teachers was taken with the help of purposive convenient sampling technique. A questionnaire was used to collect data and was done quantitatively. The Analysis showed that teachers from back ward areas and with poor qualification faced more problems as compared to those who live in the major cities. It was recommended that teachers should be provided institutional support and provided financial perks to enhance interest of the teachers in professional development programme.

Key Words: Professional Developments, Barriers, Administration, Perks, Impede.

INTRODUCTION

The role of professional development (PD) cannot be overlooked as it has become imperative to develop teachers' pedagogical skills and practice of available resources in the classroom. Many teachers admit that with PD incorporates educational opportunities. Professional development enables teachers to implement innovative skills into their teaching practices (Davis, Preston and Sahin, 2009). Some educational institutions are underway offering on-line professional development (PD) for their teachers (Ullman2010). Professional development, as defined by the National Professional Development Council in 2000, is "a lifelong collaborative learning process that nourishes the growth of individuals, teams, and the school through a daily job-embedded, learner-centered, focused approach" (DuFour, Eaker and DuFour, 2006).

There are various instances which provide enough evidence that teacher instruction is now directly related to the varying nationwide policy. In UK, the administration has endorsed the development of partnerships among the total State service provision, which include teacher education (Furlong et al. 2008). Regarding professional development of teachers, the stress on change has revealed itself in various ways in many countries around the world (Hammersley-Fletcher and Qualter 2009). Professional development has a significant impact on teachers' teaching practices displayed in the classroom. The Conservative and Liberal-Democratic coalition government published in the government White Paper, 'The Importance of Teaching' (Department for Education 2010) which indicated a substantial move to more school-led, school-based teacher education (Childs 2013) which has formed a new direction regarding changes in understanding teachers', their identity, and everyday jobs. In Scotland, the Donaldson report, Teaching Scotland's Future (Donaldson 2011) clearly set out a positive interpretation of the professional development throughout the teaching career, thus making Scotland a more positive place for working and researching on teachers' professional education (Menter 2011).

Recently, Australia declared the Smarter Schools-Improving Quality National Partnership programme (Department of Education Employment and Workplace Relations 2010) which intends to fascinate, formulate, and retain quality teachers and school managers in school. It has become prerequisite for educational institutions to develop their teachers professionally for several reasons. Language teachers of O level are required to develop themselves continuously in the global village because everything around them is rapidly changing (Richards and Farrell 2005; Bailey 2004). One may not be able to get opportunities for PD due to certain barriers such institutional policies, economic constraints, lack of interest of authorities, unscheduled load shedding and poor infrastructure of ICT. Brady and Schuck (2005) are of the view that teachers of the 21st century can exchange their experiences with other teachers during PD programme but they fail to avail these opportunities due to various barriers confronted by them.

The literature review presents different research works that examine the barriers of professional development of O level English language teachers in Pakistan. A preview of previous empirical research presents a tangible picture of professional development barriers and how they can be resolved. According to Little and C (2011) several teachers face problems in developing their professional skills because they experience complications of institutional support, financial problems and lack of ICT skill. In the 21st century, professional development (PD) of O level teachers in Pakistan has become imperative. The series of PD barriers of O level language teachers in Pakistan is very wide. It is detailed in the European Commission's communication 'Rethinking Education', that the restructuring of education and (CPD) is indispensable to achieving higher institutional output and the delivery of highly skilled human resources. Johnson (2011) finds the negative approach of the school administration towards English teachers' professional development. According to Memon (2007), the quality of teachers in Pakistan is poor. There are estimated 1.4 million teaching force in Pakistan. They are employed in government institutions from primary to higher education. In the modern era, it has become an admitted fact that the learner should have greater breadth and depth of knowledge. This demand has made teaching more demanding and multipart, and in turn this calls for boosted teachers to ensure effective teaching in the teaching sphere (Livingston2014). Professional development of teachers plays a key role just as the professional skills of teachers affect the results of pupils (Linda Darling-Hammond 2000; Murray 2014). Basically, three primary factors become barriers in the professional development of teacher namely: time and resources, professional support, and financing.

Many studies specify that several teachers criticize that they do not have enough time for their PD. They must undergo extended working hours with substantial teaching loads (SmithK 2005; Murray 2008; Dinkelman et al. 2006). Regarding to professional backing, Smith (2010) recommends that in a viable setting, people keep quiet about professional challenges. During the last two decades, it is evident that teachers lack professional development (PD) provision in the international setting. Griffiths et al. (2013) show that educators in countries of the European Union as well as in the United States frequently enter in the teaching without any formal grounding, and often face compressions and hitches. In Australia and America, Williams and Ritter (2010) and Dinkelman et al. (2006) emphasis that the changing professional characteristics are not helped by official support. Hence, professional provision for

teachers is a global question and is not simply at the country level. Thirdly, teachers experience economic barriers. One of the barriers which teachers face is that teachers are provided with such material and courses as they do not need. In addition, professional development programme do not comprise of practical issues of classrooms rather than theories of learning. These problems impede effective professional development.

Objectives of the Study

The objectives of the study were to
To identify the problems of professional development of O level language teachers
To find out the remedial measures (solutions) of O level teachers' professional development

Research Questions

(i) What are different types of professional development barriers?
(ii) Which are possible limitations of GCE O level language teachers that hamper their professional development?
(iii) What measures should be taken to overcome the barriers of professional development?
(iv) Which facilities can help teachers to develop them professionally?

METHODOLOGY

Survey approach was used for data collection and analysis. Analysis was based on quantitative method of research. A questionnaire was developed to collect data. It was administrated to the respondents personally. The study was descriptive in nature. From the thirty-six (36) districts of Punjab, nine (09) district headquarters (DHQs) were selected. Out of these selected district headquarters, (108) O level schools were selected using purposive convenient sampling technique. Four English language teachers and two Urdu language teachers were conveniently selected from each of the O level school. There were three divisional headquarters (D.G. Khan, Sahiwal and Sargodha) where number of teachers was less as compared to other six divisional headquarters. So, the researcher had to select two English language teachers and one Urdu language teacher from each O level school. Sample size and percentage was dissimilar at different divisional headquarters because, the number of students as well as subject teachers varied from one divisional head quarter to another one. The study was conducted with GCE O level English and Urdu language teachers in the province of Punjab.

The above tables show the respondents' characteristics in terms of gender, academic qualification, professional qualification and teaching experience regarding teachers as respondents. The male teachers constituted 162 (41.12%) while the female teachers comprise of 232 (58.88%). This shows that most of the essential data was obtained from female respondents. Moreover, it is evident that the number of male teachers in O level schools is much lower as compared to females. The professional qualification of respondents listed in the above table shows that 143 (36.20%) of teachers are master's degree holders. While 171 (43.40%) of teacher respondents are double master's degree holders i.e., M.A, M.Ed. 80 (20.25%) had higher degrees i.e., M. Phil or higher degree holder teachers. This implies that the teachers had enough academic and professional qualification. Therefore, it could be assumed that the sample teachers could be the sources of reliable and essential information for the study. According to the work experiences of the respondents, 147(37.31%) of teachers' respondents had 1-5 years of service as teachers. Whereas 140 (35.44%) teachers had 6-10 years teaching experience. The smaller number of teacher respondents 107 (27.08%) possessed 11 to 15 years' experience. This shows that the O level schools have experienced and highly qualified teachers. Likewise, most of schools supported the researcher in carrying out classroom observation. The trained section heads and coordinators also conducted the observation of the teachers on behalf of the researcher..

Pilot Testing

Before the conduction of research, pilot testing was conducted in that area which was a part of the population but not included in the sample. The tools were further simplified and reorganized after the pilot testing. Reliability of the questionnaire items was checked by using SPSS software.

Validity and Reliability

A tool is said to be valid only if it can quantify what is planned and for which intent it was devised (Wallen and Fraenkel 2001). An important step of a research is the reliability and validity of the questionnaire. A perfectly reliable questionnaire produces consistent reactions. Great dependability of responses can be attained if all respondents are provided with the same set of questions. According to Suskie (2004), reliability and validity can be increased if the researcher takes certain precautionary measures. For the intent of authentication of the elements of the research instruments, the team of seven (7) experts was requested to refine the instruments. The experts revised and modified questions and made necessary changes in the research instruments and were incorporated. These experts refined the elements in arrangement and language to place reports in a more rational order. To validate the questionnaire, the tool was distributed among male and female language teachers of O level schools of (9) DHQs. Validity is more significant than reliability. It makes a rational linkage to the stability of the collected data (Wallen and Fraenkel 2001). The validity and dependability of the questionnaire is considered as a key footstep of the research. The researcher used the Cronbach alpha to assess the reliability of the instrument. It was .706 which means the tool was reliable and acceptable (Gay 2000). Therefore, the research instrument was suitable to answer the questions of the study.

Before carrying out the investigation, the researcher did a preliminary testing of the tool, with an aim to validate the questionnaire. It was circulated among one hundred O level teachers, both male and female, in the districts Faisalabad and Lahore. After gathering the reactions of the relevant sample areas, the questionnaires were rearranged on a bigger scale on the foundation of preliminary testing. The questionnaire was developed in accordance to teachers' mental level and was pilot tested with ten (10) teachers of The Aims Cambridge School System, The Lahore Lyceum and Divisional Model College, Faisalabad. These schools were not included in the study. All the surveys were returned from the pilot test as the researcher went personally to collect data. Respondents easily followed all items and none of the items was found to be ambiguous. The experts in the field of test and measurement verified face validity and content validity of the instrument. Face validity was also checked by three subject specialists who identified some minor changes keeping in view the grammatical nature of the test items. Kasomo, (2007) wrote about validity and says that validity means to check whether the tool contain sufficient traits expected to measure that domain. Validity of an instrument is related to the appropriateness and effectiveness of the conclusions made by the researcher upon the source of the data that was collected (Wallen and Fraenkel 2001). Content validity is decided upon on the appropriateness of the tool's content. Moreover, there are three basic principles to improve the validity of the content:

1) Select a broader sample, rather than a narrow one.
2) Emphasize essential material
3) Make such questions which quantify the related ability.

These three skills were kept in mind and were addressed while writing the survey items. In this study, content, pattern and format validity was checked by having the instrument reviewed by the university professors whose recommendations were incorporated to review the instruments. All the experts were experienced researchers. They reported that the content of the tool was clear, relevant to the objectives and valid for this study. Moreover, no ambiguity was reported, so the tools were reported that they had content validity.

Presentation and Analysis of Data

According to Kalsoom and Mumtaz (2013), a questionnaire is a multipurpose tool which helps to collect data both in qualitative and quantitative research. They further added that open ended questions can also be included in a questionnaire. A questionnaire was used to collect data from English and Urdu language teachers. The detail is as under: A questionnaire was developed for O level English and Urdu language teachers. The questionnaire comprised of different items regarding barriers of professional development which impeded teachers' PD. Each item in the survey was linked with the research questions of this study. The questionnaire was developed on five point likert scale, the most frequently used rating scales for surveys often employed to assess the degree of intensity from best to worst of opinions, ranging from "Strongly Agree" to "Strongly Disagree" in which the participants were requested to mark the opposite box.

In current study, the investigator requested to the principals informing them with the purpose to collect data for the research, consent was granted, and the researcher informed the teachers through telephone contacts that he will visit their school to collect data. He also briefed them about the research study. The researcher then visited teachers as per fixed schedule to collect data from the respondents. The data were collected from (108) O level schools from 9 divisional headquarters of the province of Punjab. A sample of 394 teachers (230 English language and 164 Urdu language Teachers) were selected for this study. These teachers were selected by using purposive convenient sampling from nine (9) DHQs. The data collected from questionnaire, provided answers to the research questions of this study. For data collection from the respondents by employing the above stated tools, the investigator went to all the schools taken as sample personally and served the questionnaires to the sample teachers e.g. English and Urdu O level teachers who had professional development during last five years. The researcher methodically elaborated the procedure of filling in the questionnaires to the respondents. The filled questionnaires were collected by the investigator personally. The investigator personally went to the sample schools that were chosen for the intent of data collection.

For the process of data analysis, explanatory statistics were calculated on the independent variables. Elemental analysis was carried out to conclude the internal reliability of each element as well as subscale. Cronbach 's *Alpha* was employed to check internal stability. Inferential statistics were used to draw assumptions and generalize about the features of population based on data collected from the sample. Independent t-test and simple analysis of variance (ANOVA) technique was employed. Results were used to deduce inferences and findings**.** The 0.05 rejection level was used for all tests. It was found that the problems faced by O level teachers could be solved by taking certain remedial measures. Three hundred and ninety-four (394) O level English and Urdu language teachers from one hundred and eight (108) O level schools were selected. These teachers and schools were in the nine-divisional headquarters of Punjab. The data was collected from a total of 362 respondents because thirty-two respondents did not complete and returned the questionnaires. A total of 394 copies of questionnaires were distributed, out of these, 352 (89.34%) were filled in and collected. 42 (10.66%) of the questionnaire were ineffectual since there were problems of clarity and were not filled properly. Finally, the research analyses were prepared based on the data obtained from the remaining 352 (89.34%) of the questionnaires. Total number of sample school was 108 but due to institutional policy, eight schools did not allow to conduct observation of their teachers. Sample teachers responded to all questionnaire items. These items were computed and analyzed using frequency, percentage, standard deviation, mean scores and Independent t-test. Percentage was utilized for easy presentation of frequency distribution and for comparison of the degree of the prevailing practices and barriers which teachers experience. Mean scores of each response were calculated. For analysis, the mean values of each item and dimension were interpreted as follows.

Table 3 shows data regarding professional development barriers which both male and female English teachers face regarding their professional development programme. The most frequently barrier faced by teachers is 'poor infrastructure of ICT. The mean score of male English teacher (61.3 %) is higher as compared to female teachers (60.0 %). Both teachers agree that they frequently face this problem. The mean score of male English teachers (62.5 %) as

compared to female teachers is (58.4 %) regarding unscheduled load shedding, the mean score of male English teachers is (58.8 %) and female English teachers (53.6 %) regarding financial constraints from school authorities. Teachers had to face these barriers frequently as compared to all other barriers. Table 4shows the results of professional development barriers faced by male and female teachers during their teaching career. Female teachers agreed that they face more problems regarding financial constraints, slow internet speed, forced to sacrifice weekends and face poor infrastructure. Male teachers also agreed that they faced more problems as compared to female teachers regarding little interest of school authorities, unscheduled load shedding and poor infrastructure. It can be concluded that both male and female teachers face professional development problems regarding their professional development. But both faced different type of problems.Table 5 shows descriptive statistics, weighted score (WS) and rank for professional development barriers which hamper teachers' professional development. The mean score of financial constraint of teachers from school authorities is (4.03) and is ranked as no:1. Majority of the teachers face this rier before joining professional development programme. Another barrier which is most frequently faced by the teachers is 'unscheduled load shedding. The mean score of this barrier is (3.75) and is ranked as no:2. Majority of the teachers also live over to slow internet speed. The means score of this barrier is (3.70) and is ranked as no: 3.Observing the mean score and ranking, it is concluded that majority of the teaches face different types of professional development barriers during and before their professional development programme which hamper their development.

Table 6 shows descriptive statistics, weighted score (WS) and rank for professional development barriers which hamper teachers' professional development. The mean score of financial constraint of teachers from school authorities is (4.09) and is ranked as no:1. Majority of the teachers face this barrier before joining professional development programme. Another barrier which is most frequently faced by the teachers is 'unscheduled load shedding. The mean score of this barrier is (3.72) and is ranked as no:2. Majority of the teachers also live over to slow internet speed. The means score of this barrier is (3.69) and is ranked as no: 3. Observing the mean score and ranking, it is concluded that majority of the teaches face different types of professional development barriers during and before their professional development programme which hamper their development. Table 7 shows descriptive statistics, weighted score (WS) and rank for professional development barriers which hamper teachers' professional development. The mean score of financial constraint of teachers from school authorities is (3.96) and is ranked as no:1. Majority of the teachers face this barrier before joining professional development programme. Another barrier which is most frequently faced by the teachers is 'unscheduled load shedding. The mean score of this barrier is (3.78) and is ranked as no:2. Majority of the teachers also live over to slow internet speed. The means score of this barrier is (3.72) and is ranked as no: 3. Observing the mean score and ranking, it is concluded that majority of the teaches face different types of professional development barriers during and before their professional development programme which hamper their development

RESULTS

This section deals with the interpretation of results. It presents analysis on the different barriers of O level teachers faced by them with reference to their professional development and how these barriers could be solved. The present study aimed to identify professional development barriers faced by O level teachers. These problems were sub-divided in three categories; technical problems, Language competency problems and institutional problems. Cronbach's alpha is mostly used to measure internal consistency. In this survey, questions were asked to identify the barriers of PD at secondary level in O level schools. With the sample used here and the way the survey was applied, the conditions for good reliability have been fulfilled. The tables above summarize the descriptive data collected. In the study, teachers of (9) divisional headquarters were the sample of the study who had professional development. All these teachers positively responded and agreed and strongly agreed that they face various barriers which impede their professional development. Statements related to institutional barriers were also agreed by the respondent teachers. They agreed that institution should support them financially and facilitate them. Department should frame

supporting policy for the professional development (PD) of teachers. It is also concluded that professional development barriers could be solved by taking certain preventive and remedial measures. The effectiveness of PD could be enhanced and enriched if teachers are provided with certain facilities. Financial support could also enable teachers to perform better after their professional development. Institutional support could also facilitate teachers and their interest level could be developed by taking certain measures. In the light of findings of this study, it is also recommended that institutions should consistently provide professional development programs to bring their teachers at national and international standards because professional development of O level teachers enhance their professional skills, knowledge and pedagogical skills.Cumulative comparison between English and Urdu Teachers (Male N: 205 Female N: 147) regarding professional development barriers which both male and female teachers face during and before their professional development programme. The most frequently faced barrier is 'financial constraints from school authorities. The mean score of English teachers (4.09) is higher as compared to Urdu teachers (3.96). The calculated t-vale of both English and Urdu teachers regarding financial constraints is (1.312).

Cumulative comparison between male and female Urdu Teachers (Male N: 64 Female N:83)regarding professional development barriers which both male and female teachers face during and before their professional development programme. The most frequently faced barrier is 'financial constraints from school authorities. The mean score of male teachers (4.09) is higher as compared to female teachers (3.86). The calculated t-vale of both male and female teachers regarding financial constraints is (1.460). The mean score of female teachers (4.09) as compared to male teachers (3.86), regarding financial constraints from school, the mean score of both male and female teachers (3.78) regarding unscheduled load shedding, and the mean score of both male and female teachers (3.72) regarding slow internet speed shows that these barriers were most frequently faced by the teachers. Teachers were unable to develop their competencies and skills due to these barriers of professional development. The calculated t-value of financial constraints is (1.460), unscheduled load shedding (-0.017) and slow internet speed (-0,027) reveal that teachers faced these barriers more frequently as compared to all other barriers. Cumulative comparison between male and female English teachers (Male N: 80 Female N: 125) regarding PD barriers data regarding professional development barriers which both male and female teachers face during and before their professional development programme. The most frequently faced barrier is 'financial constraints from school authorities. The mean score of female teachers (4.10) is higher as compared to male teachers (4.08). The calculated t-vale of both male and female teachers regarding financial constraints is (-0.173).

Both male and female teachers faced different barriers which impede their professional development. Teacher need support to grow more and develop their potential and it is possible through their institutional support. They should not be forced to sacrifice their weekends. School authorities should take interest in the professional development of their teachers. They should be supported with high speed internet and latest ICT infrastructure. Teachers should enjoy backup power system to avoid load shedding problem which wastes their time. The mean score of female teachers (4.10) as compared to male teachers (4.08), regarding financial constraints from school, the mean score of male teachers (3.76) and female teachers (3.61) regarding poor infrastructure of ICT, and the mean score of male teachers (3.75) and female teachers (3.70) regarding unscheduled load shedding shows that these barriers were most frequently faced by the teachers. They were unable to develop their competencies and skills due to these barriers of professional development. The calculated t-value of financial constraints from school authorities (-0.173), poor infrastructure of ICT (1.337) and unscheduled load shedding (0.434) reveal that teachers faced these barriers more frequently as compared to all other barriers. An overview shows that both male and female teachers faced different barriers which impede their professional development. Teachers need support to grow more and develop their potential and it is possible through their institutional support. They should not be forced to sacrifice their weekends. School authorities should take interest professional development of their teachers. They should be supported with high speed internet and latest ICT infrastructure. Both English and Urdu teachers faced different barriers which impeded their professional development. Teacher need support to grow more and develop their potential and it is possible through their institutional support. They should not be forced to sacrifice their weekends. School authorities should take interest in the professional development of their teachers. They should be supported with high speed

internet and latest ICT infrastructure. Teachers should enjoy backup power system to avoid load shedding problem which wastes their time. The mean score of English teachers (4.09) as compared to Urdu teachers (3.96), regarding financial constraints from school, the mean score of English teachers (3.72) and Urdu teachers (3.78) regarding unscheduled load shedding, and the mean score of English teachers (3.69) and Urdu teachers (3.78) regarding slow internet speed shows that these barriers were most frequent. Teachers had to face these barriers frequently as compared to all other barriers. Teachers were unable to develop their competencies and skills due to these barriers.

DISCUSSION

The current research attempted to identify the barriers of professional development and to find out their solutions. There are certain barriers which hamper teachers to improve their competencies through professional development programme. Majority of the teachers agreed and strongly agreed that they wanted to join professional development programme but due to institutional policies, financial constraints, poor infrastructure and slow internet speed barriers, they were not able to improve their skills and competencies.The questionnaire discusses barriers of professional development. It is clear from cumulative data that male teachers slightly dominated over female teachers' opinion regarding financial constraints, slow internet speed, little interest of school authorities in professional development of teachers, weekend sacrifice and unscheduled load-shedding. Both male and female teachers agreed that these barriers hamper their professional development. Their competency can be enhanced and enriched if these barriers are resolved by taking supporting measures. Another barrier which hamper teachers' professional development is poor infrastructure of ICT. Both genders agree that poor infrastructure of ICT hamper teachers' professional development.The evidence regarding teachers' opinion about awareness of Wiki and Blog etiquettes shows that both male and female were not familiar with them.

CONCLUSION

The education scene round the global village is rapidly moving and educational institutions are at the forefront of this transformation. Teachers are key players in maintaining quality and their professional development is pertinent to quality assurance. This study focused professional development barriers which impede them.It was found that teachers face many barriers such as they face financial constraints from school authorities, live over with slow internet speed, School authorities take little interest, forced to sacrifice my weekends for professional development, unscheduled load shedding, unfamiliar with Wiki and Blog etiquettes, unfamiliar with basic skills in Online professional development and poor infrastructure of ICT.This study identified those barriers which impede professional development of in-service teacher and propounded their solutions.It was found that theseberriers could be resolved if authorities take interest, support financially and facilitate teachers in their professional development.
Following recommendations are made which can be useful for school principals, teachers, policy makers and educationists in the pyramid of education.

Recommendations

- Teachers may be given more incentives and facilities to boost their interest in professional development and to motivate them to work with zeal.It has been observed that some talented and qualified teaching lots leave the profession for some other field due to financial constraints. So, it is recommended that teachers may be given incentives and higher grades if they engage themselves in professional development programmes.
- Slow internet speed is another barrier in the professional development of some teachers, so it is strongly recommended that teachers may be provided with high speed internet so that they work speedily and complete their assignments within given time frame.

- Teaching is a noble and respectable profession which needs equal attention, so all the teachers may be provided opportunities for their professional development. Teachers' interest may also be boost up towards professional development.
- The institutions should provide sufficient funding to develop the quality of teachers by providing enough expenditure for their educational development. They may be offered special perks and compensation for their participation in professional development.
- Due to frequent load shedding and failure of power, there may be arrangement for back up power supply. UPS and generator supply can support teacher in their PD as such support can save their time.
- Teachers should be guided and facilitated by IT experts who solve their problems while using internet and in browsing relevant material.
- To provide a high-quality education, institutions may be committed to ensure that every teacher is provided high quality ICT facilities for professional development.
- There is a dire need to develop a professional culture to continually improve teachers' effectiveness. Better infrastructure can also boost up interest of teachers towards professional development programmes. Authorities should pay special attention towards this aspect.

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Table. 1The Distribution of the Sample according to Gender, Qualification and Experience

Classification		Number	Percentage
1	Male	162	41.12%
2	Female	232	58.88%
1	M.A	143	36.20 %
2	M.A. B. Ed / M. Ed	171	43.40 %
3	M. Phil or Higher	80	20.25 %
1	1-5 years	147	37.31%
2	6-10 years	140	35.44%
3	11-15 years & Above	107	27. 08 %
Total		**394**	99.83 %

Table 2. Reliability Coefficient of the Questionnaire

Sub-categories	No of items	Serial number in Scale	Cronbach Alpha Reliability
Barriers of Professional Development (pd)	08	1-8	.706

(Cronbach alpha of Barriers of Professional Development (pd): 0.706

Table. 3. Comparison between Male and Female English Teachers' Responses Regarding Barriers of PD

Barriers of Professional Development	Male					Female				
	SDA	DA	UD	A	SA	SDA	DA	UD	A	SA

Q.1 I face financial constraints from school authorities.	0.0%	8.8%	3.8%	58.8%	28.8%	0.0%	9.6%	4.0%	53.6%	32.8%
Q.2 I live over with slow internet speed.	0.0%	13.8%	21.3%	43.8%	21.3%	0.0%	12.0%	24.8%	48.0%	15.2%
Q.3 School authorities take little interest in my professional development.	1.3%	32.5%	1.3%	55.0%	10.0%	3.2%	28.8%	.8%	52.0%	15.2%
Q.4 I am forced to sacrifice my weekends for professional development.	0.0%	25.0%	23.8%	45.0%	6.3%	0.0%	23.2%	26.4%	46.4%	4.0%
Q.5 I experience unscheduled load shedding.	0.0%	5.0%	23.8%	62.5%	8.8%	0.0%	8.8%	22.4%	58.4%	10.4%
Q.6 I am not familiar with Wiki and Blog etiquettes.	0.0%	21.3%	58.8%	17.5%	2.5%	0.0%	24.8%	52.0%	16.8%	6.4%
Q.7 I am not familiar with basic skills in Online professional development.	27.5%	43.8%	6.3%	15.0%	7.5%	35.2%	44.8%	5.6%	12.8%	1.6%
Q.8 I face poor infrastructure of ICT.	0.0%	10.0%	16.3%	61.3%	12.5%	0.0%	13.6%	19.2%	60.0%	7.2%

Table 4. Comparison between Male and Female Urdu Teachers' Responses Regarding Barriers of PD

Barriers of Professional Development (PD)	Male					Female				
	SDA	DA	UD	A	SA	SDA	DA	UD	A	SA
Q.1 I face financial constraints from school authorities.	0.0%	9.4%	9.4%	43.8%	37.5%	0.0%	16.9%	9.6%	44.6%	28.9%
Q.2 I live over with slow internet speed.	0.0%	12.5%	23.4%	43.8%	20.3%	0.0%	14.5%	18.1%	48.2%	19.3%
Q.3 School authorities take little interest in my professional development.	3.1%	25.0%	3.1%	57.8%	10.9%	1.2%	33.7%	3.6%	47.0%	14.5%
Q.4 I am forced to sacrifice my weekends for professional development.	0.0%	26.6%	21.9%	48.4%	3.1%	1.2%	31.3%	14.5%	49.4%	3.6%
Q.5 I experience unscheduled load shedding.	0.0%	4.7%	20.3%	67.2%	7.8%	0.0%	3.6%	24.1%	62.7%	9.6%
Q.6 I am not familiar with Wiki and Blog etiquettes.	0.0%	26.6%	51.6%	17.2%	4.7%	0.0%	28.9%	47.0%	18.1%	6.0%
Q.7 I am not familiar with basic skills in Online professional development.	29.7%	40.6%	9.4%	17.2%	3.1%	31.3%	42.2%	8.4%	15.7%	2.4%
Q.8 I face poor infrastructure of ICT.	0.0%	9.4%	21.9%	57.8%	10.9%	0.0%	10.8%	22.9%	57.8%	8.4%

Table5: Over all Descriptive statistics, weighted score (WS) and rank for Barriers ofPD (N:352)

Barriers of Professional Development	Min	Max	Mean	SD	WS	Rank
Q.1 I face financial constraints from school authorities.	2	5	4.03	0.908	1420	1
Q.2 I live over with slow internet speed.	2	5	3.70	0.918	1303	3
Q.3 School authorities take little interest in my professional development	1	5	3.44	1.118	1211	5
Q.4 I am forced to sacrifice my weekends for professional development.	1	5	3.29	0.913	1158	6
Q.5 I experience unscheduled load shedding.	2	5	3.75	0.705	1319	2
Q.6 I am not familiar with Wiki and Blog etiquettes.	2	5	3.02	0.795	1064	7
Q.7 I am not familiar with basic skills in Online professional	1	5	2.15	1.124	758	8

development.						
Q.8 I face poor infrastructure of ICT.	2	5	3.67	0.799	1291	4

Table 6. English Teachers' Descriptive statistics, weighted score (WS) and rank for PD Barriers (N: 205)

Barriers of Professional Development	Min	Max	Mean	SD	WS	Rank
Q.1 I face financial constraints from school authorities.	2	5	4.09	0.847	838	1
Q.2I live over with slow internet speed.	2	5	3.69	0.908	756	3
Q.3 School authorities take little interest in my professional development.	1	5	3.44	1.126	706	5
Q.4 I am forced to sacrifice my weekends for professional development.	2	5	3.32	0.892	680	6
Q.5 I experience unscheduled load shedding.	2	5	3.72	0.738	763	2
Q.6 I am not familiar with Wiki and Blog etiquettes.	2	5	3.03	0.776	622	7
Q.7 I am not familiar with basic skills in Online professional development.	1	5	2.13	1.126	436	8
Q.8 I face poor infrastructure of ICT.	2	5	3.67	0.809	752	5

Table 7. Urdu Teachers' Descriptive statistics, weighted score (WS) and rank of PD Barriers (N: 147)

Barriers of Professional Development	Min	Max	Mean	SD	WS	Rank
Q.1 I face financial constraints from school authorities.	2	5	3.96	0.985	582	1
Q.2I live over with slow internet speed.	2	5	3.72	0.935	547	3
Q.3 School authorities take little interest in my professional development.	1	5	3.44	1.111	505	5
Q.4 I am forced to sacrifice my weekends for professional development.	1	5	3.25	0.942	478	6
Q.5 I experience unscheduled load shedding.	2	5	3.78	0.657	556	2
Q.6 I am not familiar with Wiki and Blog etiquettes.	2	5	3.01	0.823	442	7
Q.7 I am not familiar with basic skills in Online professional development.	1	5	2.19	1.125	322	8
Q.8 I face poor infrastructure of ICT.	2	5	3.67	0.788	539	4

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RESEARCH ARTICLE

Biosynthesis and Characterization of Zinc Oxide Nanoparticles from *Gomphrena globosa* and Analysis of Strutural Parameters in *insilico* Studies

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ABSTRACT

Edible plant from the family Amaranthaceae. *Gomphrena globosa* used as a herbal medicine .It is also known as Globe amaranth, makhmali, vadamalli, etc. Here, the plant *Gomphrena globosa* used to synthesis the Zinc oxide (ZnO) nanoparticle. The plant *Gomphrena globosa* fixes the carbon through the C4 pathway. The flower and whole plant used in treatment of Respiratory disease, oliguria, jaundice, urinary system condition and kidney problems. It has a potential application like anti-bacterial, anti-fungal, anti-diabetic, wound healing and anti-inflammatory properties. The secondary metabolites of plant extract is used in the responsible for the synthesis of Nanoparticles. Zinc nitrate [$Zn(NO_3)_2$] solution used as precursor solution of synthesis of the Zinc oxide (ZnO). In some cases, the synthesis of zinc oxide mediated by the solution like Zinc sulphate, Zinc acetate. The synthesis of nanoparticles was confirmed by the color change may be pale(or)light yellow by heat treatment of the solution using oven. The characterization of nanoparticles by using UV-spectrophotometer. The Zinc oxide (ZnO) is in the white powder were characterized by XRD analysis, FTIR. The normal ZnO nanoparticles formed in the range 85-98nm. Some other nanoparticles formed in range 500nm. Many applications are in the Zinc oxide nanoparticles such us commercial application, field of medicine, agriculture etc. In this process, the structural parameters were analysized by insilico technique.

Keywords: *Gomphrena globosa,* medicinal plant, zinc nitrate, synthesis of zinc oxide, characterization, phytochemical, parameters in *insilico* studies.

INTRODUCTION

The plant *Gomphrena globosa* used for synthesis of ZnO. The family of Amaranthaceae, otherwise the *Gomphrena globosa* is known as vadamalli. The plant grows approximately upto 24 inches in height. This plant should be heat tolerant and fairly drought resistant. But grows best in full sun and regular moisture(silva et al 2012). The flower of the *Gomphrena globosa* in round shape it has many colors such as magenta, purple, red, orange, white, pink and lilac. The true flowers are small and inconspicuous.The flower head should be approximately 4cm long. The origin of the plant *Gomphrena globosa* is Central America. The plant also grown globally included Tamil Nadu. The plant is pollinated by butterflies, bees and insect (jiang et al 2011). The plant has at least 27 phytochemicals that like 6 phenolic acids, 15 specific flavonoids. Major phenol found kaemferol 3-o-rutinoside. The phenol based on chromatographic and mass spectrometry techniques (Roriz et al 2014).

Scientific classification

Kingdom: Plantea, Order: Caryophyllales, Family: Amaranthaceae, Genus: Gomphrena, Species: *G.Globosa*. The leaves, stem, root and fruit indicates the presence of different phytochemicals. The zinc oxide Nanoparticles used for many purposes such as new light emitting devices, solar cells, biosensor and photo catalysts (M.Manokari et al 2016). The activate biological compound present in plant parts like enzyme itself act as a reducing and capping agent. In addition to other biomolecular such as flavanoids, terpenoids, glycosides, alkaloids, inositols, resins, saponins, terpenes, volatile oil, tannanins, steroids, quinine also act as reducing/ capping agent (sudhankumar chaudhuri et al 2017). ZnO used in many applications in solar energy conversion, sensor, catalysis etc (elizabath et al, (2015)). The nanoparticles ZnO used in many field from the plant extract. The synthesis of ZnO from plant extract is mediated by zinc nitrate. Finally the zinc powder was obtained in white color. And then characterized by UV-Vis, FTIR and XRD. zinc oxide is used wide range of cosmetics and personal carer products including makeup, nail products, baby lotion, bath soap and food powders. Also used in skin protectants, such as diaper rash ointment and sunscreen product. Zinc oxide is the metal zinc that has been oxidized. 1 Zinc atom and 1 oxygen atom held together by an ionic bond. Here the, bioinformatics used as the application for the biotechnology. In this research, concluded with the help of insilico method. The bioinformatics represent by the correlation of the biotechnology, microbiology, biochemistry. The bioinformatics defined as to solve the biological problems using computer. There are many databases in the insilico technique. The structural prediction of nanoparticles should predicted by the structural databases. There are 2 databases in the structural databases, PDB (protein data bank) and MMDB (macro molecule data bank). All biomolecule structure should be collected in the databases. Structure should be PDB in the format. In PDB have 2D and 3D structures. In structure prediction have a linear, secondary, tertiary structures and etc. The secondary and 3D structure should easy to bind and identified. In x-ray crystallography,90% used in structural databases NMR, AAS. Finally the nanoparticles were synthesized from plant extract, characterized and structure predicted by the insilico method.

MATERIALS

Soxhlet apparatus

A soxhlet extractor is a kind of laboratory equipment made up of glass. Franz von soxhlet invented in 1879. It has a flask, an extraction chamber, and a condenser. It can be used for solid-liquid extraction.

Magnetic stirrer

Magnetic stirrer is a laboratory equipment, by a stir bar also called “flea” rapidly spinning in a glass container, the liquid or semi-liquid can be equably mixed well. Stir bar is an elongated permanent magnet, which is

usually coated by PEFT(poly tetra fluoro ethene, also known as Teflon) (ACTTR, 2015). On a magnetic stirrer with heating function, the material can accept the highest temperature, up to 270degree Celsius. The spinning of the stir bar is brought by the spinning magnetic field. Magnetic coils are arranged in a definite pattern and AC current is passed through them. In the regular stirrers, it's usually a plate of mu-metal arranged in the center axis, which can concentrate the longitudinal magnet flow lines of the coils interacting with which the rotor rotation and enhance the magnetism.A magnetic stirrer is common laboratory equipment with simple structure. It is usually used in the chemical and biological experiments. The frequency of usage is more than the traditional mechanical stirrer, because of its quieter and more efficient characteristic. When there is no exposed mechanical transmission component, it won't be damaged easily. In addition, the chemical reactions are occurred majorly in the glass vessel. A magnetic stirrer or magnetic mixer is a laboratory device that employs a rotating magnetic flied to cause a stir bar or(flea) immersed in a liquid to spin very quickly. The main functions of the magnetic stirrer is to agitate the liquid for speeding up the reactions or improving mixtures. A magnetic stirrer is often used with hot plates.

Zinc nitrate

Zinc nitrate is a colorless crystalline solid. Noncombustible, but accelerates the burning of combustible materials. If large quantities are involved in a fire or the combustible material is finely divided, an explosion may result. Prolonged exposure to fire or heat may result in an explosion. Toxic oxides of nitrogen are produced in fires involving this material. It is used as a catalyst in the manufacture of other chemicals, in medicine, and in dyes. Zinc nitrate has no large scale application but is used on a laboratory scale for the synthesis of coordination polymers; its controlled decomposition to zinc oxide has also been used for the generation of various ZnO based structures, including nanowires. It can be used as a mordant in dyeing. Zinc Nitrate is a highly water soluble crystalline Zinc source for uses compatible with nitrates and lower (acidic) pH. Nitrate compounds are generally soluble in water. All metallic nitrates are inorganic salts of a given metal cation and the nitrate anion.

METHDOS

The plant *Gomphrena globosa* were collected from Ramanathapuram District. The collected leaves from the plant were thoroughly washed under tap water several times to remove dust particles and then dried at room temperature still complete drying. leaves were powdered using home mixture blender and stored in an air-tight container at room temperature till further use. Now, the powder was dissolved in the distilled water. The 50g of powder were weigh in the weigh machine then extract should be produced by using sahlet apparatus. The extract should be cooled and filtered through the whatman filter paper no 1. After filteration, the solution was stored at 4degree Celsius. The precussor solution for the synthesis of zinc oxide nanoparticles is zinc nitrate. The zinc nitrate added to the plant extract is the precussor for synthesis ZnO nanoparticles. For the synthesis of nanoparticles, 50 ml leaf extract were boiled at 60-80 degree Celsius in the stirrer. 5g of zinc nitrate was added to the extract solution till the temperature reached 60 degree Celsius for 2 hours.Then boiled it until reduced to deep yellow paste. The paste should be collected and heated in air heated furnace at 400 degree Celsius. After heated in furnace, the powder should be obtained. The zinc oxide powder was white in color. The white ZnO nanoparticles were used in the man fields. The powder were collected and stored for characterization.

UV-Vis spectra analysis

Electromagnetic radiation such as visible light is commonly treated as a wave phenomenon, characterized by a wavelength or frequency. Wave length of the left below, as the distance between adjacent peaks and may be designated in meters, centimeters or nanometers (10-9 meters). Visible wave lengths cover a range from approximately 400 – 800 nm. UV visible spectroscopy has proved useful in biochemical analysis, environmental studies, in forensic science, drug kinetics, food quality, etc .,(p.ramesh et al 2014). Ultra violet and visible

absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be t a single wavelength or oven an extended spectral range.

The absorption spectrum

When a sample is exposed to light energy that matches the energy difference between a possible electronic transition within the molecule, fraction of the light energy would be absorbed by the molecule and the electrons would be promoted to the higher energy state orbital. A spectrometer records the degree of absorption by a sample at different wavelengths and the resulting plot of absorbance (A) versus wavelength (lambda) is known as a spectrum.

FTIR analysis

FTIR was used to identify the possible functional groups involved in the reduction of zinc ion and capping of reduced zinc oxide nanoparticles. FT-Raman Spectra were obtained on a Bruker RFS- 100 Instrument. It is equipped with an ND: YAG laser (1064 nm line) and the laser power can be controlled using the OPUS software. The main Instrument is connected to a microscope using which small areas of samples can be analyzed. In the analysis of ZnO-NPs, the powder is placed under the microscope the energy of the laser photons being shifted up or down. The shift energy gives information about the vibration modes in the system. (G.Bhumi and N.Savithramma, 2014).Infrared refers to electromagnetic waves between the wavelength of visible light (0.78μm) and microwave (1000μm). Because molecules absorbs specific frequencies of infrared light, and transit to an excited state. Therefore, by reading the spectrum, we can realize the molecular structure. This is the basic principle of the infrared spectrometer. Infrared spectrometer method, which is a kind of non-destructive analysis. The original structure of the sample won't be changed or damaged, after the measurement of IR spectrometer NCBI pub chem Database(2018).

X- Ray diffraction

The formation and quality of compounds were investigated by X-ray diffraction technique. The scanning was done in the region of 2θ from 20° to 80°. X-ray powder diffraction is rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The size of the ZnO-NPs was determined by Debye Sherrer's equation. D = 0.94l/b cos q. Sadhan kumar chaudhuri, lalit malodia (2017), G.bhumi and N.savithramma (2014).

Phytochemical Analysis

Preliminary phytochemical analysis was carried out for all the extracts as per standard methods described by brain and turner 1975 and evans 1996.

Detection of Alkaloids

Extract were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

Marer's test

Filtrate was treated with mayer's reagent.Formation of a yellow cream precipitates indicates the presence of alkaloids.

Mayer's test

Mercuric chloride (1.358g) is dissolved in 60ml of water and potassium iodine (5g) is dissolved in 10ml of water. The 2 solution are mixed and made upto 100ml with water.

Wagner's test

Formation of brown/ reddish brown precipitate indicates the presence of alkaloids. Wagner's reagent: Iodine (1.2g) and potassium iodide (2g) is dissolved in 5ml of water and made up of 100ml with distilled water.

Detection of Steroids

Liebermann burchard test

2ml of acetic anhydride was added to 0.5g of the extract, each with 2ml of H_2SO_4, The color change from violet to blue or green in some sample indicates the presence of steroids.

Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthroquinoes

Borntrager's test

About 0.2g of the extract was boiled with 10% HCL for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrates. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicates the presence anthroquinoes.

Detection of Phenols

Ferric chloride test

Extract was treated with few drops of acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Leade acetate test

Extract was treated with few drops of acetate solution formation of yellow color precipitate indicates the presence of phenol.

Detection of Saponnins

Froth test

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistence of small bubbles) shows the presence of saponnins.

Detection of Tannins

Ferric chloride test

A small quantity of extract was mixed with water and heater on water bath. The mixer was filtered and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

Detection of Carbohhydrates

Fehling's test

0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solution a and b. A red precipitate indicates the presence of sugar.

Fehling's solution A

Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

Fehling solution B

Potassium sodium tartrate (173g) and sodium hydroxide (50g) is dissolved in water up to 500ml.

Detection of Oils and Resins

Stop test

Test solution applied on filter paper. It develops a transparent appereance on the filter paper. It indicates the presence of oils and resins.

RESULTS AND DISCUSSION

The results obtained from the various in vitro and in vivo studies during the course of experiment are given in this chapter. The yellow colour detects the presence of ZnO Nanoparticles synthesis in our plant *Gomphrena globosa*. The change of yellow colour should indicate the Zno Nanoparticles synthesis. It should be confirm the presence of ZnO Nanoparticles synthesis. Here the ZnO Nanoparticles synthesis characterized in the UV absorbtion. The Nanoparticles synthesis is in white powder should be characterized in UV and the extract also characterized in various methods. The phytochemical test were analyzed and the structural parameters also analyzed by using bioinformatics.

Analysis of phytochemical compounds

Phytochemical constituents like alkaloids, flavonoids, carbohydrates, phenol, tannins, terpenoids, steroids, oils, anthroquinones and saponins of leaf of *Gomphrena globosa* were analysed by qualitatively and reported in table 3. In this investigation, the phytochemical analysis confirm the presence of alkaloids, carbohydrates, steroids, saponins, terpenoids, flavonoids, oils and absence of phenol, tannins and anthroquinones. Pharmacological activity of the plant is attributed to the presence of these compounds.

Analysis of Structural parameters in *insilico* studies

In this study, the docking studies between the compound Salbutanol and *Bacillus cereus* and docking between *E.coli* and Salbutanol. The Salbutanol is the ligand and it is responsible for the *E.coli* and *Bacillus cereus*. The docking between the Bacillus cereus with Salbutanol and *E.coli* with Salbutanol is detected in the fig-3 and fig-5.And the parameters should be detect in the table -2. The binding affinity should vary in various compounds and depend on the docking. Here, the comparison between the *Bacillus cereus* and *E.coli* is detect by the binding affinity should be shown in table-2. Ligand should enhance the binding affinity between the *Bacillus cereus* and *E.coli*.The structures of the *Bacillus cereus*, ligand salbutanol and *E.coli* shown in fig-1,2 and 4.Finally, the structural parameters are analyzed

and shown in table-2. It should be analyzed by docking studies with the compound which is detect in the UV-absorption. The ligand Salbutanol is responsible for the binding with such compounds are *Bacillus cereus* and *E.coli.*

CONCLUSION

Here, we are successfully synthesis the ZnO nanoparticles and confirmed by color change into yellow by Zinc Nitrate. The plant extract and nanoparticles were characterized using XRD, UV-Vis and FT-IR.In this project, we are successfully analyzed the phytochemical test and confirm the presence of some compounds like alkaloids, flavonoids, saponins, terpenoids, tannins, phenol, oils, anthroquines, carbohydrates and steroids and the structural parameters should be analyzed by bioinformatics using docking studies. The docking is concerned by the compound present in the UV-absorption.

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Table 1. Data analysis of XRD Report

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
20.9231	42.22	0.0010	4.24582	53.43
21.8333	26.08	2.8800	4.06745	33.01
22.6363	79.02	0.6089	3.92820	100.00
23.4130	72.93	0.6421	3.79962	92.30

Table 2. Data of FT-IR

Wavelength in cm^{-1}	**Functional group**	**Name of the functional group**
3386.79	-OH,Ph-OH	Alcohol &phenols
2923.56	C-H	Alkanes
1630.26	C-H	Alkenes
1439.07	C-H	Alkanes
1384.23	-CHO	Carboxylic acids
1245.71	$-NH_2$/-OH&Ph-OH	Amines /alcohol&phenols
1153.53	$-NH_2$	Amines
1101.46	$-NH_2$	Amines
1075.88	$-NH_2$	Amines
1048.28	$-NH_2$/-OH&Ph-OH	Amines/alcohol&phenol
1032.11	$-NH_2$/-OH&Ph-OH	Amines/alcohol&phenol

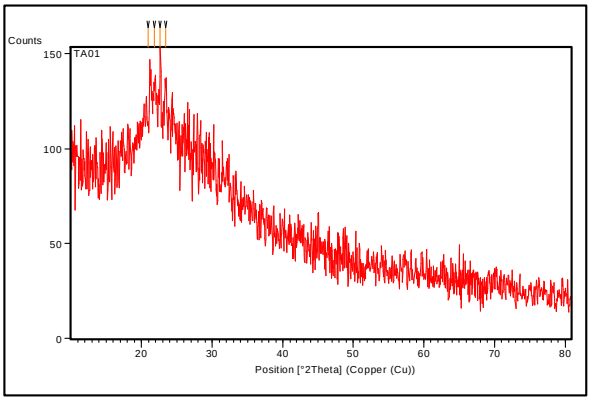
Table 3. Presence of Phytochemical elements

Name of phytochemical	**Presence of phytochemical**
Alkaloid	++
Flavanoid	+
Phenol	Nil
Tannins	Nil
Terpenoid	+++
Saponin	+++
Carbohydrate	+
Oils and resins	+++
Steroid	+++
Anthroquinones	Nil

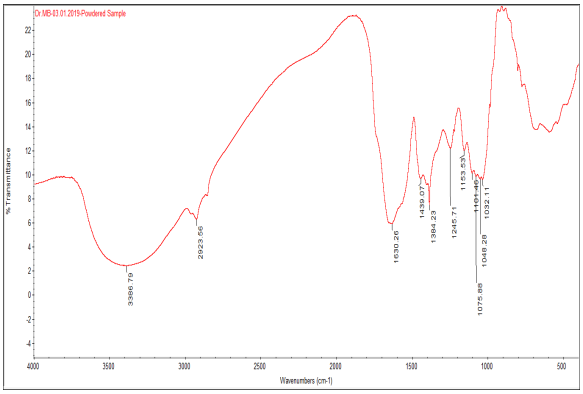
+++ Present in high concentration, ++ Present in medium concentration.
+ Present in low concentration, Nil- Not present in sample.

Table 4. Doking studies in bioinformatics

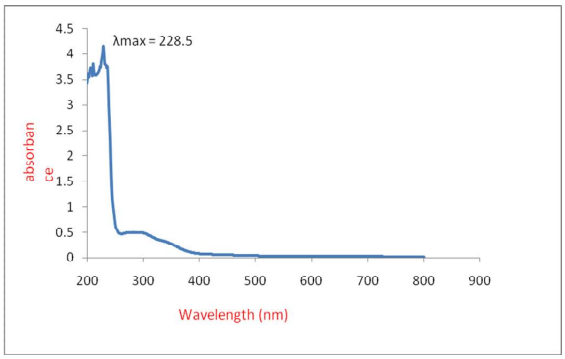
Receptor – Ligand Interaction	E total	Eshape	Emax	Emin	Net charge		Net Formal Charge	Atoms in the Ligand	Binding Affinity
					+ve	-ve			
Bacillus Cereus	-610.26	-610.26	9905.79	-610.26	37	39	-2	4696	12.03
E.Coli	-662.99	-662.99	7142.21	-722.47	108	99	9	4696	22.21



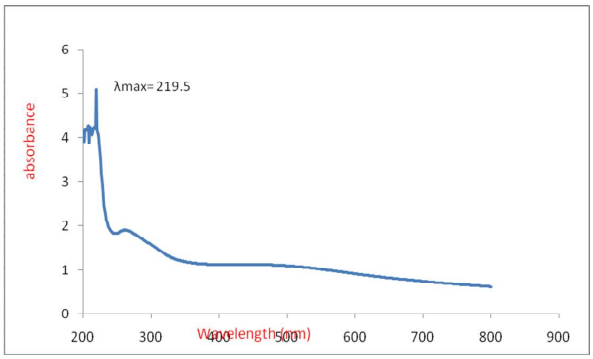
Graph 1. XRD Report of *Gomphrena globosa*



Graph 2. FT-IR Report of *Gomphrena globosa*



Graph 3. UV Graph for *Gomphrena globosa* Extract



Graph 4. UV graph for ZnO NPs

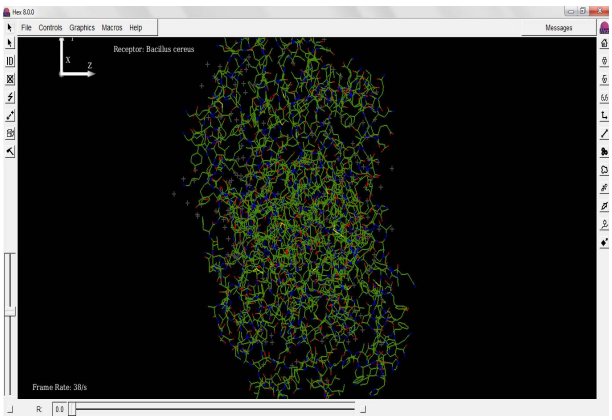


Fig.1. Structure of *Bacillus cereus*

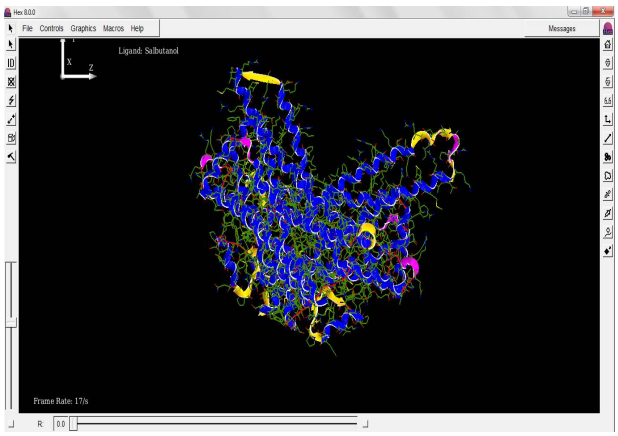


Fig.2. Structure of the Ligand Salbutanol

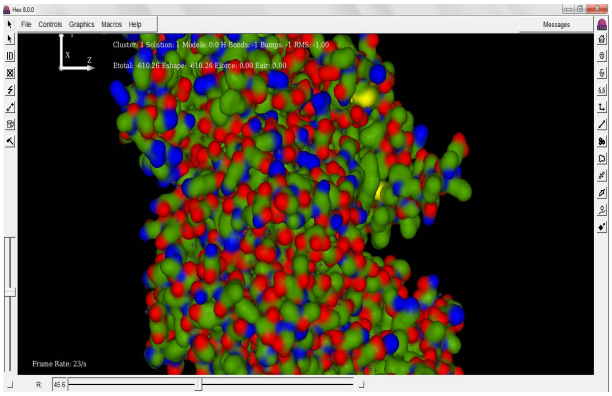


Fig. 3. Docking of *Bacillus cereus* and Salbutanol

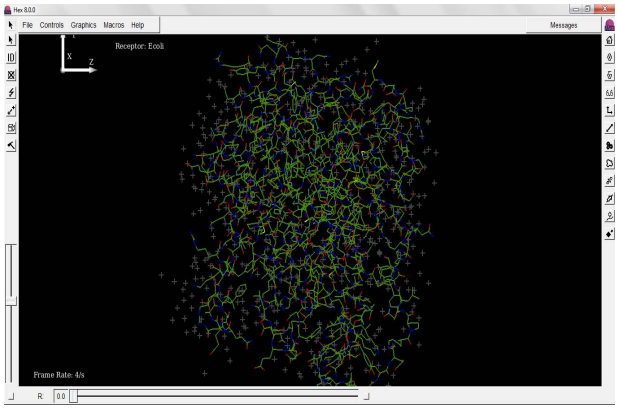


Fig. 4. Structure of *E.Coli*

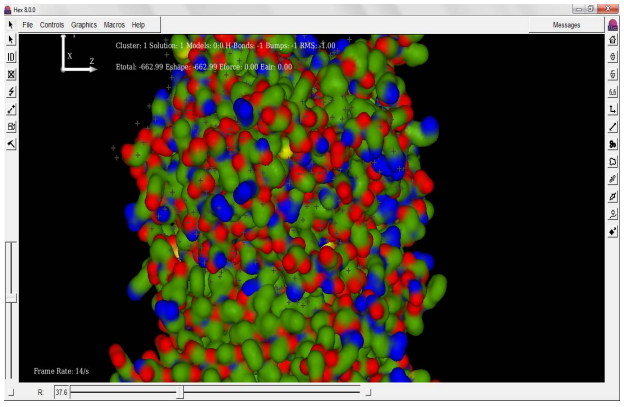


Fig .5. Docking of *E.coli* and Salbutanol

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RESEARCH ARTICLE

Determination the Infiltration Rate by Using a Double - Ring Infiltrometer in AL-Jadwal Al-Gharbi District / Karbala / Iraq

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ABSTRACT

The infiltration means the movement of water downwards and interring the soil through its surface. The measurement and determination of the infiltration rate has a great need for the planning and design of water resources projects, agricultural projects, calculating the amount of runoff to avoid flooding and the transfer of pollutants to surface water sources. The present study is a field work to measure the infiltration rate by using a double ring infilitrometr. Field experiments were carried out at three different locations of the soil in a Technical Institute of Karbala that located in AL-Jadwal Al-Gharbi district within the borders of Karbala city in Iraq. Repeated water depth readings were taken at intervals of (1,1,1,2,5,5,5,5,5,10,10,10,20,20, and 20) minutes. Depending on the filed results, Horton's equation in the three locations were determined. Soil texture was analyzed and was found to be Silty Sand (SM) to Clayey Sand (SC).The results of field measurements showed that the values of constant infiltration rate were; 6, 4.2 and 2.1 cm / hr in the locations; 1, 2 and 3 respectively. Based on the ratio of the filed values of initial infiltration rate to steady state infiltration rate, the soil in the three locations were classified as high permeable soils. The Horton's model gives a good fittings with the filed infiltrations results and the correlation coefficient (R^2) were (0.8001,0.9167,0. 9253) in the locations ; 1, 2 and 3 respectively. The result showed that the steady infiltration rate (F_C) increase with increase of effective diameter (D_{10}), and that there is a significant correspondence between the field measurements values ; ($F_{C(Filed)}$) with their the calculated from the Horton equation; ($F_{C\ (Horton)}$) and those calculated values according to (ASTM D 422) ; ($F_{C\ (ASTM\ D\ 422)}$) that based on the effective diameter (D_{10}) in the three locations of the study area.

Keywords: AL-Jadwal Al-Gharbi District, Karbala , Iraq, infilitrometr, Horton's model , infiltration rate.

INTRODUCTION

Infiltration is one of the major parts of the hydraulic cycle and by which the water inters vertically into the soil from the ground surface. The rate of infiltration is the water depth that inflirated into the soil from its , surface per unit time (Gayatri Das and Mimi Das Saikia , 2016 ; Amreeta Champatiray, 2014). On the relatively long term time the infiltration rate will be constant, then its called the Steady infiltration state (C. L. Jejurkar and Dr. M. P. Rajurkar, 2012). The infiltration capacity can be known as the maximum inflitration rate for a particular soil at a given time. Usually it is expressed in the unit length per unit of time. The Soil texture , degree of temperature, vegetation cover, dgree of compaction in the soil surface ,the rainfall intensity, and soil water content are the most important factors affecting infiltration capacity. (Gayatri Das and Mimi Das Saikia, 2016; Amreeta Champatiray, 2014). Water runoff and recharge to subsurface soils represent the main hydrological components resulting from the water arriving the soil surface (from rainfall or irrigation). In other words, there are two possibilities for water reaching the surface of the soil , once it reaches the rivers, streams and lakes by runoff or infiltrate into the soil section. Surface runoff may cause soil erosion, flooding and deteriorate water quality. Water infiltrated across the soil surface can be considered one of the important source of water to maintain the growth of plants and replenish the groundwater supply to wells and springs (Manoj Gundalia, 2018 ; Ellen R. Turner, 2006) . Based on this concept, the infiltration process can pass through three states: the first is entry of water to the surface of the soil, the second is the arrival of water to the unsaturated area, and the third state is recharge the groundwater reservoirs (Anjaneya Prasad M., *et al.,* 2015) .

The determination of the amount of infiltration is of the important fundamentals in many hydrological concepts such as; the prediction of surface runoff, estimation of groundwater feeding, watershed management, assessment of the availability of water for plants growth, describing of the permeability of soil, designing for Irrigation and drainage projects, prediction of flooding and erosion, and determination the amount of runoff and peak water discharge. The values of maximum runoff represents a fundamental variable in the design of various hydraulic structures. (C. L. Jejurkar and Dr. M. P. Rajurkar, 2012 ; Ellen R. Turner,2006 ; Anjaneya Prasad M., et al., 2015 [6] ; Tarek Silem, 2011 ; Srinivasan.K and Poongothai.S , 2013). The field methods are usually used to determined the infiltration rate. The most common of these methods are ; flood of water in basins or furrows, sprinkler of water and double or single-ring infiltrometers.(Kobra S. Leiveci , et al., 2016) Ring infilitrometr are commonly used in situ measurements to determine the hydraulic properties of the soil such as determination of the infiltration rate into the soil. The Single ring infilitrometr gives estimates of infiltration rates higher than the real values most often (Tarek Silem, 2011 ; Gregory, J. H., et al., 2005). This is because the movement of of water from the bottom of the cylinder is in two directions, vertically and laterally. The errors in the inflitration measurements associated with the use of the single ring inflitrometr were greatly reduced by the use of the double ring inflitrometr. Field research has shown that infiltration measurements using the double ring inflitometr were more reliable and represent the real state because the head of water in the external ring increased the infiltration in vertical direction in the internal ring (ASTM, 2003) . Kostiakov, Horton and Holtan empirical well -Known equations, and Green - Ampt and Philip approximate equations can be applied to extract infiltration rate in field studies. (Manoj Gundalia, 2018) .

Ellen R. Turner (2006) conducted a study to measure infiltration rates from rainfall simulation events at two different locations in Maryland, USA. In this study, five equations were compared, including Kostyakov, Horton, Holten, Phillip, and Green-Ampt to determine which ones give results approaching field values. The Horton equation was shown to have the best results. Galawezh et al. (2010) conducted a study to determine the amount of infiltration rate of some soils in different locations in the central part of the Erbil plain in the Kurdistan region of Iraq using a double cylinder infilitrometer and apply the Horton equation on the results. In this work the sudy area was classified into three categories; Moderate – High , Moderate and low – Moderate, according to the results of the infiltration rate mesurments. F. Haghighi et al. (2010) presented a study to determine the final infiltration rate of soil at eight different sites in the Taleghan region of Iran using double ring infiltrometer .The seady state infiltration rate was compared with the cofficent of pearmablity of soil. In this study, infiltration models such as Kostiakov-Lewis, Philips, and

Horton were evaluated to fit the filed infiltration data. The results showed that Horton's model estimated infiltration rates were having closer values to that of field measured .Tariq Selim (2011) presented a field study using a double ring inflitrometr. He investigated the infiltration rate for heavy soil in diffrent locations in the El-Salam Canal near of Suez Canal , Egypt . Galawezh B. Bapeer (2011) conducted a study to find infiltration rate in various locations of the Quesengq region in the Kurdistan region / Iraq using a double cylinder infilitrometer. In the most parts of the Quesengq region , the infiltration rates ranged from (moderate - high) to (low- moderate), and its values were low in the southern parts because of the increase in the percenteg of clay in the soil. In (2012), Jagdale S. D. and Nimbalkar P. T. conduted different expermintal works of infiltration by using the double-ring inflitrometer of different soil types under different soil conditions. In this study the measured field data were compared with the results of a group of empirical equations . They showed that the results of Horton and GreenAmpt equations gave a good correlation with the expermintal measurements. C. L. Jejurkar and Dr. M. P. Rajurkar (2012) presented a filed infilitration study in different conditions at different locations in Village Brahmangaon , India.

This study showed that inflitration rates are significantly affected by the type and density of vegetation.Various infiltration equations such as Kostyakov, Kostyakov, Horton, and Phillip have been applied to verify the extent to which their results are close to field results. Dr. Avinash S. Kadam (2016) presented a study to find the infiltration rate resulting from artificial water recharge at selected sites in Degloor Tahsil in theNanded region of India. In his study, he also showed the effect of various physical factors on the value of infiltration rate . Manoj Gundalia (2018) conducted a study to find the constant infiltration rates for different soil by double-ring inflitrometer in the Ozat watershed in Gujarat (India). In this study, a new mathematical model was develobed to determine the infiltration rate and compare the results obtained by this model with the values calculated from many empirical infiltration models. The Present study is an experimental work to assess the infiltration rate of the soil of agricultural fields in the Technical Institute of Karbala which located in AL- Jadwal Al-Gharbi District / Karbala / Iraq by using the Double - Ring Infilitrometer. The Horton equation was also applied to determine the correlation between its results and the field infiltration measurements.

STUDY AREA

Karbala city is located at central zone of Iraq, about 100 km south of Baghdad, which is the capital city of Iraq (Al-Mohammed and Mutasher , 2013).The study area is an agricultural fields of the Technical Institute of Karbala which is located in AL- Jadwal Al-Gharbi district, at the coordinates (N 32 ◦ 34' 38.62", E 44 ◦ 10' 17.02.2") ; 20 km south of Karbala city along the main road (Karbala- Hilla) within the borders of Karbala governorate as shown in Fig.(1). The clamatic state for the study area is ; A dry hot summers and cold winters, the temperature during summer is usually reached over 43 ◦ C and between 16 ◦ C and 2 ◦ C during winter, and the annual mean rainfall intensity is about 2.5 mm. So,the study area can be considerd is arid to semi-arid area. The study area is also characterized by dense palm groves, fruits and citrus trees, in addition to the cultivation of some fodder crops ; alfalfa and corn (Al-Mohammed and Mutasher , 2016). Soil texture was analyzed and was found to be Silty Sand to Clayey Sand (USCS , 2015) . In order to conduct field experiments and find the amount of infiltration rate, three locations were selected in three different sectors in the study area at the coordinates as shown in Table (1).

MATERIALS AND METHODS

Measurements of Infiltration Rates

The tests of Infiltration rates were carried out at the three locations in the study area shown in fig.(1) for a period of 120 min for each test. Double ring infiltrometer consists of two iron rings (inner and outer rings) with 2 mm plate thicknesses, (30 and 60) cm diameters and 80 cm in height was used in these mesurments. The two rings have bevel sharp edges to facilitate their penetration into the ground surface of the soil (ASTM, 2003) . Before the start of the

experiment, a layer of surface soil was removed to a depth of about 30 cm without disturbing the soil surface. Firstly, the outer ring shall be placed on the soil, then a wooden piece (10 cm x 10 cm) and a long of 1 meter shall be placed on its upper surface and then hammered with a hand iron hammer until the cylinder is firmly installed into the soil to a depth of 20 cm. To ensure the entry of this ring into the soil equally from all sides, a manual bubble level tool has been used to this purpose. In the same way the inner ring is installed exactly in the center of the outer ring and penetrated the soil to a depth of 10 cm. A pieces of nylon were placed on the soil before water was poured when filling the rings to prevent the erosion and disturbance of soil. The gap between the two rings was filled with water first to a specific mark. Then the inner ring was filled to the same mark, a metal ruler was inserted vertically into the inner ring. The test begins where the water drop (infiltration) is measured in centimeter during specific time intervals (1,1,1,2,5,5,5,5,5,10,10,10,20,20 and 20 min) using a stopwatch. It should also be noted that a constant level of water has been maintained in the two rings by refill both rings to the mark after each reading and compensating for the drop of water during the test.Throughout the test period, the water depth is maintained constant in the outer ring so that the soil is saturated in the area surrounding the inner ring and thus the vertical infiltration will increased through the soil surface below this ring .

Soil Texture Analysis

Sieve analysis were carried out on soil samples taken from the three locations of the study area in the laboratories of the Department of Civil Techniques of the Technical Institute of Karbala. From these results, the gradient curves of the soil were plotted in different locations as shown in Fig. (2). From these curves, the percentages of gravel, sand, (clay and silt) and the effective diameter (D_{10}) were extracted, and then the results were recorded in Table (2). Since the percentage of sand ranged from (87-90) % in the three locations, it can be said that the soil in general as sandy soil, and when conducting a detailed analysis of the soil of the study area based on the unified soil Classification System it was found to be Silty Sand (SM) to Clayey Sand (SC) (USCS, 2015).

Infiltration Model

In this study the equation that proposed from Horton in 1940 was applied as infiltration model on the filed measurements of infiltration rate in the study area .This equation described the infiltration rate [F t] with varying time (Galawezh B. Bapeer , 2011).

$$F_t=F_c+(F_0-F_c)e^{-Kt} \quad \text{.....(1)}$$

Where;
F_t : infiltration rate. (cm/hr)
F_c : Final steady state or constant infiltration. (cm/hr)
F_0: Initial infiltration rate. (cm/hr)
K: Horton's constant. l / (hr)
t: time of infiltration. (hr)

The values of (F_C) and (F_0) are extracted from the infiltration curve, which represents the relationship between the infiltration rate and the accumulative time. The F_0 is the intersection point of the curve with the Y axis and the F_C value represents the constant or stable final value of the infiltration rate when the curve starts to turn into a straight line. The value of the coefficient (K) is determined is as follows; (John. J. M. and Peter A. A., 2010).

1.Rearranging Equation. (1) as below:

$$(F_t-F_c)=(F_0-F_c)e^{-Kt} \quad \text{.....(2)}$$

2.Taking the logarithm of Equation.(2):

$\text{Log}(F_t - F_c) = \text{Log}(F_0 - F_c) - Kt\ \text{Log}(e)$ ……(3)

3.Equation (3) above can be expressed as a straight line equation ; Y = Mx + C ,

where ;
C = log (F0-FC); which is the intercept point with the Y-axis,
x = (t) which is a variable.
M = –K Log (e) ; which is the slope of straight line , then

$K = -M / \text{Log}(e)$ ……(4).

Hence we can determined the value of the coefficient (K) by plotting the relationship between the value of (F_t -F_c) on the Y axis on the logarithmic scale and the cumulative time on the X axis on a natural scale, and then extracting the slope value (M) for the best straight line resulting from this graph and by the applying the equation (4) above we can obtained the value of (K).

RESULTS AND DISCUSSION

Infiltration results

The field measurements of infiltration in (cm) during specific time intervals (1,1,1,2,5,5,5,5,5,10,10,10,20,20, and 20 min) by using the double ring at the three locations in the study area were recorded . The infiltration rate in (cm/min) was obtained by dividing the infiltration in (cm) on the time interval in (min) and then converted to (cm/hr) unites, and; accumulative time (min) and accumulative infiltration (cm) also calculated. All results were recorded in Table (1) –Appendix (1).Depending on these results the infiltration curves of infiltration rate (cm/hr) verses time (min) were plotted for the three locations as shown in Fig.3. Fig .(3); shows that the infiltration curves for locations 1, 2, and 3 start with high infiltration rate values ; (60, 36 , 30 cm / hr). These values gradually decrease until they reach to a constant values (F_c); (6 , 4.2 , 2.1 cm / hr) respectively. The values of (F_0) are extracted from the infiltration curves shown in fig.(3); which are the intersections of these curves with Y axis. The values of (F_C) and (F_0) for the three locations in the study area were recorded in the table.(3). And based on the measured data the Horton's coefficient (k) can be determined from equation (4) above.

$$M = \left(\frac{\log(30) - \log(1.8)}{(0-1)}\right) = -1.223$$

but from Eq.(4) above ; **$K = -M / \log e$**

∴K=–(–1. 223) / 0.4343= 2.82)

In the same way, the coefficient (K) can be calculated in the other two locations, where these values are (3.6, 5.7) in the two locations (2, 3) respectively. Hence the Horton's equations can be written for the three locations in the study area.(Table.3). The values of the infiltration rate (cm / hr) were calculated according to the Horton equation for each location . These results were recorded in (Table 1) –Appendix .(1). The infiltration curve was plotted according to the results of the Horton equation and compared to the curve that was drawn according to the field values of the three locations in the study area as shown in Fig.5 . It is clear that the results of the Horton equation have a good correlation with the field-measured values in the three locations. This correlation between the two curves appears to be more in locations (2 , 3) than in the location (1).

When drawing the field values of the infiltration against the values calculated from the Horton equation, and when the linear regression was carried out as shown in fig.6, The correlation coefficient (R^2) at the two locations (2,3) was (88.64%, 90.25%) respectively, which is more than the value of the correlation coefficient at the location (1) where it was (80.4%). Overall the value of (R^2) between 70% to 100% are considered to have a solid bond (Amreeta Champatiray, 2014), So in this study we can say that the Horton's model gives a good fittings with the filed infiltrations results. The measurement of permeability capacity was determined according to the classification as shown in Table.(4), that depending on the F_o / F_c ratio which is computed from the results in the field (Ashikin B. Shaari et al, 2016) . Overall, the present study shows that the soil of the study area have a high permeability in the three locations as shown in Table. (5). The soil analysis results were recorded in table.(2) above show that there is a relationship between the infiltration rate and soil texture. This may give us an acceptable explanation for the high value of the infiltration rate at the first location due to the high percentage of coarse grains in its soil if compared to other locations. And based on the values of the effective diameter values (D_{10}), the long- term or design infiltration rate (Steady state or constant infiltration rate) (F_c) was calculated according to (ASTM D422), Table (6). (Joel W. Massman, 2003; Derek C. Godwin and Marissa Sowles, 2011). The results are summarized in Table (7).

From Table (7) it is clear that there is a significant correspondence between the calculated values of (F_c) based on the effective diameter (D_{10}) with their field value and those calculated from the Horton equation in three locations .And also the results showed that the infiltration rate increases with the increase of the effective diameter in the three locations of the study area as shown in Fig. (7 and 8).

CONCLUSIONS

The infiltration indicate to irrigation or rainfall water that penetrating the soil surface.This process represents the first phase of the movment of water in the soil. In the present study a double ring in- filtrometer was used to determine the filed infiltration rate in three locations of soil of the Karbala Technical Institute. From the present study there are many result have been recorded

1. From the field measurements the initial infiltration rate (F 0) were found (64, 38, 32 cm/hr) while the steady state infiltration values; (F_C) were found (6, 4.2 and 2.1 cm / hr) in the locations (1 ,2 and 3) respectively in the study area.
2. The values of the infiltrations rate that calculated from the Horton equation gave a good match with the field-measured values, and the correlation coefficients values (R^2) were; (0.8001, 0.9167.0, 9253) in the locations (1 , 2 and 3) respectively. That indicate to a good fittings of the Horton model with the filed result.
3. The soil for the three locations have a high permeability depending on the (F_o / F_c) ratio which is calculated from the filed results.
4. The results showed that the soil infiltration rate was closely related to the granular gradient of the soil and since there was a positive relationship between the infiltration rate and the effective diameter (d_{10}).
5. The results recorded a highest value of the constant infiltration; (F_C) was (6 cm / hr) in the location (1) when effective diameter was (0.132 mm), while the lowest value was (2.1 cm / hr) in the location (3) when the effective diameter of (0.05mm).
6. There is a significant correlation between the measured field steady state infiltration values: ($F_{C(filed)}$) and their values were calculated according to the Horton equation ($F_{c\ (Horton)}$) and those calculated according to (ASTM $_{D422}$) ; ($F_{C\ (ASTM\ D422)}$) depending on the effective diameter (d_{10})

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Table. 1. The coordinates of the locations of the infiltration field measurements

Locations	Coordinates			
Loc.1	N	32°	34'	29.02"
	E	44°	10'	25"
Loc.2	N	32°	34'	27"
	E	44°	10'	19.4"
Loc.3	N	32°	34'	30.84"
	E	44°	10'	17.04"

Table.2. Particle size distribution analysis

Location	Gravel %	Sand%	(Silt+ Clay)%	Soil texture classification (USCS, 2015).	Effective diameter (D_{10}) (mm)
Loc.1	5.78	87.54	6.64	Silty Sand (SM); (sand-silt mixtures) with gravel	0.132
Loc. 2	2.79	92.61	4.6	Silty Sand (SM) ; (sand-silt mixtures) with gravel	0.085
Loc.3	0.00	87.00	13	Clayey Sand (SC) ; (sand-clay mixtures	0.050

Table.3. The Horton's equations and its parameters (F_c, F_o, K) for three Locations in the study area.

Locations	F_0 *cm/hr*	F_C *cm/hr*	K $_{(hr}$-1)	Horton equation
Loc.1	64	6	2.82	$\mathbf{F(t) = 6 + 58}e^{-2.82t}$
Loc. 2	38	4.2	3.6	$\mathbf{F(t) = 4.2 + 33.8}e^{-3.6t}$
Loc. 3	32	2.1	5.7	$\mathbf{F(t) = 2.1 + 29.9}e^{-5.7\ t}$

Table 4. Permeability indication ratio (Ashikin B. Shaari et al., 2016)

Ratio (F_0 /F_C)	Permeability Capacity
> 5	High permeability
3 – 5	Moderate Permeability
< 3	Low permeability

Table.5. Classifications of the soil permeability capacity in the study area

Location	Ratio (F_o /F_C)	Permeability Capacity
Loc.1	(64/6) = 10.67	High permeability
Loc. 2	(38/4.2) = 9.05	High permeability
Loc.3	(32/2.1) = 15.23	High permeability

Table.6. The long-term or design infiltration rate; (F_C) when D10 ≥ 0.05 mm (Joel W. Massman, 2003; Derek C. Godwin and Marissa Sowles, 2011).

D_{10} from ASTM D422 mm)	Estimated Long term Infiltration Rate F_C)	
	Inches/ hr	Cm/hr
≥ 0.4	9.0	22.86
0.3	6.5	16.51
0.2	3.5	8.89
0.1	2.0	5.08
0.05	0.8	2.032

Table . 7. The values of the (F_C) that calculated depending on (D_{10}).

Location	D_{10} (mm)	Fc (ASTMD422)	Fc (Filed measurements)	Fc (Horton Equation)
LOC.1	0.132	6.3	6	6.2
LOC.2	0.085	4.93	4.2	4.23
LOC.3	0.05	2.032	2.1	2.1

Appendix 1. Results of field measurement and estimation of Infiltration rate based on Horton model

Locations	Time interval (min)	Accuml. Time (min)	Infl. (cm)	Inf.Rate cm/min	Infl.Rate cm/hr	Accuml. Infl.(cm)	Infl.Rate cm/hr Horton
Loc.1	1	1	1	1	60	1	61.34
	1	2	0.75	0.75	45	1.75	58.80
	1	3	0.5	0.5	30	2.25	56.37

	2	5	1	0.5	30	3.25	51.85
	5	10	1.5	0.3	18	4.75	42.25
	5	15	1.2	0.24	14.4	5.95	34.66
	5	20	1.1	0.22	13.2	7.05	28.66
	5	25	1	0.2	12	8.05	23.91
	5	30	1	0.2	12	9.05	20.16
	10	40	1.5	0.15	9	10.55	14.85
	10	50	1.4	0.14	8.4	11.95	11.53
	10	60	1.3	0.13	7.8	13.25	9.46
	20	80	2*	0.1*	6*	15.25	7.35
	20	100	2*	0.1*	6*	17.25	6.53
	20	120	2*	0.1*	6*	19.25	6.2
Loc.2	1	1	0.6	0.6	36	0.6	36.03
	1	2	0.5	0.5	30	1.1	34.18
	1	3	0.4	0.4	24	1.5	32.43
	2	5	0.7	0.35	21	2.2	29.24
	5	10	0.8	0.16	9.6	3.0	22.75
	5	15	0.8	0.16	9.6	3.8	17.94
	5	20	0.7	0.14	8.4	4.5	14.4
	5	25	0.7	0.14	8.4	5.2	11.74
	5	30	0.6	0.12	7.2	5.8	9.79
	10	40	1.0	0.1	6.0	6.8	7.27
	10	50	0.8	0.08	4.8	7.6	5.88
	10	60	0.8	0.08	4.8	8.4	5.12
	20	80	1.4*	0.07*	4.2*	9.8	4.48
	20	100	1.4*	0.07*	4.2*	11.2	4.28
	20	120	1.4*	0.07*	4.2*	12.6	4.23
Loc.3	1	1	0.5	0.5	30	0.5	29.30
	1	2	0.4	0.4	24	0.9	26.83
	1	3	0.3	0.3	18	1.2	23.02
	2	5	0.4	0.2	12	1.6	20.70
	5	10	0.4	0.08	4.8	2.0	13.66
	5	15	0.4	0.08	4.8	2.4	9.30
	5	20	0.3	0.06	3.6	2.7	6.57
	5	25	0.3	0.06	3.6	3.0	4.88
	5	30	0.2	0.04	2.4	3.2	3.82
	10	40	0.4	0.04	2.4	3.6	2.77
	10	50	0.38	0.038	2.28	3.98	2.26
	10	60	0.36	0.036	2.16	4.34	2.20
	20	80	0.7*	0.035	2.10*	5.04	2.11
	20	100	0.7*	0.035	2.10*	5.74	2.10
	20	120	0.7*	0.035	2.10*	6.44	2.10

Fig.1. Study area and the locations of filed infiltrations testes

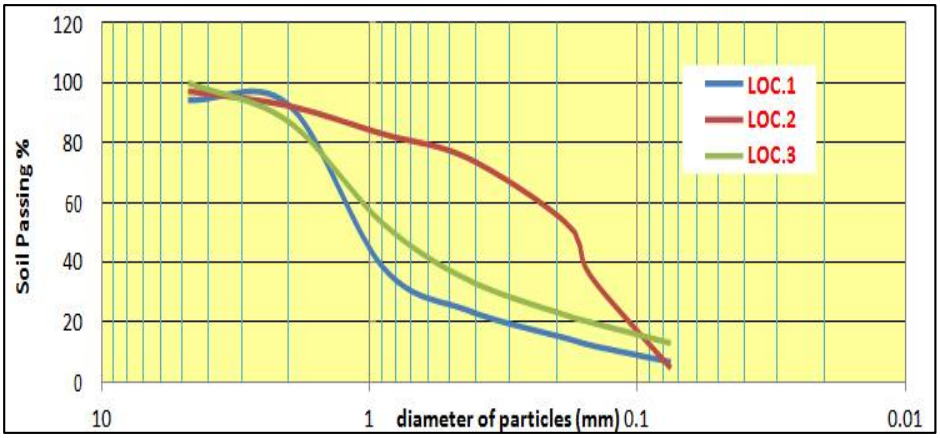

Fig. 2. Sieve analysis curves for soil symbols in three locations in the study area

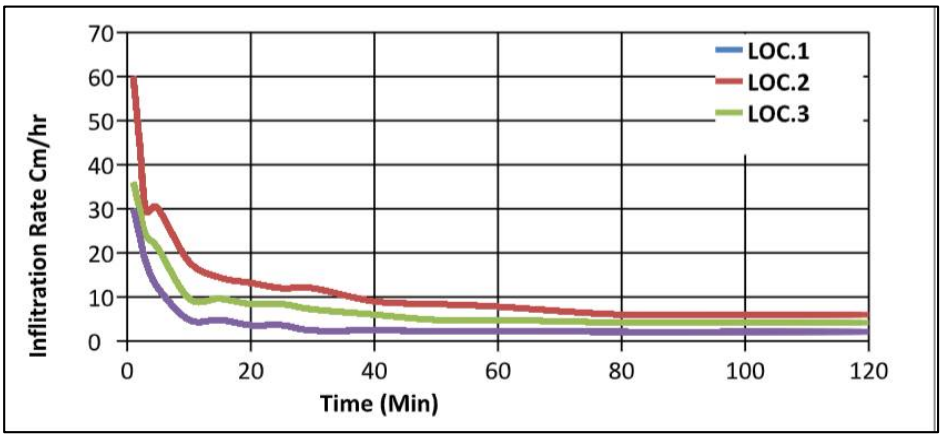

Fig.3. Infiltration curve for filed measurements in different locations in the study area

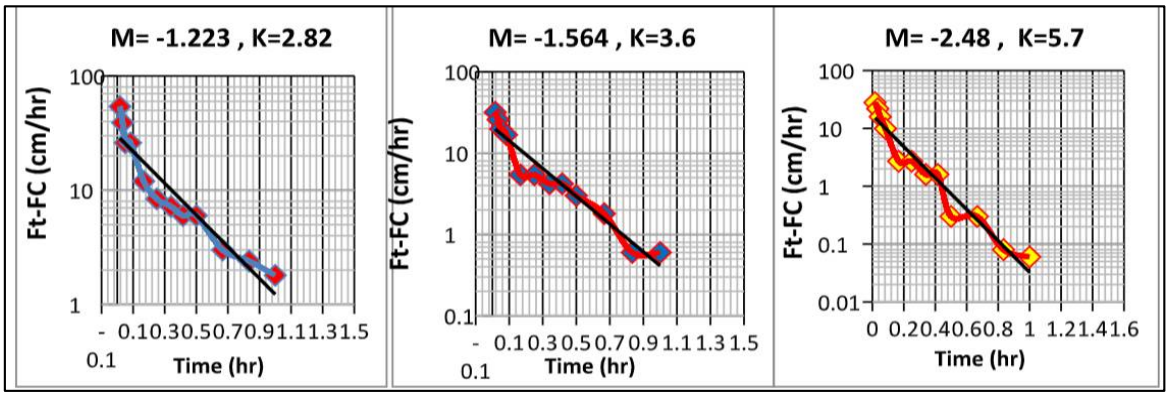

Fig.4. The determination of the Horton coefficient (K) in three locations in the study area

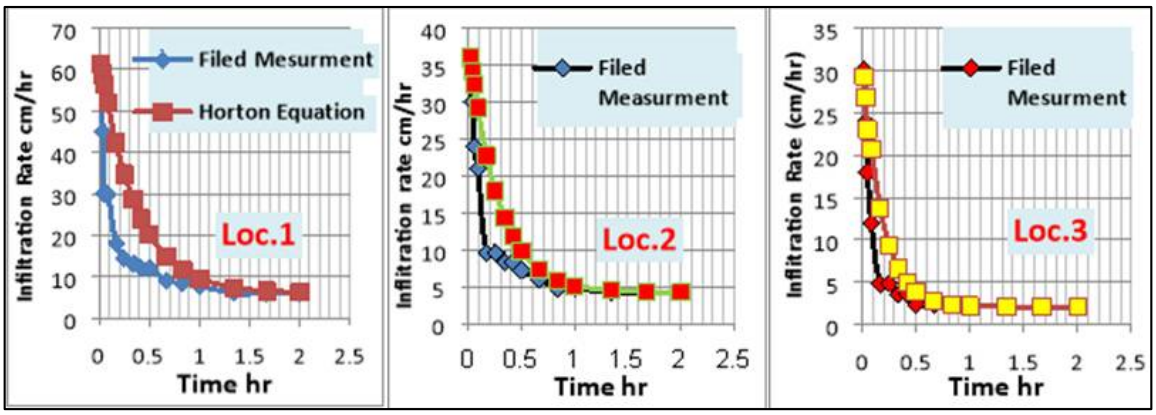

Fig. 5. Infiltration curve for filed measurement and Horton equation in Loc.1 , Loc.2 and Loc.3

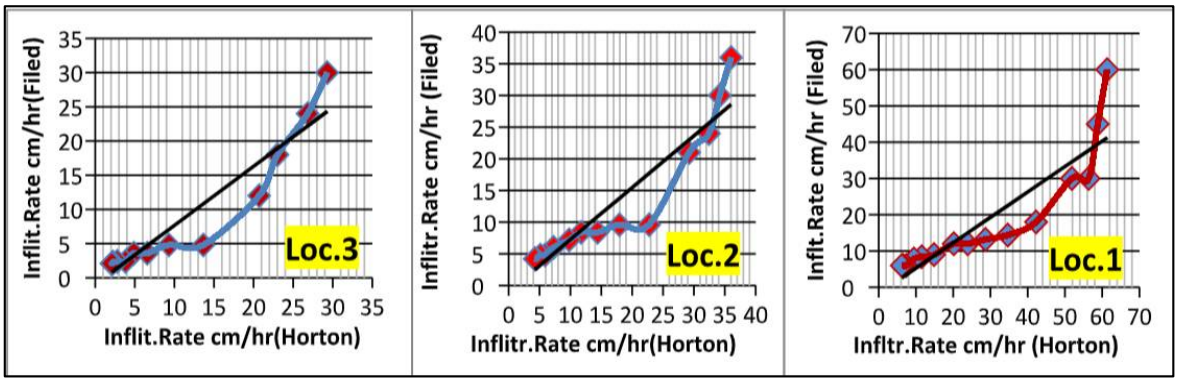

Fig. 6. The correlation between the Horton's and Filed infiltration rate values in Loc.1 , Loc.2 and Loc.3

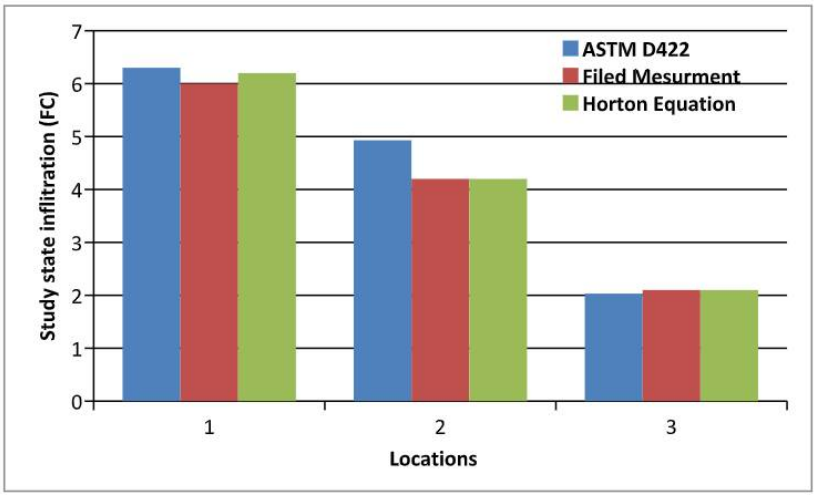

Fig.7. A comparison between ($F_{C\ (ASTM\ D422)}$) , ($F_{C\ (Filed)}$) and ($F_{C\ (Horton\ Equ.)}$) in the three locations

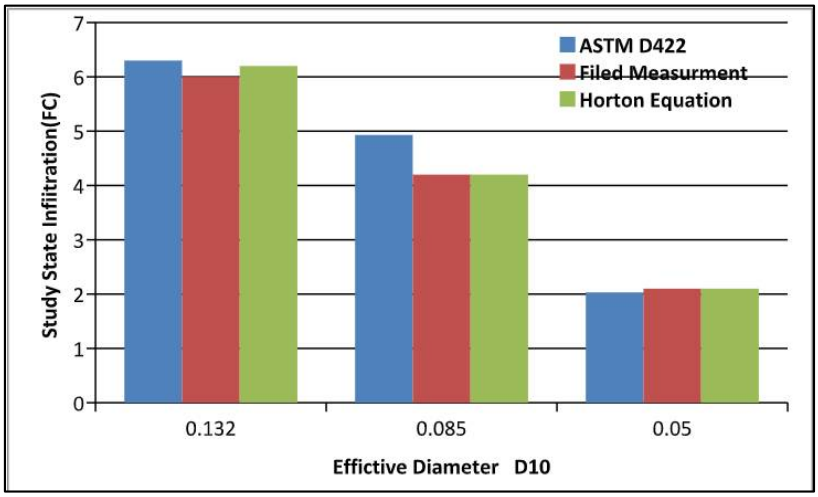

Fig. 8. Effect of the effective diameter (D_{10}) values on the (F_C) values in three locations

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REVIEW ARTICLE

Review of Photometric and Spectroscopic Properties of Blended Galaxies from Sloan Digital Sky Survey

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ABSTRACT

New observation and techniques for the astrophysical data are increasing our understanding of the evolution and formation of the different structures in the universe.Most of the physical information through the power spectrum and there is much more to explained by the statistical distribution of the random field. Estimation of the redshifts of the galaxies from broadband photometry and spectroscopy became a used in observational astrophysics. The probabilistic redshift can be derived from changes in the observed colors of the galaxies due to features of the redshifting in the spectral energy distributions of the galaxy in different of the broadband filters. Interpreting of the power spectra is estimating galaxy properties at large linear scales using likelihood methods. In this work, we review the physical properties of a sample of blended galaxies from SDSS, Data Release 14. We analysed the spectral and photometric properties of the sample in 5 passbands (u, g, r, i, z) covering from 3000 A^{o} to 10500 A^{o}at range of spectroscopic redshift of $0 \leq Z_{spec} \leq 1$. Our results of the using sample in the five recording bands show that most the blended galaxies seem to have real properties and the luminosity of the sample has a sharp Gaussian shape in r-band with a maximum number countof the galaxies in this band. The theoretical colors–redshift relation for the early or late type's galaxies has a bimodal distribution of $\mu_u - g = 1.792$

Keywords: fundamental parameters, ISM, spectroscopic redshift, photometric redshift.

INTRODUCTION

About half of the galaxies in the universe are found in groups and clusters. Group and clusters no longer expand the cosmic flow due to the mutual gravitational attraction are strong enough such that the galaxies are moving inward or passed through the core. [1].The redshift surveys are a major and growing technique in the astronomical research

field. Using of the photometric and spectroscopic redshift in the surveys make it possible to study a much larger number of the objects and to study the faint sources. The photometric redshifts are susceptible to larger random and systematic error. Both random and systematic errors can lead to systematic errors in the luminosity and mass function. [2]. The photometric redshift measurements use a photo-z algorithm which converts broad band fluxes into redshifts. This can be done by fitting to theoretical or empirical templates for galaxy spectra [3], or by completely empirical machine-learning techniques, [4] that are trained with a sample of galaxies of known spectroscopic redshift. The photometric redshifts are less accurate than the real spectroscopic redshifts, but spectroscopy is very time consuming. The spectroscopically measurements of the redshift should be significantly collect more light from the object and therefore the full spectrum could see rather than the intensity per filter, and the spectroscopic measurements are far more accurate due to a large amount of time exposure is required and therefore the photometric is a most common method. [5]

The Sample

We have written a simple SQL query to select galaxies that are blending with stars using multiple tables from SDSS using Casjobs DR14. We have downloaded the spectroscopic and photometric redshifts of 1000 objects assigned into a class 'galaxy', and their luminosities in five bandwidth filters in ugriz bands. We removed objects that have no physical meaning (i.e. -9999). The surface brightness of the disk of galaxies has adjusted to an exponential profile. We also measured the accuracy of the SDSS photometric redshift by comparing to the spectroscopic data. The accuracy determined by an R.M.S. error.

Data Analysis and Discussion of the Results

In this section, we will explain data reduction of the sample and discuss our results.
First, we will illustrate distribution of the galaxies according to their spectroscopic redshift and its correlation with the photometric redshift. Then we will discuss the luminosity distribution of the sample and study the theoretical colors –redshift relation of the sample. .

Distribution of the Galaxies in the Spectroscopic Redshift Range

The photometric and spectroscopic redshift of the blended galaxies can determine using two methods. Photometric redshift measures the average intensity of the light in a variety of different broad color filters. While the spectroscopic redshifts measured by calculating the absorption spectra of each object in the sample, then measuring the difference in wavelengths between the standard and measured lines. [6] Fig. 1 shows the normal distribution of the spectroscopic redshift in a range of $0 \leq z_{spec} \leq 1$ for 890 members in the main sample. Methods of analysis of cluster galaxies include a rich of statistical methods to group the sample data into simpler subunits. [7]. In our study, we have depended on a method of estimating the probability distribution of the objects to analyse the spectroscopic data of the cluster. [6]. We will illustrate the accurate representation of the distribution of the members in the sample by the histogram on the upper panel in Fig. 1. To specify the probability density estimation of the sample, we used a kernel density estimation method and wedependeda Gaussian Kernel. The adaptive kernel density estimation and Gaussian Kernel gives by $\mathrm{F}(z)$, [8]:

$$\mathrm{F}(z) = \frac{1}{N\,h}\sum_{i=1}^{N} K\left(\frac{z - z_i}{h}\right) \quad \text{..... (1)}$$

$$K\left(\frac{z - z_i}{h}\right) = \frac{1}{\sigma\sqrt{2\pi}}\exp\left(-\frac{1}{2}\left(\frac{|z_i - z|^2}{{\sigma_i}^2}\right)\right) \quad \text{..... (2)}$$

Where *h* is the bandwidth, *N* is total number of the objects in the main sample and *z* is the numeric values of the spectroscopic redshift for each object in the main sample, i.e.

$$z = \{z_i , z_{i+1}, \dots \dots \dots \dots z_N\}; \quad i = 1, 2, 3, \dots . N$$

The solid red line in the plot is the probability distribution as a Gaussian kernel estimate. The multiple peaks arise at , $z_{spec} \sim 0.18$ and $z_{spec} \sim 0.49$ due to the data have different colors is ugriz filters with an effective wavelength of 3590 A° - 9060 A°

Although the spectroscopic redshift is very reliable, they are time-consuming as we mentioned before. They required a long time exposure to detect the faint source which found in the deep field of observation. Furthermore, the spectroscopic observation is limited such that the range of the redshift is available to an optical spectrograph and OH lines for observation in near-infrared from the ground. The Power of observing surveys can be good enhance by using of the photometric redshift which is estimated from broadband 'galaxy'. The photometric redshifts can also be depending to show which of the less secure spectroscopic redshifts are to be correct and which are to be incorrect, [9]. For this reason and for correctly modelling galaxies evolution we estimated the photometric redshift accuracy of the sample.A standard redshift error determined by R.M.S error and given by:

$$\sigma_s = \sqrt{\overline{(\delta z)^2} - (\overline{\delta z})^2} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{(3)}$$

The plot in the lowerpanel in Fig. 1, shows the histogram of the fractional error in the photometric redshifts. The bin size is equal to 0.01. The plot shows that the typical accuracy in the photometric redshift of

$$\sigma\left(\frac{(z_{photo} - z_{spec})}{(1 + z_{spec})}\right) \approx 0.071 \text{ and the bias of } \frac{(z_{photo} - z_{spec})}{(1 + z_{spec})} = 0.01$$

The distribution is well described by a Gaussian as shown in the solid red curve in the figure with a mean of. $\mu_{\Delta z} = -0.009$ The best Gaussian fitting can express in equation 4 below. [10]

$$P(z) = \exp[-\frac{(z - \mu_z)^2}{2\sigma^2}] \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{(4)}$$

Where μ represents mean of the distribution, and σ is the standard deviation. The scatter plot of the photometric redshift against the spectroscopic redshift for the galaxies in cluster field at the five bands of photometry is shown in the upper panel in Fig. 2. We notice that there is a linear relationship between z_{spec} and z_{photo}, and the scaled intensity is proportional to the number of galaxies in each bin. The principal effects are the galaxies with spectroscopic redshift larger than 0 will be scattered up regarding to the photometric redshift. We also detected few blue shifted galaxies with$z_{spec} < 0$and neglected in this plot. The figure shows also that the galaxies with photometric redshifts less than 1 scattered down in spectroscopic redshift range. The comparison between the spectroscopic redshifts and photometric redshifts for all objects in the different bands gives a good agreement between probabilities of the redshifts and this suggests that the photometric redshifts can depend to identify an object by an object of less reliable spectroscopic redshift. [11]. The error distribution of $\frac{\Delta z}{(1 + z_{spec})}$ versus z_{spec} plottedin the lower in Fig. 2. R.M.S. error in the distribution is equal to 0.0000 ± 0.1032.

Luminosity Distribution of the Galaxies

The galaxy luminosity function is a fundamental property of the galaxy distribution, as it provides information on how visible matter is distributed among galaxies of various luminosities at a given epoch. Therefore, its evolution can be used to constrain models of galaxy evolution and structure formation. [12]. The absolute magnitude is a good indicator to measure the luminosity of the astrophysical object in the sky. We extracted the absolute magnitude of five photometric bands form the database of SDSS using 'P. ModelMag' function.Fig.3, explain the luminosity distribution functions of the sample in ugriz bands. We noticed that the absolute magnitude limits variation and the number of galaxies in each bin.As we see in this figure the distribution is similar and like to a kernel distribution in u, b, I, and z bands. While the distribution in r-band is different and has a sharp Gaussian of $\mu_r = 19.97$ and $\sigma_r = 2.58$.

Range of the luminosities in the five bands are $13 \leq \mu_u \leq 28$ in u-band, $15 \leq \mu_g \leq 25$ in g-band, $16 \leq \mu_r \leq 23$, in r-band, $14 \leq \mu_i \leq 22$ in i band, and $14 \leq \mu_z \leq 23$ in z-band. This figure shows also that the sample has a higher luminosity in u-band of $\mu_u = 22.4$ and$\sigma_u = 3.67$.The following figure (Fig. 4) illustrates the luminosity distribution function versus z_{spec}in each photometric band of SDSS. In this figure we have explained how the luminosity distribution in each band will be varying in the limits of the spectroscopic red shifts. The lower limit is set to $z_{spec} = 0.01$and the upper limit corresponds to $z_{spec} = 0.025$. the vertical dotted black line represents the mean value of the spectroscopic red shift at $z_{spec} = 0.2999$ and move toward mean values of the maximum numbers counts of the galaxies in each bin are 62, 75, 86,90,105 in ugriz bands respectively. We notice that the higher number of counts of the galaxies is in z-band and it is larger than 43 galaxies of u-band and 15 galaxies of i-band. The behaviour is approximately similar at low and high red shift limits. Due to the imaging array have an effective wavelength of 3590 A°, 4810 A°, 6230 A°, 7630 A° and 9060 A° in u, g, r, i, z filters, we see that the luminosities of the main sample arevarying in each band which leads to different apparent brightness of the sample. According to the inverse-square law, the total energy emits from a star per second on each area is proportional to the luminosity of the star. [13]As we note in Fig. 4, the main sample is brighter in z-band

Correlation among Spectroscopic Redshift and Colors

Theoretical color-redshift relation of the galaxies can be measured directly at different wavelengths. Galaxy color allows to study the wavelengths. Galaxy color allows also studying the evolutionary history of the galaxy and their stellar content. [14]. We have calculated the colors from the absolute magnitudes presented in the previous section as; u-g, g-r, r-i, and i-z. Fig. 5 shows the corresponding distribution functions of the colors bands as a function of z_{spec}. The dashed horizontal lines in each plot are mean of the color ofthe main sample, and the dashed vertical line ismean ofz_{spec} of 0.28. The error bars are the standard deviation in each luminosity bin of u-g, g-r, r-i, and i-z bands. As we see in the figure, the distribution function of u-g color is not like to other colors. The distribution function of u-g color at higher mean value of $\mu_{u-g} = 1.792$ comparable with other colors has a bimodail shape of three peaks corresponding to early or late-type galaxies. This gives a good indicator to study the physicalmeaning of the color for the early or late type galaxies depending this color. The early type galaxies dominated by the red elliptical galaxies while the late types galaxies dominated by spirals blue galaxies. The color-redshift relations are very similaraccording to g-r, r-i, and i-z colors. Fig 5 illustrates also how the different colors can be used to select objects with low redshift at $z_{spec} < 0.7$ and the majority numbers of the objects in the sample satisfying the criteria $z_{spec} < 0.7$, and we have very few objects at the high redshift range at $z_{spec} > 0.8$.We noted that we can't get reasonable values of the colors at larger redshift range.

CONCLUSIONS

The main Conclusions of this study will be summarized as follows: The probability distribution of z_{spec} forthe galaxies in the main sample has a kernel fit of two peaks at $z_{spec} \sim 0.12$ and $z_{spec} \sim 0.5$. The estimated photometric redshift accuracy has a Gaussian distribution with mean $\mu = -0.008$ and standard deviation $\sigma = 0.071$, and there is a linear relationship between z_{spec} and z_{photo}.The galaxies of $z_{spec} < 0.02$have been scattered with respect to the photometric red shift range and the comparison between and shows a good agreement between the probability distribution function in the five bands. This suggests that can be depending to identify objects of less spectroscopic red shift. The $R.M.S\ error = 0.0000 \pm 0.1032$. The luminosities of the sample have a kernel shape in all bands except in r-band has a sharp Gaussian shape, we found also the luminosities in each photometric band are changing in the range of and the maximum number counts of the galaxies is equal to 105 in z-band. In the correlation among the spectroscopic red shift and colors, we found that the distribution function of the color in the u-g band has a bimodal shape with three peaks corresponding to early or late-type galaxies and this help to study the colors of this type of the galaxies in this band.

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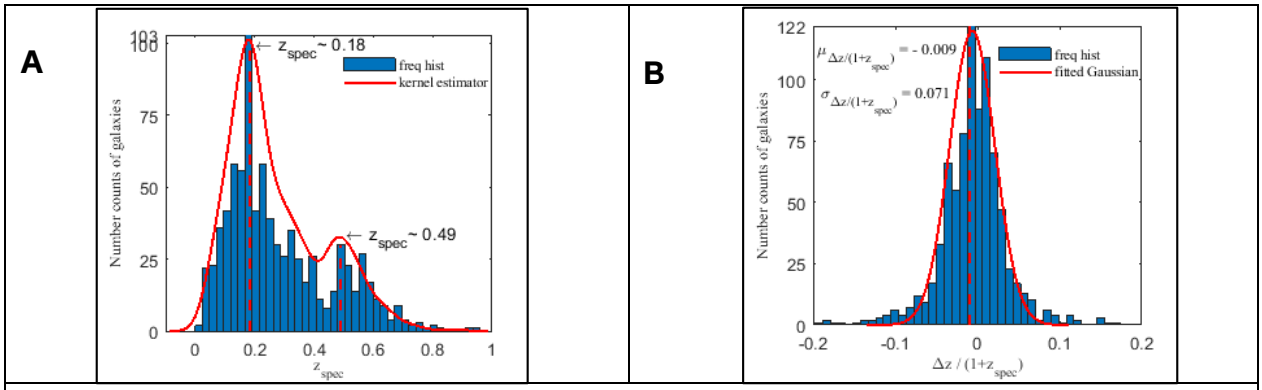

Fig.1.(A). The spectroscopic redshift distribution of the sample. (B). the distribution of $z/(1+z_{spec})$ for the objects with spectroscopic redshifts

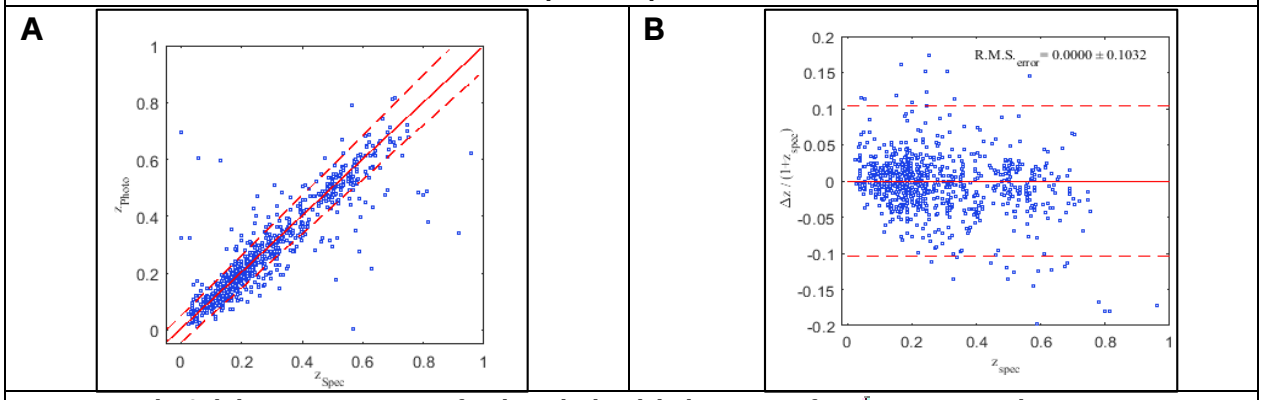

Fig. 2. (A). z_{photo} versus z_{spec} for the galaxies. (B). the scatter of $\Delta z/1+z_{spec}$ against z_{spec}

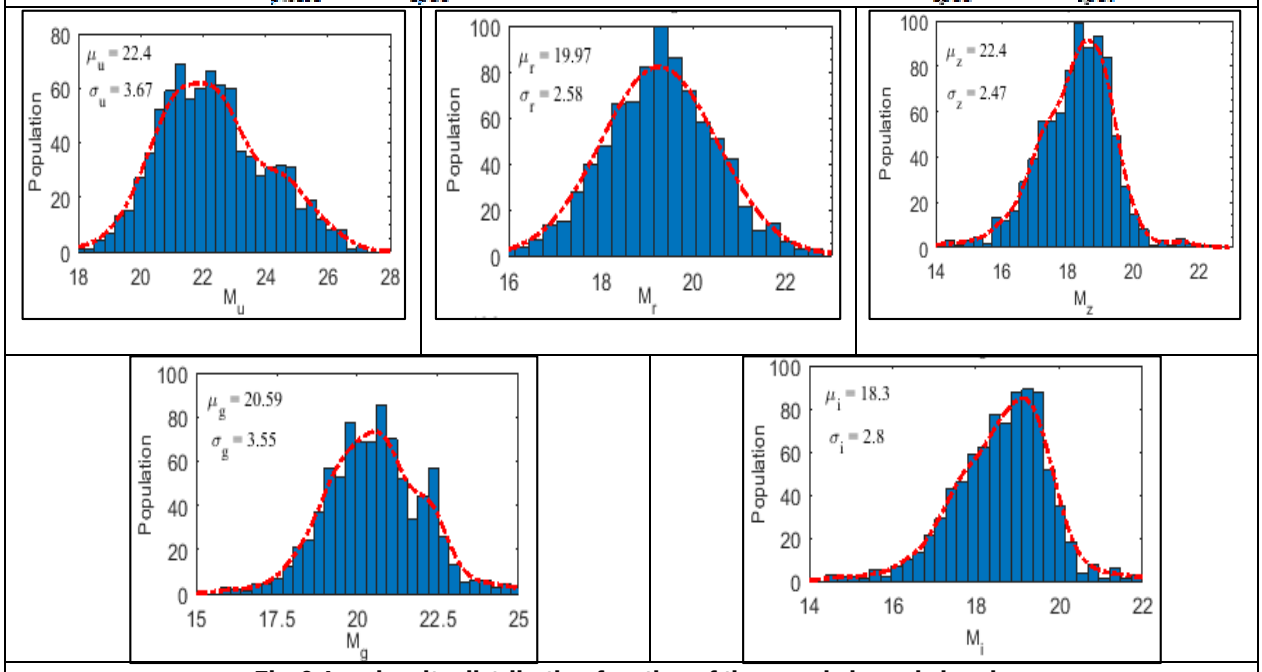

Fig. 3. Luminosity distribution function of the sample in ugriz bands

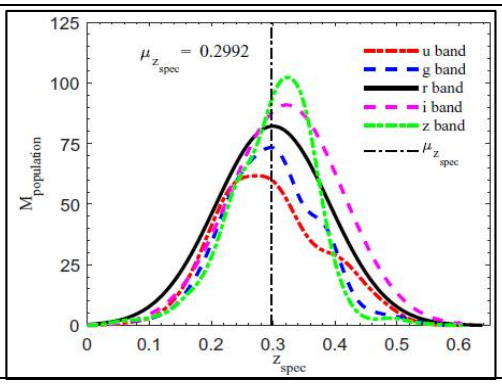

Fig. 4. Redshift distribution in all in all photometric bands of SDSS

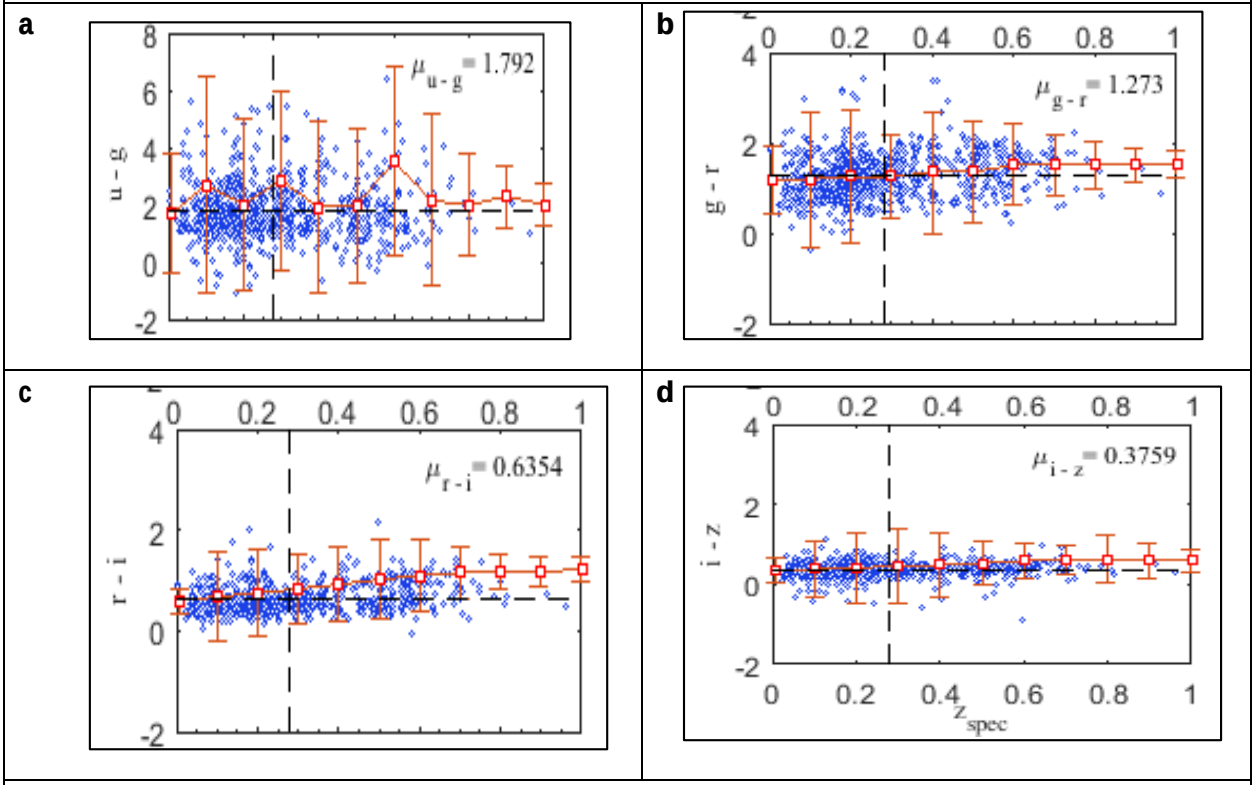

Fig. 5. Colors – redshift relation of the samplein: (a) u-g band, (b) g-r band, (c) r-i band, and (d) i-z band

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RESEARCH ARTICLE

Relational Analysis on Coconut Production Technology

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ABSTRACT

Coconut is a versatile crop. The present study on Knowledge and adoption of coconut production technology by the coconut growers in Thiruvananthapuram district of Kerala state with a sample size of 120 farmers selected from 12 different villages of six talukas. The selected variables like age, experience in coconut cultivation, land holding, social participation, extension contact and mass media were positively significant with the knowledge, whereas, education was negatively significant and family size, area under coconut cultivation, annual income and risk orientation were non-significant with the knowledge about coconut production technology. Whereas experience in coconut cultivation, social participation and extension contact were positively significant with the adoption, whereas, land holding, area under coconut cultivation and annual income were negatively correlated and age, education, family size, mass media exposure and risk orientation were non-significant relation with the adoption of coconut production technology.

Keywords: Knowledge, Adoption, Significance, Coconut growers.

INTRODUCTION

Coconut venerated as 'the tree of life' or 'the tree of heaven'- in Indian classics, bestows multiple benefits to human kindis grown in more than 93 countries of the world including Indonesia, Philippines, and India as the major producing countries of the world. India is the third largest coconut producer in the world having 19.02 percentage of the area under coconut production (J. Nehru Naik, 2017). The coconut palms are grown in most of the zones, except sub tropic and temperate regions, which includes 19 states and 3 union territories in the country. However, they are favourably adapted to coastal Agro ecosystem-having coastlines. India is often described as an agricultural economy

with majority of the population depending on agriculture for their livelihood. But the agriculture sector often faces setbacks owing to diverse factors.Coconut can be grown as a plantation crop as well as homestead crop and can be cultivated both as a major crop and as a minor crop.

METHODOLOGY

The study was conducted in Thiruvananthapuram district of Kerala State. All the talukas of Thiruvananthapuram district such as Thiruvananthapuram, Chirayinkeezhu, Neyyattinkara, Kattakada, Varkala and Nedumangad were selected for the study. Two villages were selected purposively from each taluka considering the maximum area under coconut cultivation. Thus in total, twelve villages were taken for the study and a total of 120 respondents were selected. The exploratory research design was used for the study. The data were collected in face-to-face situation by the personal interview method with the help of structured interview schedulecontaining the questions on knowledge and adoption of coconut production technology by the coconut growers. The data were tabulated, analyzed and the results were interpreted as on Table 1.

RELATIONAL ANALYSIS

It is evident from Table 1 that amongst the personal, socio-economic, communication and psychological characteristics of respondent coconut grower's age, experience in coconut cultivation, social participation and mass media were significant and positively correlated with the knowledge about coconut production technology at 0.01 level probability. Whereas the characteristics land holding and extension contact was significantly and positively correlated with the knowledge about coconut production technology at 0.05 level probabilities. The education of the selected respondents was negatively significant with the knowledge about coconut production technology at 0.05 level probability. The remaining characteristics such as family size, area under coconut cultivation, annual income and risk orientation were non-significantly correlated with the knowledge about coconut production technology.These findings are in conformity with the findings of Thippeswamy (2008), Koli (2014) and Afrin *et. al* (2016)

It is also indicated from Table 1 that amongst the characteristics of respondent coconut growers experience in coconut cultivation, social participation and extension contact were significant and positively correlated, whereas, land holding and area under coconut cultivation are correlated as negatively significant with the adoption of coconut production technology at 0.01 level probability. The characteristic such as annual income was negative and significantly correlated with the adoption of coconut production technology at 0.05 level probabilities. The remaining characteristics such as age, education, family size, mass media exposure and risk orientation were non-significantly correlated with the adoption of coconut production technology.These findings are in conformity with the findings of Sivanarayan (2000), Manjula*et al.* (2007), Thippeswamy (2008), Sherief*et. al* (2010), Koli (2014) and Vikas (2018).

CONCLUSION

In the present study, it was observed that majority of the coconut growers were above 50 years having education between 8th to 12th standard. However, coconut growers gain knowledge in coconut production through their vast experience in coconut cultivation (70 per cent having 17 to 43 years of experience). Hence the observed negatively significant correlation between education and knowledge might be observed as coconut growers gain knowledge due to their experience in coconut cultivation and not due to education. In general opinion, education of respondents favours the acquisition of knowledge and widens the horizon of knowledge by proper understanding of the importance of coconut production technology by getting exposed to the mass media, social participation and extension contact by them. More land holding and experience in coconut cultivation could also motivate the coconut growers for acquiring the information required.

It was obvious that increase in annual income with availability of land holding, more area under coconut cultivation and proper extension contact encourage the corresponding respondents to adopt the coconut production technology.

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Table 1. Correlation coefficient of selected characteristics of the respondents with their knowledge and adoption of coconut production technology

Sl. No.	Variables	Knowledge 'r' values	Adoption 'r' values
1	Age	0.2602**	-0.1543^{NS}
2	Education	-0.1995*	0.0189^{NS}
3	Family size	0.0767^{NS}	-0.0404^{NS}
4	Experience in coconut cultivation	0.2881**	0.2452**
5	Land holding	0.2078*	-0.3154**
6	Area under coconut cultivation	0.0675^{NS}	-0.3152**
7	Annual income	0.1689^{NS}	-0.2114*
8	Social participation	0.6765**	0.2561**
9	Extension contact	0.2213*	0.2614**
10	Mass media exposure	0.3309**	0.1364^{NS}
11	Risk orientation	0.02803^{NS}	-0.0021^{NS}

*** Significant at 0.01 level of probability NS- Non significant*

** Significant at 0.05 level of probability*

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RESEARCH ARTICLE

Knowledge and Adoption of Coconut Production Technology by Coconut Growers in Thiruvananthapuram District of Kerala State

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ABSTRACT

The coconut palms are grown in most of the zones, except sub tropic and temperate regions, which includes 19 states and 3 union territories in the country.The present study on Knowledge and adoption of coconut production technology by the coconut growers in Thiruvananthapuram district of Kerala state with a sample size of 120 farmers selected from 12 different villages of six talukas. Vast majority of the selected respondents for the respective study had knowledge about the recommended practices such as for selecting a good quality planting materials and mother palms, proper management of the nursery, planting seedlings in the main field, important pest attacking coconut palm and important disease attacking coconut palm. In overall, 55.83 per cent of coconut growers had medium level of knowledge about coconut production technology.The adoption of control measures for pest and diseases may vary from one pest and disease to another. In overall, most (60.83%) of coconut growers had medium level of adoption of coconut production technology.

Keywords: Knowledge, Adoption, Coconut growers.

INTRODUCTION

Coconut commonly Kalpavriksha is grown in more than 93 countries of the world including Indonesia, Philippines, and India as the major producing countries of the world. Coconut is a traditional plantation crop of India and assumes the status of a high value commercial crop. In India, most of the acreage under coconut palm (90%) lies in the four southern states i.e., Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. Today, the top coconut suppliers are struggling to meet the increasing demand of the global economy. Nonetheless, the top global coconut producers

must learn from the current situation, and take steps to ensure that their farms are sustainable enough to stand the test of time and meet future demands. The adoption of plant protection measures also found to be very low due to low level of knowledge, scarcity and high cost for engaging coconut climbers. Knowledge is a pre-requisite for understanding field problems, taking best possible decision for adoption and ensuring success (Anithakumari *et. al*, 2017). The study was conducted in Thiruvananthapuram district of Kerala State. All the talukas of Thiruvananthapuram district such as Thiruvananthapuram, Chirayinkeezhu, Neyyattinkara, Kattakada, Varkala and Nedumangad were selected for the study. Two villages were selected purposively from each taluka considering the maximum area under coconut cultivation. Thus in total, twelve villages were taken for the study and a total of 120 respondents were selected. The exploratory research design was used for the study. The data were collected in face-to-face situation by the personal interview method with the help of structured interview schedulecontaining the questions on knowledge and adoption of coconut production technology by the coconut growers. The data were tabulated, analyzed and the results were interpreted as on Table 1.

RESULTS AND DISCUSSION

The findings of the study had been presented under the following headings.

Knowledge of coconut growers about coconut production technology

The data in Table 1 further revealed that majority (98.33%) of the selected respondents for the respective study have knowledge about the recommended practices for selecting a good quality planting materials and mother palms and for planting seedlings in the main field with correct pit size, recommended spacing, time of planting the seedling and about recommended dose and correct time for fertilizer application. 80 per cent of the coconut growers have knowledge on recommended practices to manage nursery properly. About 100 per cent of the respondents hadknowledge on intercropping and mixed cropping to be practiced in between coconut palms, crop cafeteria for multiple cropping, regular weeding, periodical intercultural operations and some good agricultural practices like the use of green manure, cover crops, recycling of palm waste and the use of mulches. The knowledge about control measures for pest and diseases varies from respondents.

Majority (92.5%) of the coconut growers had knowledge about the important pest attacking coconut palm viz. Black headed caterpillar, Eriophyid mite, Rhinocerous beetle, Red palm weevil and Mealy bug. Majority (95.83%)of selected respondents had knowledge about control measures on Rhinocerous beetle and Red palm weevil, 90 per cent on Eriophyid mite and 89.17 per cent on Mealy bug and Black headed caterpillar. Majority (93.33%) of the coconut growers had knowledge about the important disease attacking coconut palm viz. Stem bleeding, Mahali disease- bud rot or fruit rot, Root wilt, Tanjore wilt and Grey blight. 95.83 per cent of selected respondents had knowledge about control measures on Mahali, 95 per cent on Root wilt and Grey blight, Stem bleeding (91.67%) and Tanjore wilt (89.17%).

Adoption of coconut production technology by coconut growers

The Table 1 concluded thatmajority (66.67%) of the respondents had full adoption of the recommended practices for selecting a good quality planting materials and mother palms by the selected respondents for the respective study. Only 31.67 per cent respondents had partial adoption of recommended practices to manage nursery properly such as selection on site of seed bed, type of soil where nursery is to be grown. About 74.17 per cent of the respondents had full adoption of recommended practices for planting seedlings in the main field.Only fewer respondents had fully adopted the control measures on pests such asMealy bug (10%), Rhinocerous beetle (35.83%), Red palm weevil (35.83%), Eriophyid mite (13.33%) and Black headed caterpillar (13.33%). Also fewer respondents had fully adopted

the control measures on diseases such as Stem bleeding (16.67%), Tanjore wilt (8.33%), Root wilt (21.67%), Grey blight (17.5%) and Mahali (27.5%).

Overall knowledge and adoption level

Data presented in Table 2 shows that majority (55.83%) of coconut growers had medium level of knowledge about coconut production technology, followed by 25.84 per cent in high and 18.33 per cent in low level respectively. These findings were consonance with the findings of Anitha Kumari et al. (2003), Thippeswamy et al. (2008) and Koli (2014). Table 2 also revealed that majority (60.83%) of coconut growers had medium level of adoption, followed by 18.33 per cent highand 20.84 per cent with low level of adoption of coconut production technology.These findings are supported by Shivalingaiah *et al.* (2002), Rao *et al.* (2008), Thippeswamy *et al.* (2008) and Koli (2014).

CONCLUSION

The large number of selected coconut respondents for the study had medium knowledge about coconut production technology. Most probably this might be because of two reasons; one is the positive efforts of extension contact and exposure to mass media, whereas, second may be from farmer's side, where the educated old age farmers had a greater experience in coconut cultivation since many years transferred from ancestors as a traditional occupation and also they are always ready to take risk for gaining the income. Vast respondent growers had medium adoption of coconut production technology. It had already been discussed that most of the respondents had medium and high level of knowledge; similarly here also large number of respondents had medium followed by high level of adoption. Hence, it implied that extension agencies had to tailor the extension activities to convert the knowledge of farmers in to the decision of adoption.

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Table 1. Distribution of respondents according to the recommended practice wiseknowledge and adoption of coconut production technology

Sl. no.	Recommended practices	Knowledge		Adoption		
		Yes	No	FA	PA	NA
A	**Selection of planting materials**					
1	Type of planting material required (Cultivars, Hybrids)	118 (98.33%)	2 (1.67%)	80 (66.67%)	37 (30.83%)	3 (2.5%)
B	**Selection of mother palms**					
1	Yield capacity (nuts/annum) (not less than 80 nuts)	118 (98.33%)	2 (1.67%)	77 (64.17%)	40 (33.33%)	3 (2.5%)
2	Number of fully opened leaves (more than 30 leaves with strong petiole and wide base firmly attached to stem)	118 (98.33%)	2 (1.67%)	77 (64.17%)	40 (33.33%)	3 (2.5%)
3	Number of bunches of nuts per annum (at least 12 bunches of nuts with strong bunch stalk)	118 (98.33%)	2 (1.67%)	78 (65%)	39 (32.5%)	3 (2.5%)
4	Age (20 years or more)	118 (98.33%)	2 (1.67%)	78 (65%)	39 (32.5%)	3 (2.5%)
5	Nut size (medium size and oblong shape)	117 (97.5%)	3 (2.5%)	84 (70%)	33 (27.5%)	3 (2.5%)
6	Weight of husked nut (in gram) (not less than 600 g)	117 (97.5%)	3 (2.5%)	84 (70%)	33 (27.5%)	3 (2.5%)
7	Mean copra content (gram/nut) (150 g/nut or more)	117 (97.5%)	3 (2.5%)	84 (70%)	33 (27.5%)	3 (2.5%)
C	**Maintenance of nursery**					
1	Site of seed bed (shaded area)	96 (80%)	24 (20%)	38 (31.67%)	33 (27.5%)	49 (40.83%)
2	Type of soil (well drained with light textured soil)	96 (80%)	24 (20%)	38 (31.67%)	33 (27.5%)	49 (40.83%)
3	Spacing between nuts (30x30 cm with 4 to 5 rows per bed)	96 (80%)	24 (20%)	38 (31.67%)	32 (26.66%)	50 (41.67%)
4	Periodic weeding	96 (80%)	24 (20%)	38 (31.67%)	32 (26.66%)	50 (41.67%)
5	Irrigate the beds regularly	96 (80%)	24 (20%)	38 (31.67%)	32 (26.66%)	50 (41.67%)
D	**Selection of seedling from nursery**					
1	Collar girth of seedling (10-12 cm)	95 (79.17%)	25 (20.83%)	38 (31.67%)	32 (26.66%)	50 (41.67%)
2	Age (9 to 12 month old)	95 (79.17%)	25 (20.83%)	38 (31.67%)	32 (26.66%)	50 (41.67%)
E	**Planting of seedling in the main field**					
1	Pit size according to soil type	118	2	89	20	11

	(Sandy soil-0.75m x 0.75m x 0.75m; Loamy soil- 1m x 1m x 1m and Laterite soil-1.2m x 1.2m x 1.2m)	(98.33%)	(1.67%)	(74.17%)	(16.67%)	(9.16%)
2	Spacing (Triangular planting- 7.6m; Square- 7.6m to 9m; Single hedge- 5m x 9m and Double hedge- 5m x 5m (rows) 9m (between rows).)	118 (98.33%)	2 (1.67%)	84 (70%)	21 (17.5%)	15 (12.5%)
3	Time of planting (may- onset of pre-monsoon; April- under assured irrigation and September- after occasion of heavy rain.)	118 (98.33%)	2 (1.67%)	89 (74.17%)	20 (16.67%)	11 (9.16%)
F	**Maintenance of established plants**					
1	Fertilizer application					
	i) Recommended dose (1/10th of full dose- 3 MAP; 1/3rd of full dose- 1 year; 2/3rd of full dose- 2 year; Full dose- 3 year onwards), Average NPK -0.34: 0.17: 0.68 kg/palm/annum and Good NPK -0.50: 0.32: 1.20 kg/palm/annum	118 (98.33%)	2 (1.67%)	81 (67.5%)	34 (28.33%)	5 (4.17%)
	i) Method of application (split dose)	118 (98.33%)	2 (1.67%)	81 (67.5%)	34 (28.33%)	5 (4.17%)
	ii) Time of application (April- June or Sept- Oct)	118 (98.33%)	2 (1.67%)	81 (67.5%)	34 (28.33%)	5 (4.17%)
2	Pests of coconut					
	A. Important attacking pests in coconut (Black headed caterpillar, Eriophyid mite, Rhinocerous beetle, Red palm weevil and Mealy bug)	111 (92.5%)	9 (7.5%)	55 (45.83%)	33 (27.5%)	32 (26.67%)
	B. Control measures for pests					
	i) Mealy bug- 0.05% quinalphos, 2% neem oil garlic emulsion	107 (89.17%)	13 (10.83%)	12 (10%)	18 (15%)	90 (75%)
	ii) Eriophyid mite- 2% neem oil garlic emulsion, field sanitation	108 (90%)	12 (10%)	16 (13.33%)	18 (15%)	86 (71.67%)
	iii) Rhinocerous beetle- use beetle hook, 250g neem cake	115 (95.83%)	5 (4.17%)	43 (35.83%)	51 (42.5%)	26 (21.67%)
	iv) Red palm weevil- field sanitation, 1% DDVP	114 (95%)	6 (5%)	43 (35.83%)	51 (42.5%)	26 (21.67%)
	v) Black headed caterpillar- release parasitoid, field sanitation	107 (89.17%)	13 (10.83%)	16 (13.33%)	18 (15%)	86 (71.67%)
3	Diseases of coconut					
	A. Important attacking diseases in coconut (Stem bleeding, Mahali disease- bud rot or fruit rot, Root wilt, Tanjore wilt and Grey blight)	112 (93.33%)	8 (6.67%)	54 (45%)	34 (28.33%)	32 (26.67)

	B. Control measures for diseases					
	i) Stem bleeding- 5 kg/palm neem cake, hexaconazole @ 25ml in 25 litre of water.	110 (91.67%)	10 (8.33%)	20 (16.67%)	18 (15%)	82 (68.33%)
	ii) Mahali- 1% Bordeaux mixture, 2g/L Copper oxychloride	115 (95.83%)	5 (4.17%)	33 (27.5%)	53 (44.17%)	34 (28.33%)
	iii) Root wilt- apply NPK, rogue out diseased palm	114 (95%)	6 (5%)	26 (21.67%)	25 (20.83%)	69 (57.5%)
	iv) Tanjore wilt- 5 kg/palm neem cake drench the basin with 40 litres of any copper fungicides to soak soil up to 15 cm in depth at quarterly intervals	107 (89.17%)	13 (10.83%)	10 (8.33%)	15 (12.5%)	95 (79.17%)
	v) Grey blight- 1% Bordeaux mixture, propiconazole @ 1ml /L	114 (95%)	6 (5%)	21 (17.5%)	45 (37.5%)	54 (45%)
4	Irrigation					
	i) Time of irrigation (summer- by flood or basin irrigation)	120 (100%)	0 (0%)	68 (56.67%)	35 (29.17%)	17 (14.16%)
	ii) Method of irrigation (Drip irrigation)	58 (48.33%)	62 (51.67%)	20 (16.67%)	28 (23.33%)	72 (60%)
5	Intercropping and mixed cropping (Cereals, Legumes and pulses, Tubers, Fruits, Spices and condiments, Beverage crops and Fodder grasses)	120 (100%)	0 (0%)	106 (88.33%)	14 (11.67%)	0 (0%)
6	Crop cafeteria for multiple cropping (Annual crops and Perennial crops)	120 (100%)	0 (0%)	106 (88.33%)	14 (11.67%)	0 (0%)
7	Good practices (Use of green manure and cover crops, Recycling of palm waste, Use of mulches and Husk burial for moisture conservation)	120 (100%)	0 (0%)	106 (88.33%)	14 (11.67%)	0 (0%)
8	Regular weeding (ploughing, digging, raking or forming mounds)	120 (100%)	0 (0%)	106 (88.33%)	14 (11.67%)	0 (0%)
9	Periodical intercultural operations	120 (100%)	0 (0%)	106 (88.33%)	14 (11.67%)	0 (0%)

Table 2. Distribution of respondents according to their level of overall knowledge and overall adoption of coconut production technology

Sl. No.	Index Level	Respondents (n=120)			
		Frequency	Percentage	Frequency	Percentage
1.	Low (Up to 33.33)	22	18.33	25	20.84
2.	Medium (33.34 to 66.66)	67	55.83	73	60.83
3.	High (Above 66.66)	31	25.84	22	18.33
	Total	**120**	**100.00**	**120**	**100.00**
	Mean	**60.06**		**47.97**	
	SD	**24.37**		**15.93**	

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RESEARCH ARTICLE

Constraint Analysis of Coconut Growers during Coconut Production Technology

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ABSTRACT

Coconut is a traditional and homestead crop. The present study on Knowledge and adoption of coconut production technology by the coconut growers in Thiruvananthapuram district of Kerala state with a sample size of 120 farmers selected from 12 different villages of six talukas. In case of constraint almost all of the respondents (90%) faced the vigorous pest and disease attack on coconut, high labour cost (45%), non-availability of labours (40.83), drastic decline in production loss (32.50%), lack of getting subsidies for coconut trees (24.17%), facing the arrest of water scarcity during summer periods (23.33%), low profit and lack of IT accessibility (16.66%), lack of awareness about improved technology (14.17%), lack of timely credit availability (8.33%), non-availability of needed fertilizers and other inputs (6.67%), the trees become resistant to the chemicals used against pest and diseases (5%) and the losses occurred during flood (3.33).

Keywords: Constraints, Coconut growers.

INTRODUCTION

Coconut is a traditional plantation crop of India and assumes the status of a high value commercial crop. Coconut is grown in 20.99 lakh hectare in the country with an estimated production of 16812 million nuts during 2017-18 (Horticulture Statistics, 2018). Coconut is a versatile crop; each and every part of the coconut palm is functionally useful. The products of commercial importance are copra, oil, cake, desiccated coconut, fibre, neera and a number of post-harvest products such as coconut chips etc. India is often described as an agricultural economy with majority of the population depending on agriculture for their livelihood. The growth registered in the agriculture sector indicates a diminishing trend due to diverse factors.

With modern agricultural practices, there are many reasons which are attributed to low productivity and in some cases even loss of the palm. Most important among them are highly fragmented holdings, pest and diseases, unstable price, unsteady market, lack of scientific management practices, lack of irrigation facilities etc.

METHODOLOGY

The study was conducted in Thiruvananthapuram district of Kerala State. All the talukas of Thiruvananthapuram district such as Thiruvananthapuram, Chirayinkeezhu, Neyyattinkara, Kattakada, Varkala and Nedumangad were selected for the study. Two villages were selected purposively from each taluka considering the maximum area under coconut cultivation. Thus in total, twelve villages were taken for the study and a total of 120 respondents were selected. The exploratory research design was used for the study. The data were collected in face-to-face situation by the personal interview method with the help of structured interview schedulecontaining the questions on knowledge and adoption of coconut production technology by the coconut growers. The problems related to various aspects of adoption of coconut production technology by coconut growers were identified by obtaining response of individual respondent. The relevant data in this regard has been presented in Table 1.

CONSTRAINTS ANALYSIS

It is evident from Table 1 that, the vast majority of respondents (90%) faced the constraints of vigorous pest and disease attack on coconut, followed by high labour cost (45%), non-availability of labours (40.83), drastic decline in production loss (32.50%), lack of getting subsidies for coconut trees (24.17%)and facing the arrest of water scarcity during summer periods (23.33%). Only less of the respondents come across with the constraints of low profit and lack of IT accessibility (16.66%), which points out to the less use of improved technology. The lack of awareness about improved technology (14.17%), 12.5 per cent come across with the getting of disease affected planting materials distributed by agriculture departments and high damage of palms by pig attack (10%). The 8.33 per cent of the corresponding coconut growers had lack of timely credit availability when and where financial support is required and also cause great damage by monkeys attacking the coconuts and the intercrops, the non-availability of needed fertilizers and other inputs (6.67%), the trees become resistant to the chemicals used against pest and diseases (5%) and at last faces the losses occurred during flood (3.33). These findings were conformity to the findings of Thamban (1998), Sivanarayana (2000), Mahadik*et. al* (2009) and Koli (2014).

CONCLUSION

Today, the top coconut suppliers are struggling to meet the increasing demand of the global economy. Coconut has been a cash crop for decades and even with the stiff competition from other vegetable oils, it continues to be a profitable venture in the future. Though various control measures are used by farmers to tackle some of the major diseases, the efficient technological methods have to be integrated to derive greater benefit.The other agencies can solve this problems by providing special management practices for coconut tree and providing technical guidance to coconut growers. There is tremendous scope for improving the productivity in our country. If we make serious efforts India can lead the world in production and productivity of coconut.

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Table 1.Distribution of respondents according to constraints faced by them in adoption ofcoconut production technology

Sl. No.	Constraints	Frequency (n=120)	Percentage
1	More vigorous pest and disease attack	108	90%
2	Demanding high labour cost which is unaffordable by the financially poor farmers	54	45%
3	Non-availability of labours in required time	49	40.83%
4	Production loss year after year	39	32.50%
5	Lack of subsidies for coconut crop when compare with other crops	29	24.17%
6	Greater water scarcity during summer	28	23.33%
7	Lack of IT access by growers having low use of digital technologies	20	16.66%
8	Less profit for selling coconuts nowadays when compare with buying it	20	16.66%
9	Lack of awareness about improved technology by growers having less social mingling.	17	14.17%
10	Distribution of the disease effected planting materials by the agriculture departments	15	12.5%
11	Pig attacking the coconut palms by pushing down the trees from the roots with their head and destroying the entire plantation in a night	12	10%
12	Lack of timely credit availability to the farmers by government through krishi-bhavan	10	8.33%
13	Monkey attacking coconut palms by plucking and throwing the undeveloped nuts	10	8.33%
14	Non-availability of needed fertilizer and other inputs at the recommended time for application of it.	8	6.67%
15	Trees become resistant to the chemicals used against pest and diseases	6	5%
16	Loss in flood happened in 2018	4	3.33%