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## **MADINAT AL-HIKMAH**

### **City of Education, Science and Culture**

Shaheed Hakim Mohammed Said (1920-1998), a scion of the renowned South Asian Hamdard family, decided in 1948 to make the newly created Pakistan his home. He settled down in Karachi and by untiring, single minded devotion and commitment, braving all handicaps, created Hamdard Pakistan. He developed it into the leading pharmaceutical complex of Eastern Medicine in the country, run on the latest modern lines and techniques, supplying drugs of high quality and purity, backed with free clinical consultations to help ailing humanity. Hamdard Pakistan, under his leadership, also emerged as the leading philanthropic organization, and also tried to motivate people through dialogue, conferences, and journals like the *Hamdard Medicus*. In addition, he provided help to various institutions and academic bodies.

He was restless to do more, and during one of his *Hajj* pilgrimages, he envisioned the creation of a comprehensive City of Education, Science and Culture: the Madinat al-Hikmah. Work on it was initiated and funded by the Hamdard Foundation Pakistan in 1981, in the picturesque surroundings of Bund Murad Khan, 35 kilometers away from the city centre. Today it is a beehive of activity, with children and youth engaged in academic, technical and sports activities, devoted to promoting learning and culture, and through it, help achieve moral and physical welfare, peace and progress. The Bait al-Hikmah Library, Hamdard Public School, free Hamdard Village School, Centre for Horticulture and the Hamdard University are the major institutions found here. The Hamdard University's mission provides value-based education to all students in its constituent institutions. Its prestigious institutions – some based at the main campus and some in the city and some in Islamabad - include Hamdard Institute of Management Sciences (HIMS), Hamdard College of Medicine and Dentistry (HCMD), Hamdard Al-Majeed College of Eastern Medicine (HACEM), Shifa ul-Mulk Memorial Hospital, Hamdard University Hospital – Naimat Begum Mother & Child Care Unit (part of the Hamdard University Centre of Excellence), Hamdard Institute of Education and Social Sciences (HIESS), Dr Hafiz Mohammad Ilyas Institute of Pharmacology and Herbal Sciences (HMI-IPHS), Hamdard School of Law, Faculty of Pharmacy and Faculty of Engineering Sciences and Technology (FEST). The Madinat al-Hikmah continues to develop and grow.

# HAMDARDMEDICUS

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# HAMDARD MEDICUS

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## Clinical Efficacy of Unani Herbal Formulation *Garlina* Against Primary Hyperlipidemia

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### Abstract

Primary hyperlipidemia and atherosclerosis are correlated to each other and these are major contributors of coronary artery diseases. The objective of the study was to evaluate the efficacy of polyherbal Unani medicine (*Garlina*) a well-known herbal formulation to be used for Primary hyperlipidemia. This was an open, randomized, prospective, multicenter study on human subjects of 6 months duration. After the laboratory investigations for fasting plasma lipids of the suspected subjects, 25 subjects were identified, randomized and received the drug. The subjects were diagnosed and classified according to the international recommended levels of plasma lipids (PL) as total cholesterol levels were borderline high (200-239 mg/dL), triglycerides (TGs) were high (200-499 mg/dL), high density lipoprotein (HDL) were desirable but not optimal (41-59 mg/dL) while low density lipoproteins (LDL) were borderline high (130-159 mg/dL). After 8 weeks administration of *Garlina* tablets 500 mg twice-a-day, the total cholesterol levels were reduced to 166.48±35.59 mg/dL from 224.04±9.16 mg/dL, triglyceride levels were also reduced to 144.28±37.11 mg/dL from 217.28±73.54 mg/dL at the base line, low density lipoprotein levels were reduced to 87.52±40.17 mg/dL from 133.96±

54.00 mg/dL while high density lipoprotein level was increased to 56.20±8.66 mg/dL from 48.28±10.57 mg/dL. The findings from this study demonstrated that there was significant difference ( $p<0.05$ ) before and after treatment with *Garlina* (500 mg) as mean lipid profiles of test group administered 1 tablet b.d. after meals. This is clearly evident that *Garlina* possesses a significant hypolipidemic therapeutic value for the treatment of primary hyperlipidemia.

### Keywords

Herbal formulation, Primary hyperlipidemia, *Garlina*, Clinical trial.

### 1. INTRODUCTION

The primary hyperlipidemia is one of the burning issues worldwide that leads to dysfunction of other body organs too particularly GIT heart and kidneys. Although there are many medicines available in market to reduce serum lipid levels but various serious side effects like liver upsets, rhabdomyolysis are associated with them. In the present study a polyherbal Unani medicine *Garlina* was used for the management of primary hyperlipidemia.

Hyperlipidemia refers to elevated total cholesterol and low density lipoprotein cholesterol (LDL-C) and low levels of high

density lipoproteins (HDL-C) <35 mg/dL. The plasma lipid levels recommended by National Cholesterol Education Program (NCEP), USA should be LDL-Cholesterol (mg/dL) <100 optimal, 100-129 near optimal/above optimal, 139-159 borderline high, 160-189 high,  $\geq 190$  very high. HDL-Cholesterol <40 low,  $\geq 60$  high. Triglycerides <150 normal, 159-199 borderline high, 200-499 high,  $\geq 500$  very high. Total Cholesterol <200 desirable, 200-239 borderline high,  $\geq 240$  high (Peter, 1998).

Healthy cholesterol levels do not interfere with the blood flow while high levels of cholesterol particularly the low density lipoproteins owing to their adhesive nature disturbs the blood circulation by slowing it down thus, enhancing the peripheral vascular resistance. As a consequence it leads to the development of hypertension and atherosclerotic changes in the vasculature, triggering the atheroma formation in different sensitive arteries like coronary, cerebral, renal or even retinal arteries.

Hypercholesterolemia is strongly associated with the incidence of ischemic heart disease in experimental and epidemiological studies. Moreover, many intervention studies have clearly shown the role of low density cholesterol (LDL-C) in the cardiovascular mortality and morbidity (John *et al.*, 1999) while high density cholesterol (HDL-C) is regarded as good cholesterol helping in the reverse cholesterol uptake (Jafar and Qadri, 2008).

Hamdard Laboratories (Waqf) Pakistan has developed herbal formulation based on Unani medicines *Garlina* in tablet dosage form indicated for hyperlipidemia. It is a new formulation composed of specific morphological parts of Unani medicinal plants shown in Table 1. The individual medicinal plants have been used and prescribed individually or in compound formulations since long time for

balancing the cholesterol levels along with other beneficial healthy effects. The acute and sub-chronic toxicology of *Garlina* in *Oryctolagus cuniculus* rabbit showed that it is non-toxic (Sadaf *et al.*, 2016). The clinical trial study was conducted at 2 sites: 1) Shifa-ul-Mulk Memorial Hospital for Eastern Medicine, Hamdard University, 2) Amna Unani Hospital, New Karachi, an affiliated teaching hospital to Hamdard University.

The use of this polyherbal Unani medicine has been incorporated in *Garlina* formulation. However, *Garlina* provides evidence that a combination of medicinal plants is effective in lowering plasma lipids because these plants possess pharmacological properties to lower plasma cholesterol shown in Table 1. Therefore, this study was designed to find out the clinical efficacy of *Garlina* for primary hyperlipidemia. Although conventional medicines i.e. statins are available to control hyperlipidemic condition but due multiple side-effects such as liver upsets, altered liver function tests, renal issues and muscular spasmodic conditions like rhabdomyolysis etc., after their long term use. Moreover, the cost of treatment is high in prolonged therapy. Thus, there is a need to develop an alternative Unani Medicine formulation that should be a safe and cost-effective alternative choice for the hyperlipidemic subjects and the Unani practitioners as well.

In its preliminary stages, high cholesterol generally occurs without any symptoms. For this reason, screening through routine blood tests is crucial for early detection. In its advanced state, however, high cholesterol may result in the tendinous and skin fat depositions (called xanthomas), enlarged liver and spleen (which the healthcare provider may feel on examination), severe abdominal pain as a result of pancreatitis (occurs if triglycerides deposit in the pancreas having triglyceride level is around

Table 1: Composition of Polyherbal Unani Medicine *Garlina*

S.No.	Unani medicinal name	Botanical name	Parts used in formulation	Traditional uses	References
1.	<i>Muqil</i>	<i>Commiphora mukul</i> Hook. ex Stocks.	Gum	Laxative, Hypolipidemic	Singh <i>et.al.</i> (1994)
2.	<i>Lehsan</i>	<i>Allium sativum</i> L.	Bulbets (Dried powder)	Anti-inflammatory, anti-allergic, antithrombotic	Block <i>et.al.</i> (2002)
3.	<i>Zanjbeel/ Sonth</i>	<i>Zingiber officinale</i> (Willd.) Rosc.	Root (Dried powder)	Hypolipidemic	Paranipe <i>et.al.</i> (1990)
4.	<i>Tukhm-e-Pyaz</i>	<i>Allium cepa</i> L.	Seeds (Dried powder)	Hypolipidemic	Sebastian <i>et.al.</i> (1979)
5.	<i>Kalongi</i>	<i>Nigella sativa</i> L.	Seeds (Dried powder)	Hypolipidemic	Memon <i>et. al.</i> (2012) Najmi <i>et.al.</i> (2008)

800 mg/dL or higher), chest pain and even a heart attack and patients having very high level of triglycerides above 2000 mg/dL may have lipemia retinalis.

## 2. METHODOLOGY

This study was an open, randomized, prospective, multicenter study, which was conducted to evaluate the clinical for the treatment of primary hyperlipidemia efficacy and adverse drug reaction of herbal coded Unani medicine *Garlina*. All the subjects having following criteria were inducted in the study. 25 patients were randomized and received the

protocol required study drug exposure and required processing after screening the baseline investigations. The entire duration of treatment was 8 weeks.

### 2.1. Diagnosis

The main diagnosis was made on 14 hours fasting lipid profile. The other investigations were also included i.e., fasting blood sugar, liver function tests, serum urea and creatinine along with complete blood count.

### 2.2. Inclusion Criteria

Participants having moderate to high

**Table 2: Frequency of Patients with Age Range**

Age (years)	Frequency	Total (n)	Age range (years)	Percent of 25 Subjects
35	3	4	35-40	12.0
40	1			4.0
43	1	7	43-45	4.0
44	3			12.0
45	3			12.0
48	1	5	46-50	4.0
49	1			4.0
50	3			12.0
52	1	3	51-55	4.0
54	2			8.0
56	1	2	56-60	4.0
60	1			4.0
61	1	4	61-65	4.0
63	2			8.0
65	1			4.0
Total	25	25		100.0

plasma lipid levels, subjects over 35 years of age in both genders and informed consent.

### 2.3. Exclusion Criteria

- Pregnant women
- Particular in other investigational team.
- Having hyperlipidemia alone or along with diabetes.
- Participants suffering from chronic liver diseases and kidney failure or taking any hypolipidemic medication or raised levels of alanine aminotransferase (ALT) >3 times the upper limit of normal range.
- Any type of carcinomal patients were excluded from the study.
- Patients having the history of myocardial infarction/severe ischemic heart diseases/valvular heart disease/coagulation disorders/severe neurological disorders/alcoholism/hypothyroidism.
- Currently taking any antibiotics, contraceptive pills.
- Patients having history of adverse drug reaction or currently undergone any surgery.

Each participant had been screened for 14 hours fasting lipid profile, Fasting blood sugar, liver function tests, Urea, Creatinine and Complete blood count. After drug administration patients data was recorded to find out the (1) Significant variation in plasma lipid levels, (2) plasma urea and Creatinine levels and (3) any change in plasma liver function tests. Patients had been followed for 4 months. In case of premature withdrawal from the subject side all follow-ups data were recorded and maintained. Phone calls had been made to find out the actual reason of withdrawal to the subjects or next of kin. After simple randomization, the subjects were prescribed tablet *Garlina*. Subjects were advised to collect their prescribed drug after ten days again up to

8 weeks till the completion of trial. All patients were called for their blood testing after 4 weeks upto three months (two followups and four week washing period). Any side effects or severe adverse effects were reported in clinical trial proforma. The difference of the lab findings from the baseline up to the final report were recorded and analyzed for the result and discussion. The obtained data was analyzed by the software SPSS version 17 and the statistical tests were applied to find out the significant difference between the pre-treatment and post treatment groups.

On each visit all patients were assessed for the adverse effects. They will be properly physically examined to find out any abnormal sign during the treatment along with their blood samples which were also taken for the follow up tests. Any change in the liver function tests, complete blood count, increase urea and creatinine more than expected were noticed. The protocol, clinical trial proforma, informed consent was approved by the Ethical committee of Faculty of Eastern Medicine, Hamdard University, Karachi.

### 2.4. Statistical Analysis

The data has been analyzed by paired *t*-test with unequal variance using MS excel,  $p \leq 0.05$  is considered to be significant.

## 3. RESULTS AND DISCUSSION

This was an open, randomized, prospective, multi-centered clinical study carried out to document the clinical efficacy of herbal coded test medicine *Garlina* (a research product of Hamdard Laboratories [Waqf] Pakistan) for controlling moderate to high serum lipid levels in subjects with primary hyperlipidemia.

For the purpose of clinical trial of *Garlina*, the preliminary screening data of 148 patients was collected during October 4<sup>th</sup>, 2013 to

August 4<sup>th</sup>, 2014 at Shifa-ul-Mulk Memorial Hospital for Eastern Medicine, Hamdard University and Amna Unani Hospital, New Karachi respectively. Out of 148 patients 14 were male and 11 were female in test shown in Table 3.

All selected cases were thoroughly examined and clinical history was recorded in the clinical proforma which was specially designed for this purpose. Therapeutic evaluation of the drug was made on the basis of improvement in the lipid profile, i.e. (i) reduction in total cholesterol, (ii) triglycerides, (iii) LDL-cholesterol and (iv) increase in HDL-cholesterol levels at initial enrollment stage then at periodic intervals of 4 weeks during the 8 weeks of the course of study.

After exclusion of drop-outs (changes in accordance with the exclusion/inclusion criteria), the sample population comprised of 25 patients (males: n=14 and females: n=11), who had fulfilled the criteria at baseline 25 patients were enrolled to use *Garlina* as test drug so they were enrolled to use *Garlina* as a test drug or at follow up were inducted in this study. The patient's gender, age, and baseline clinical features at the time of enrolment were recorded. A monthly record of lipid profile and liver

function tests was maintained to analyze the gradual improvement in hyperlipidemic condition and any changes occurred in the report of liver function tests. All statistical data was prepared by applying Paired samples *t*-test and the level of significance was applied to validate the efficacy of *Garlina* as the test drug.

### 3.1. Patient Description

The mean age of males (n=14) in test group was 47.92 years while the age of females (n=11) was 50.81 years. The mean age of 25 patients (both males and females) in *Garlina* treated test group was 49.2 years. The distribution of patients was categorized in different class intervals ranging from 35 to 65 years shown in Table 3.

### 3.2. Treatment Assignment and Follow-up

After examination and establishing diagnosis (n=25) patients were consented to be participated in the trial. Their pre-treatment lipid profiles were recorded and were administered the test drug *Garlina* i.e. 1 tablet b.d. after meals for 8 weeks. Their follow up was recorded for the changes in lipid profiles and any observed side effects. All cases were clinically studied and completed the assigned therapy.

**Table 3: Mean Urea, Creatinine, Alkaline Phosphate and GPT Profile of Subjects Before and after *Garlina* Treatment**

Parameters	Before treatment (mg/dL)	After treatment (mg/dL)
Mean Urea	26.92	25.48
Mean Creatinine	0.61	0.62
Mean Alkaline Phosphate	256	253.4
Mean Serum GPT	35.4	27.52

### 3.3. Response of Test Drug *Garlina* on Lipid Profile

Total cases (25) were administered with the test drug *Garlina* who initially met the inclusion criteria for duration of 2 months. The follow-up was made after every 4 weeks up to 8 weeks and the lipid profiles were noted accordingly. *Garlina* tablet (500 mg) twice a day was administered to them with having mean cholesterol level  $224.04 \pm 45.82$  mg/dL at the base line. After 8 weeks, total cholesterol level was reduced to  $166.48 \pm 35.59$  mg/dL. The mean triglyceride level was  $217.28 \pm 73.54$  mg/dL at the base line. After 8 weeks drug administration

the mean triglyceride level was also reduced to  $144.28 \pm 37.11$  mg/dL. The mean high density lipoprotein (HDL) level was  $48.28 \pm 10.57$  mg/dL at the base line. After 8 weeks drug administration the mean high density lipoprotein (HDL) level was increased to  $56.20 \pm 8.66$  mg/dL. The mean low density lipoprotein (LDL) level was  $133.96 \pm 54.00$  mg/dL at the base line. After 8 weeks drug administration the mean low density lipoprotein (LDL) level was reduced to  $87.52 \pm 40.17$  mg/dL. The effect of *Garlina* on lipid profile of hyperlipidemic patients before and after treatment is presented in Fig. 1.

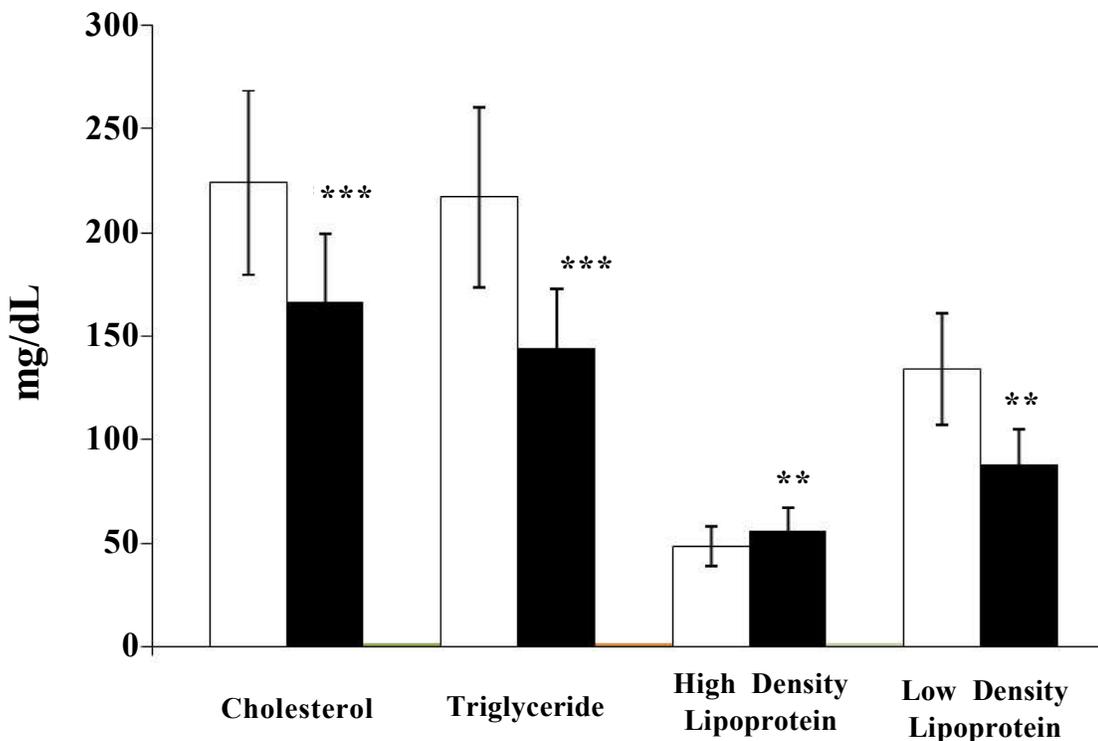


Fig.1: Effect of *Garlina* on lipid profile of hyperlipidemic patients before and after treatment

The values of lipid profile (mg/dL) represent mean±S.E.M of 25 subjects.

Before (□) and after (■) treatment with *Garlina* (500 mg b.d). \*\*, \*\*\* represents  $p \leq 0.01$  and  $0.001$ , respectively.

### 3.4. Adverse Effects Profile

All patients enrolled in the study were evaluated for safety. Side effects were defined as sign and symptoms that first occurred or became more severe during the course of treatment. The majority of adverse events were assessed as mild in severity and self-limiting in nature.

Three patients treated with the test drug experienced dryness of mouth, bloating of abdomen and mild sweating which disappeared within two to three days where the treatment was kept continued while two patients had discrepancy in eating habits temporarily due to any reason traveling out of the city and taken fatty food in official dinners. Their plasma lipid levels were raised for few days but later normalized after adjustments their diet. There were no significant changes in the alkaline phosphate, SGPT, urea and creatinine levels shown in Table 3 while the complete blood count of most of the patients were not altered and showed no significant changes. Furthermore, none of the patients withdrew from the study due to these adverse events. No significant adverse effects were recorded.

The plants had been in use by the Unani practitioners/Hakims for centuries not only as a drug but also as food agents too. The literature review and previous clinical studies provides sufficient support for this study. *Allium sativum* L. has been reported to decrease total cholesterol (CH) by 7% and low density lipoprotein cholesterol (LDL) by 10% in hypercholesterolemic men compared with subjects on placebo in a randomized, double blinded, placebo-controlled interventional study (Yeh and Lijuan, 2001). Similarly epidemiological studies in the past ten years have also shown the antithrombotic activity, antiplatelet aggregation and antioxidative properties, and to stimulate the phagocytotic function of

macrophage and lymphocyte proliferation (Block *et al.*, 2002). *Allium sativum* L. contains the sulphur containing compounds allinin, ajoene, diallylsulphide, dithiin, S-allylcysteine, vitamin B, proteins, flavonoids, saponins and minerals. These compounds behave as antioxidant, anti-inflammatory, anti-allergic, antithrombotic activity by inhibiting cyclooxygenase (Block *et al.*, 2002). Allicin affects atherosclerosis through modification of lipoprotein and as an inhibitor of HMG-coA reductase activity (the rate limiting enzyme of cholesterol biosynthesis pathway).

*Nigella sativa* L. along with *Trigonella foenum-graecum* L. has shown the hypolipidemic effects on serum triglycerides, high density lipoprotein and creatinine levels in type two diabetes mellitus patients in study. The study showed the decreases in triglyceride levels from mean  $171.00 \pm 10.75$  to  $169.00 \pm 8.33$  mg/dl and increase in HDL levels from mean  $36.70 \pm 0.70$  to  $38.16 \pm 0.86$  mg/dl after three month intervention (Memon *et al.*, 2012). Similarly oil of *Nigella sativa* L. has shown the mean percentage reduction in total cholesterol by  $26.87 \pm 6.75$ , the mean percentage reduction in triglyceride by  $12.02 \pm 0.55$ , the mean percentage increase in HDL cholesterol by  $15.89 \pm 2.15$  and the mean percentage reduction in LDL cholesterol by  $23.88 \pm 7.29$  in comparison with the standard group with standard regimen (Najmi *et al.*, 2008).

The compounds isolated from *Commiphora mukul* Hook. ex Stocks. like cembrenoids, a bicyclic diterpene, guggulsterone derivatives, myrrhanonol derivatives, myrrhanol derivatives, and a lignan, inhibit the lipid peroxidation by 79, 57 and 58% respectively (Jayaraj *et al.*, 2004). In a randomized, double blind study *Commiphora mukul* Hook. ex Stocks. was proved to be hypolipidemic and decreased the total cholesterol level by 11.7%,

the LDL-Cholesterol by 12.5%, triglycerides by 12.0% and the total cholesterol/HDL-Cholesterol ratio by 11.1% from the post diet levels compared with the placebo group (Singh *et al.*, 1994). In six randomized clinical trials of *Commiphora mukul* Hook. ex Stocks., involving 388 patients with different diagnosis, five in India and one in United States, 4 placebo-controlled and 1 compared guggul with 2 reference compounds reduced the total cholesterol from 10% to 27% and significant decrease in lipid peroxide levels (Joanna *et al.*, 2003). According to another study, guggulsterone, inhibit LDL oxidation mediated by either catalytic copper ions, free radicals generated with the azo compound 2, 2-azobis-(2-amidinopropane) dihydrochloride (AAPH), soybean lipoxygenase enzymatically, or mouse peritoneal macrophages reducing the chances of atherogenesis (Wang *et al.*, 2004). In another study of twenty patients of hyperlipidemia, purified gum of *C. mukul* Hook. ex Stocks. in two divided doses of 4.5 gm daily for 16 days reduced serum cholesterol and triglyceride levels at the end of 4<sup>th</sup> and 8<sup>th</sup> weeks (Verma *et al.*, 1988).

*Allium cepa* L. in one study has shown the significantly reduction in serum, liver and aortic triglycerides and serum and liver proteins as well. While liver free amino acids have been significantly increased in the onion treated groups as compared to the sucrose fed control. These effects are thought due to its sulphur containing principles which oxidize thiol compounds present free or in combined state with a protein and NADPH that are necessary for lipid synthesis (Sebastian *et al.*, 1979). Regular consumption of onions is reported to lower cholesterol levels and preventing incidence of atherosclerosis and diabetic heart disease due to speculated effect by the organo-sulphur compounds that lower high homocysteine levels, risk factors for heart attack and stroke (Janssen *et al.*, 1998).

*Zingiber officinale* Rosc. used in combination with other herbs significantly reduced body weight, skin thickness, waist/hip circumference accompanied with the reduction of serum triglyceride and cholesterol in diabetic and hyperlipidemic patients (Paranjpe *et al.*, 1990). Similarly ethanolic extract of *Z. officinale* Rosc. has shown hypolipidemic effects in cholesterol fed rabbits. There was significant reduction in the rise of serum and tissue cholesterol, serum triglyceride, serum lipoproteins and phospholipids of 10 week of cholesterol fed rabbits after the administration of ethanolic extract of ginger and results were compared with Gemfibrozil, a standard orally effective hypolipidemic drug (Kamal and Aleem, 2009).

Since the efficacy of *Garlina* has been proven by this clinical trial so this study should be extended to various locations in Pakistan and comparative studies with the conventional allopathic drugs like Statins e.g. atorvastatin or simvastatin should be done. Moreover the *Garlina* can be subjected for studying weight reduction as it contains very powerful and effective antioxidant ingredients that can hamper the fat synthesis in liver hepatocytes and other tissues like adipose tissues as it was felt during the research. To meet the international standards of efficacy, certain animal model studies should be done to validate its phase zero trial which is very much essential for the process of drug development internationally. With the help of such data international market can be captured to get more revenue and profit to Hamdard Laboratories (Waqf) Pakistan.

Similarly the ratios of the ingredients should be readdressed because there are many chances that any ingredient which in higher ratio can be more helpful and synergistic for other ingredients so one by one the ratio of ingredients should be increased and tested so that the best

ratio for the formula can be achieved through more study projects. This should be done also because there are many other herbal/Unani medicine companies who are in the business for the same therapeutic indication as competitors.

There is potential gap in the herbal or Unani medicine industry regarding good quality and efficacious medicinal products for cardiovascular diseases. *Garlina* can be one step forward for Hamdard Laboratories (Waqf) Pakistan in light of its clinical findings and toxicological manifestation it could lead as a desirable option regarding Unani medicine for cholesterol management.

#### 4. CONCLUSION

The finding from this research study demonstrated lipid profiles that there was statistically significant difference in the before and after treatment with *Garlina* (500 mg) 1 tablet b.d. after meals suggesting that *Garlina* possesses a great hypolipidemic potential for the treatment of primary hyperlipidemia due to its chemical and pharmacological significance.

Paired Samples t-test was applied by using SPSS version 17.0 to analyze the statistical difference. The statistical results of clinical response it was concluded that *Garlina* is effective for the treatment of primary hyperlipidemia, the effect being confirmed by the physicians and patients alike. The promising result with 'p-value' i.e. less than 0.05 was indicated its statistical significance of hypolipidemic condition.

*Garlina* is a product of common culinary herbs. There were no untoward or significant side effects associated with the use of *Garlina* that proved its acceptability by the patients. Moreover there was subjective feeling of wellbeing regarding breathlessness and chest

tightness or pressure among the patients. This makes an added advantage to focus on this effective alternative drug for primary hyperlipidemia.

#### Conflict of Interest

The authors declare there is no conflict of interest concerning the publication of the manuscript.

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## Assessment of Antibacterial Activity of Herbal Formulations Used in Typhoid Fever, Available in Local Markets of Twin Cities of Pakistan

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### Abstract

Herbal formulations are used for so many years in various countries including Pakistan for the treatment of different diseases. Thus six herbal formulations were purchased from local market of Islamabad and Rawalpindi in order to assess antibacterial activity like minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) on *Salmonella typhi* ATCC (14028). The results revealed that only two formulations showed activity against *S. typhi*. Two herbal formulations failed to show any antibacterial activity. While remaining formulations had weak activity. To promote the role of alternative medicines, appropriate measures should be taken by Regulatory authorities to promote standard and good quality herbal products.

### Keywords

Minimum Inhibitory Concentration (MIC), Zone of Inhibition (ZOI), Muller Hilton Agar (MHA), American Type Cell Culture (ATCC), Colony Forming Units (cfu).

### 1. INTRODUCTION

*Salmonella enteric* is a Gram-negative facultative intracellular anaerobe of worldwide importance causing as many as 1.3 billion

cases of disease annually. Over 2500 serovar of *S. enterica* have been identified belonging to six subspecies. Subspecies are further subdivided into serovars that are differentiated by their flagellar, carbohydrate and lipopolysaccharide (LPS) structures. *S. enterica* species are typically orally acquired pathogens that cause one of four major syndromes: 1) Enteric fever (typhoid), 2) Enterocolitis/diarrhea, 3) Bacteremia and 4) Chronic asymptomatic carriage (Coburn and Grass, 2007). In developed countries, typhoid cases are stable but non-typhoidal cases are increasing worldwide. Usually 5 to 30% mortality rate has been observed in developing countries due to typhoid infection. WHO has estimated about 16 to 17 million cases annually of which 600,000 deaths occur. The mortality rates differ from regionally (Mehboob *et al.*, 2013).

In Pakistan, epidemiology of typhoid fever within the country is heterogeneous both seasonal and location wise. Cases of typhoid fever are greater in areas where rainfall is greater like Rawalpindi and Faisalabad as compared to areas where rainfall is less and moderate like Multan and Lahore (Mehboob *et al.*, 2013). In recent years, an increasing rate was observed regarding the use of herbal drugs in our country. Herbal

and all other forms of drugs are considered as complementary and alternative medications having less or no side effects as compared to allopathic formulations. Herbs are generally available as standardized, dried extracts (pills, capsules, or tablets), teas, or tinctures/liquid extracts (alcohol extraction, unless otherwise noted) or sometimes herbal extracts are mixed with popular beverages. Dose for teas is 1-2 teaspoonful/cup of water steeped for 10-15 minutes (roots need longer).

Herbal formulations are being marketed to cure typhoid. Unlike allopathic medications which pass the entire required quality control tests, there are few herbal formulations which contain the stated amount of active agent in them. They may not contain the active drugs and possibly a major reason for being ineffective or develop resistance.

## **2. MATERIALS AND METHOD**

### **2.1. Assessment of Antimicrobial Activity of Herbal Brands**

Six herbal formulations were purchased from the local markets of Islamabad and Rawalpindi for assessment of antibacterial activity. Respective Codes were given to herbal formulations. Bacterial strain of *Salmonella typhi* ATCC 14028 was purchased from a reputable supplier.

### **2.2. Determination of Zone of Inhibition Preparation of Media**

Muller Hinton agar (6.5 g, MHA) was dissolved in distilled water (500 mL) and simultaneously heated until boiled. The media was poured in a conical flask and along with glass Petri plates was autoclaved at 121°C at 15 psi for 15 minutes. Under aseptic conditions these Petri plates were filled with MHA and allowed to solidify.

### **Inoculation and Incubation**

After solidification of the medium, sterile cotton swab was dipped in the bacterial inoculum that was spread over the medium on plate. Sterile cork borer of 8 mm diameter, wells were made and sealed with medium and approximately 100 µL of the stock solutions of the standard drug and six herbal formulations of herbal and ciprofloxacin were added into the wells. These plates were divided into four portions and labeled. After allowing the drug to diffuse for about 30 minutes, these plates were incubated at 37°C for 24 hours (Uduma *et al.*, 2011). Zone of inhibition was measured for all herbal formulations by using vernier caliper (Muhammad *et al.*, 2011).

### **2.3. Minimum Inhibitory Concentration Determination**

Minimum inhibitory concentration (MIC) is the lowest concentration or amount of the drug at which it shows the highest activity against microorganisms (Chakraborty and Ahmed, 2013). MIC was determined by broth dilution method. All six herbal formulations along with the standard ciprofloxacin were assessed and their MIC was determined.

### **2.4. Preparation of Inoculum**

Standard stock culture of bacterial isolate was prepared by suspending a loop full of microbial growth in distilled water (4 mL). It was incubated at 37°C for 12 hours and its turbidity was compared with 0.5 McFarland's standard giving a bacterial load of about  $3.6 \times 10^2$  cfu/mL (Chakraborty and Ahmed, 2013).

### **2.5. Preparation of Antimicrobial Solutions**

One tablet (500 mg) was dissolved in sterile water (10 mL) to final concentration of

50 mg/mL. It was further diluted through two-fold dilution method and serial dilutions were prepared ranging from 1 to 12 µg/mL (Uduma, 2011). Similarly herbal formulations were diluted using sterile water.

### 2.6. Preparation of Media

Muller Hinton Broth media was prepared using manufacturer's guidelines. It was autoclaved at 121°C at 15 psi pressure for 15 minutes and allowed to cool. Finally media

was poured in sterile test tubes under aseptic conditions in laminar flow hood.

### 2.7. Inoculation and Incubation

Bacterial isolate suspension (1 µl) was inoculated in the test tube containing broth media. Each dilution (1 ml) of the antimicrobial agents of herbal formulations was poured in the test tube. These test tubes were incubated at 37°C for 18-24 hours. Herbal formulations used is given in Table 1.

**Table 1: Local Herbal Formulations Used for Assessment of Anti-bacterial Activity**

Codes for herbal preparation	Brands	Company	Batch No.	Date
1	Taifax	Kamal Laboratories	L-0135	—
2	Fever-X	Qarshi Industries	25101012	10/2015
3	Bukhar Rok	Ashraf Laboratories	01309008	09/2016
4	Typhex Plus	Qarshi Industries	090312	03/2015
5	Johar Khaksi Compound	Javaid Unani Laboratories	004	11/2014
6	Khameera Marwarid Khas	Ashraf Laboratories	1309085	09/2018
	Reference Standard	Ciprofloxacin		

### 3. RESULTS AND DISCUSSION

Among six herbal formulations only two brands showed inhibition of bacterial growth at 12.5, 25, 50 and 100% dilutions. Herbal preparation 3 and 6 formulations demonstrated

remarkable activity against the bacterial strain while Herbal 4 and 5 was active only at 100% dilution. Herbal 1 and 2 were inactive at all tested dilutions. The MIC of standard ciprofloxacin was observable at 1.2 µg/mL.

**Table 2: MIC Against *Salmonella typhi* at Various Concentrations**

Codes for herbal preparation	Minimum inhibitory concentration MIC ( $\mu\text{g/ml}$ )					
	1	5	12.5	25	50	100
Herbal-1	-	-	-	-	-	-
Herbal-2	-	-	-	-	-	-
Herbal-3	-	-	+	+	++	++
Herbal-4	-	-	-	-	-	+
Herbal-5	-	-	-	-	-	+
Herbal-6	-	+	+	++	++	+++
Ciprofloxacin standard	+++	+++	++++	++++	+++++	+++++

Negative (-), weak (+), mild (++) , moderate (+++) and remarkable (++++).  
Serial dilution in terms of percentage.

The anti-typhoidal activity of the herbal formulations were assessed by agar well diffusion method and presented in Table 3. Only Herbal 6 showed marked zone of inhibitions almost at all dilutions. Herbal 3, 4 and 5 had lesser ZOI as compared to

Herbal 6. While Herbal 1 and 2 showed no antibacterial activity against *Salmonella* strains. The ZOI of ciprofloxacin at 2, 4, 6, 8, 10 and 12  $\mu\text{g/mL}$  was found to be 14, 19, 20, 21, 23, 25 mm in diameter respectively.

**Table 3: Zone of Inhibition Against Various Concentration of Herbal Formulations**

Codes for herbal preparation	( $\mu\text{g/ml}$ )				
	5	10	25	50	100
1	0	0	0	0	0
2	0	0	0	0	0
3	0	3	5	8	10
4	0	0	0	0	5
5	0	0	0	0	6
6	0	5	7	10	12

Serial dilution in terms of percentage.  
Zone of inhibition (mm, ZOI).

Herbal drugs are considered safe and having minimum or no side effects, due to which most community people prefer them. On the other hand they are cost effective as compared to allopathic formulations. Therefore, it is expected that antibacterial activity for herbal formulations are of similar magnitude to allopathic formulations. All herbal formulations mentioned in Table 1 have some plants extract commonly which included extract of *Glycyrrhiza glabra*, *Sisymbrium irio*, *Zizyphus lotus*, baptisia, *Melia azadirachta* and *Fumaria indica*. Earlier, methanolic extract of *Glycyrrhiza glabra* showed significant antibacterial activity against *Salmonella typhii* from 6 mm to 17 mm in disc diffusion method (Gul *et al.*, 2006). Aqueous extract of *Sisymbrium irio* was comparable ineffectiveness to that of tetracycline against *Salmonella typhii* (Khan *et al.*, 2011). The etheric and methanolic extracts of *Zizyphus lotus* was reported for growth inhibition (diameters between 11 and 20 mm) of *Salmonella Typhi* (Rsaissi *et al.*, 2013). Baptisia, an extract from indigo plant root, has been proved to be highly effective ultradilute medicine for the treatment of typhoid. However, mode of action is uncertain. Due to the antigenic variations of *Salmonella* it induces immune system by activating both T and B cells by the formation of antibodies (Banerji, 2012). The ethanolic extract of *M. azadirachta* showed promising results against *S. typhii* at 40 mg/mL concentration (Kelati *et al.*, 2015). Extract of *Fumaria indica* also showed activity against different bacterias including *S. typhii* (Shinwari *et al.*, 2015).

#### 4. CONCLUSION

Due to random and excessive use of antimicrobial drugs in the treatment of contagious diseases, pathogenic bacteria have developed

resistance against prevailing antibiotics. Multi-drug resistant strains of bacteria generally spread in hospitals and are isolated from population acquired infections. Thereby, leading to severe consequences such as failure of treatment. Hence, it is imperative to explore natural products as an alternative to synthetic antimicrobial agents. In order to promote these herbal formulations as Alternative medicines, Ministry of Health and Drug Regulatory Authority of Pakistan should take appropriate measures for maintaining the quality and standard of these formulations so that they can be used as alternative to allopathic medicines. It was concluded from the study that only two products Herbal 3 and 6 showed slight antibacterial activity (minimum inhibitory concentration and zone of inhibition) at different concentrations. Two products Herbal 1 and 2 did not show any activity at any concentration used. While Herbal 4 and 5 showed slight activity at 100% concentration. Thus it appears that either the medicinal plants extracts are absent or present in insufficient amounts in these formulations and hence non-efficacious or which resulted in absence/lesser antibacterial activity of herbal formulations.

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## Investigation of *Musa paradisiaca* L. for Brine Shrimp Lethality, Phytotoxicity and Insecticidal Potential

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### Abstract

Natural plant products contain diversity of molecules that have different biological, pharmacological and toxicological properties. The present study was carried out to investigate the brine shrimp lethality, insecticidal and phytotoxic potentials by using the *Musa paradisiaca* L. bract, flower, trachea (flowering stalk) and tracheal fluid. Brine shrimp lethality assay was determined by using the *salina* species of *artemia* and results showed that all four extracts were found inactive at low concentration while little activity was found in each part at high concentration. Phyto-toxic potential was studied on the *Lemna minor* L. and dose dependent phytotoxic potential of all four extracts were observed. While insecticidal activity was determined by using the direct contact method which indicated that these parts of *Musa paradisiaca* L. possesses no such kind of activity.

### Keywords

Brine shrimp lethality assay, insecticidal, phytotoxic and *Musa paradisiaca* L.

### 1. INTRODUCTION

World population is becoming more interested to use traditional natural medicine to solve the health care problems because all new medicines are either derived from raw plant

material, crude extract or mixture. The use of traditional medicine is an integral part of many cultures (Alluri *et al.*, 2005). Weeds and plant diseases that are caused by fungi and bacteria are a major problem in agriculture because they cause a remarkable decrease in the yield of the crop and insects cause significant obstruction in production and economic performance therefore prevention and management of disease is necessary for agriculture and forestry production (Zhang *et al.*, 2011; Akkari *et al.*, 2015).

Approximately 2.5 million tons of pesticides are being used on crops now a days and damage caused by these pesticides reaches to \$100 billion annually in all over the world. The disproportionate dependence, random and indiscriminate use of chemical pesticides for crop protection have resulted in water and environmental contamination, toxic residue in treated products, phytotoxicity, development of resistance against these pesticides and a noxious risk to human and other organisms and forms the bases of natural method of pathogen control (Akkari *et al.*, 2015; Nenaah *et al.*, 2015). The conventional synthetic pesticides such as methyl bromide and phosphine that are used for the protection of stored product have very high toxicity and are banned in European Union and United States. According to the European Pesticide Regulation the pesticide should be

harmless. These regulations form the bases of bio-pesticide (Polatoglu *et al.*, 2015). Plants are screened for allelopathic potential and phytotoxic capability to use them as plant based pesticides (Ahmad *et al.*, 2012).

Plant based natural drugs are bio-friendly, eco-friendly and safe and phytochemical produce by plants have physiological action on the human body (Lalisan *et al.*, 2014). Plants are useful in crude or advanced form as a source of drug, the researchers from all over the are in search of new plant derived drug that have potency to contest the nuisance of drug resistant pathogenic microorganisms and have antitumor and anticancer activity (Mentor *et al.*, 2014).

Brine shrimp (*Artemia* sp.) lethality assay (BSLA) is used as marker for general toxicity screening as it is well known, simple efficient, inexpensive method for detection and isolation of insecticidal, pesticidal, cytotoxic, anti-neoplastic, antimalarial, and anti-feedant compounds from plant extract and is suitable method to correlates the cytotoxic and other biological properties (Lilybeth and Nuneza, 2013; Ana *et al.*, 2002).

There is an imperative requirement and strong desire for new approaches and innovative strategies to use plant based products (Nenaah *et al.*, 2015) and greener chemistry in edible horticulture crops and urban areas to control the pest that should be more toxic and environment friendly (Duke *et al.*, 2010). In order to obtained a safe treatment from plant products, intention have been drawn on toxicological and pharmacological studies of plants used by human beings because along with the important biological properties, plants also possesses lithality factors including mutagenic or genotoxic potentials due to having some content or toxic chemical components which may be produced in plants during metabolic processes toxicological are may be

due to contaminants or by the chemical compounds of plants (Mentor *et al.*, 2014).

*Musa paradisiaca* L. (Musaceae) is evergreen tropical mono herbacious plant, commonly known as *kela* (Urdu) *Kadali*, *Bali Hannu* (Hindi) and Plantain (English). It is major food crops in the humid and subhumid parts of Pakistan, Africa, India, Burma, Bangladesh, America, and Australia *M. paradisiaca* root is used as tonic for congestion of the liver and used to prevent scurvy, anaemia and veneral diseases. The leaves are used in inflammation of eye, healing wounds and ulcers. The flowers check excessive bleeding during menstruation and are used in the case of diabetes (Enye *et al.*, 2013). Objective of the study was to evaluate the various morphological parts (bract, flower, trachea and tracheal fluid) of *M. paradisiaca* for brine shrimp lethality, phytotoxic and insecticidal potential.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant Material

*Musa paradisiaca* L. plant was collected from District Muzaffar-Garh, (Punjab) Pakistan during November 2013 and authenticated by Dr. Mansoor Hameed, Associate Professor Taxonomic Laboratory Department of Botany, University of Agriculture Faisalabad. The Voucher specimens (131-2014) of each plant part material were deposited in the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi for further reference. The parts of plant [bracts, flower, flowering stalk (trachea)] were separated from plant, spread on filter paper and dried in shade at room temperature for about one month.

### 2.2. Preparation of Plant Extracts

Flowering stalk (trachea) (300 g), dried

flower (500 g) and bracts (500 g) were soaked in methanol separately. Extraction was carried out by maceration using methanol (Merck) in glass aspirator for seven days with occasional shaking at room temperature followed by filtration by using Whatman filter No 1. The filtrate were evaporated to dryness in rotary vacuum evaporator (Rotavapor R-200, Buchi) with rotation 3 rpm and pressure 0.07 MPA or 20 in Hg. The dried material were weighed, labeled and stored in refrigerator. After cutting the bunch of fruit, fluid (100 ml) obtained from floral stalk was lyophilized at  $-65$  to  $-60^{\circ}\text{C}$  with vacuum of 30-40 mili-bar in alpha 1-4 LSC Christ Germany lyophilizer. The dried material were weighed, labeled and stored in refrigerator (Sanyo biomedical freezer, MDF-U333, Japan). These stored extracts were used for brine shrimp lethality assay, phytotoxicity and insecticidal activity.

### 2.3. Brine Shrimp Lethality Assay

The procedure for BSLA was modified from the assay that described by Solis *et al.* (1993). Each extracts 4.0 mg weighing (bract, flower, stalk and tracheal fluid) was dissolved in dimethylsulfoxide (1% DMSO) and solutions of varying concentrations (10,100,1000  $\mu\text{g/ml}$ ) were obtained by the serial dilution using simulated seawater. Serial dilutions were made in the wells of 96-well microplates (Nunc, Denmark) in triplicate in sea water (120  $\mu\text{l}$ ). Control wells with DMSO were included in each experiment. A suspension of nauplii (larve) containing 30 organisms (100  $\mu\text{l}$ ) was added to each well. The plates were covered and incubated at room temperature ( $28\pm 1^{\circ}\text{C}$ ) for 24 hours. Plates were examined under the binocular microscope and the numbers of dead (non-motile) nauplii in each well were counted. Three replications were used for each concentration. A parallel series of tests with

the standard etoposide solution ( $\text{LD}_{50}=7.4625 \mu\text{g/ml}$ ) and the blank control were always conducted. The lethal concentration for 50% mortality after 24 h was calculated. The  $\text{LC}_{50}$  values greater than 1000  $\mu\text{g/ml}$  for plant extracts were considered inactive (Solis *et al.*, 1993).

### 2.4. Phytotoxic Activity

The phytotoxic activity of *M. paradisiaca* (bract, flower, stalk and tracheal fluid) samples was checked against *Lemna minor* L. obtained from H.E.J Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi. Stock solutions of the methanolic extract of bract, flower, stalk and dried tracheal fluid of *M. paradisiaca* were prepared in methanol at concentration of 20 mg/ml. Willium medium E used for the growth of *Lemna minor* L. was also prepared. Three concentrations (10, 100 and 1000  $\mu\text{g/ml}$ ) from the stock solution of samples were prepared and introduced into three separate flasks and left at room temperature till methanol was evaporated. (20 ml). The Willium medium E and twenty healthy plants with a rosette of three leaves were added to all the flasks and incubated at ( $28\pm 1^{\circ}\text{C}$ ) for 7 days. Paraquat (0.015  $\mu\text{g/ml}$ ) was used as standard growth inhibitor. Results were noted by counting the number of damaged and healthy plantlets (McLaughlin *et al.*, 1991).

### 2.5. Insecticidal Activity by Direct Contact Method

Dried extract of *M. paradisiaca* (bract, flower, trachea and tracheal fluid) was prepared at concentration of 1019.10  $\mu\text{g/cm}^2$  in acetone. Two insects namely *Tribolium castaneum* and *Rhyzopertha dominica* were obtained from H.E.J Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi,

Karachi. Permethrin ( $230.5 \mu\text{g}/\text{cm}^2$ ) and acetone were used as positive control and negative control, respectively. Aliquots (0.7 ml) of different part extract of *M. paradisiaca* at the concentration of  $1019.10 \mu\text{g}/\text{cm}^2$  were used for the impregnation of filter paper Whatman No. 1, 8.5 cm diameter. After drying during 5 min, filter papers were inserted at the bottom of the petri dishes (5.5 cm diameter  $\times$  1.2 cm) containing culture media and then ten adult unsexed *T. castaneum* and *R. dominica* were added to separate petri dish under the similar environmental conditions and six replicates were tested. The inner side of the lid was coated with vaseline to prevent insect staying on lid. The insects were considered dead when no antenna or leg movement was detected when gently touched with a brush. Mortality percentages were recorded after 24 h of treatment (Zapata and Smagghe, 2010).

### 2.6. Statistical Analysis

All the graph, calculation and statistical analysis were performed by using 5<sup>th</sup> version of graph pad prism with window seven professional (Graph pad software, San-diego California USA).

## 3. RESULTS AND DISCUSSION

*A. salina* toxicity assay method is used as a pre-evaluation model for pharmacological activities such as pediculicidal and ecotoxicological activities of compounds that have much complex structure (Vidotta *et al.*, 2013). The brine shrimp lethality assay is quick, inexpensive and simple bioassay method for investigating the plant extracts bioactivity which in some cases correlates logically with cytotoxic and anticancer properties (Krishnaraju *et al.*, 2005). Brine shrimp lethality activity of the plant extracts (bract, flower, flowering stalk and trachea) was investigated and results are

presented in Figs. 1, 2, 3 and 4, respectively. These results showed that all four parts of plant has activity only at the highest concentration at ( $1000 \mu\text{g}/\text{ml}$  and have no activity at concentration of 10 and  $100 \mu\text{g}/\text{ml}$  while the standard drug etoposide was  $\text{LD}_{50}=7.4625 \mu\text{g}/\text{ml}$ . This indicates that no significant differences in percentage mortalities between different concentrations of extracts no brine shrimp lethality as compared to that of control. This reveals that these part of *M. paradisiaca* have little cytotoxic activity at high concentration.

The use of natural plant product for understanding the dynamics of plant-plant interactions as well as searching their prospective to find biologically and environmentally secure drugs for controlling the weeds and protecting the crops in agricultural sector is of scientific and industrial significance (Ulukanli *et al.*, 2014). In this study the phytotoxic activity of four different part (bract, flower, trachea and tracheal fluid of *M. paradisiaca* was studied and result was expressed in Figs. 5, 6, 7 and 8, standard drug paraquat ( $0.015 \mu\text{g}/\text{mL}$ ) showed growth inhibitory effect.

These results indicate that the different organic extracts of *M. paradisiaca* influence germination and seedling growth of *Lemna minor* L. The inhibition of early growth and germination by natural extracts are reported in literature (El-Ayeb *et al.*, 2015). The inhibitory effect was increase with the increasing concentrations suggesting the phytotoxic activity.

In the current study insecticidal activity by direct contact method of bract, flower, flowering stalk (trachea) and tracheal fluid of *M. paradisiaca* against two insects namely *Tribolium castaneum* and *Rhyzopertha dominica* were tested. Result of insecticidal activity of bract, flower, trachea and tracheal fluid of *Musa paradisiaca* showed in Table 1.

**Table 1: Insecticidal Activity of *Musa paradisiaca* (bract, flower, trachea and tracheal fluid)**

Name of insect	Plant parts	% Mortality of permethrine (+ control)	% Mortality of (-ve)control	% Mortality of sample
<i>Triboliumc astaneum</i>	Bract	100	0	0
	Flower	100	0	0
	Trachea	100	0	0
	Tracheal fluid	100	0	0
<i>Rhyzopertha dominica</i>	Bract	100	0	0
	Flower	100	0	0
	Trachea	100	0	0
	Tracheal fluid	100	0	0

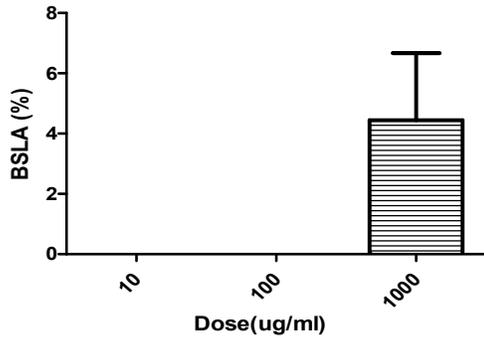


Fig. 1: Brine shrimp lethality Assay of bract

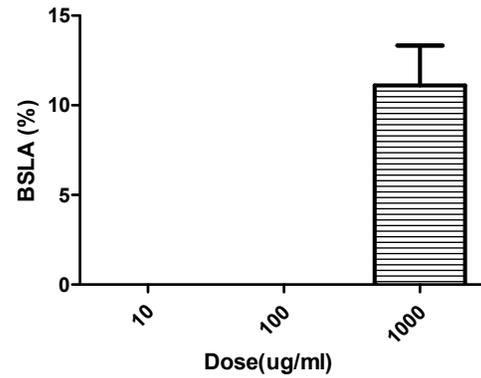


Fig. 2: Brine shrimp lethality assay of flower

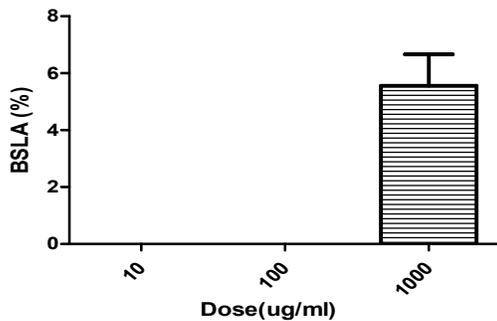


Fig. 3: Brine shrimp lethality assay of trachea

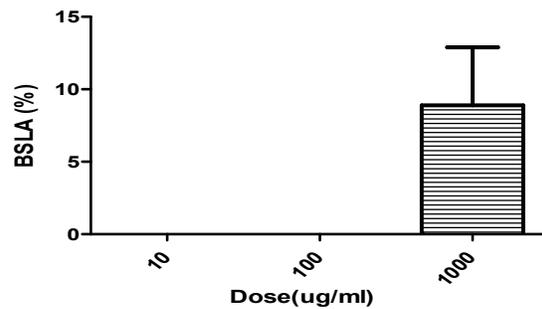


Fig. 4: Brine shrimp lethality assay of tracheal fluid

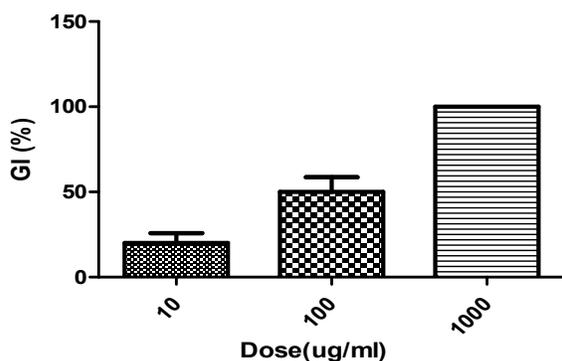


Fig. 5: Phytotoxicity bioassay of bract

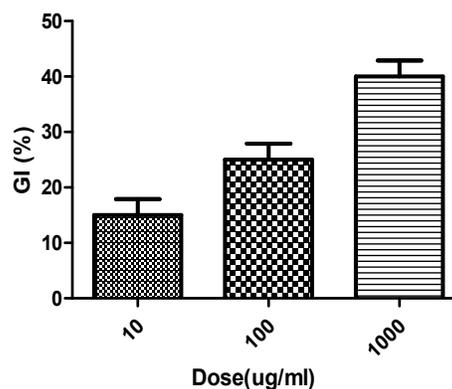


Fig. 6: Phytotoxicity bioassay of flower

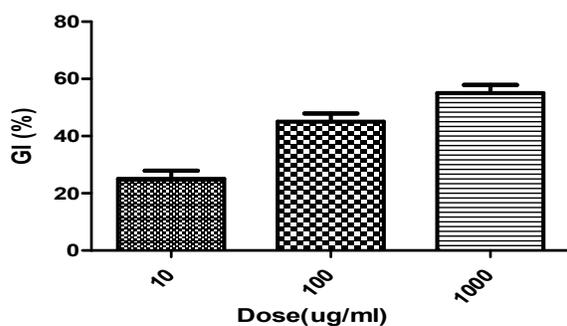


Fig. 7: Phytotoxicity of trachea

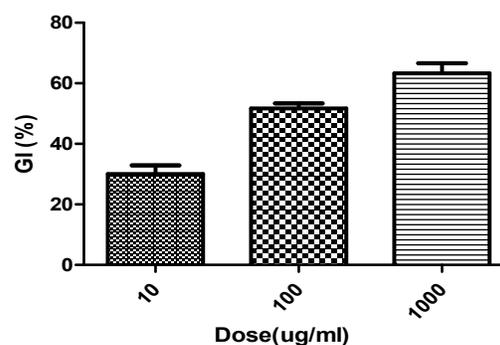


Fig. 8: Phytotoxicity of tracheal fluid

All these four plant parts i.e bract, flower, flowering stalk (trachea) and tracheal fluid of *Musa paradisiaca* at the dose of 1019.10  $\mu\text{g}/\text{cm}^2$  have no insecticidal activity whereas standard drug Permethrin at concentration of 230.5  $\mu\text{g}/\text{cm}^2$  shows 100% mortality.

#### 4. CONCLUSION

This research findings showed that bract, flower, trachea and tracheal fluid of *Musa paradisiaca* L. has no significant cytotoxic and insecticidal activity whereas phytotoxic activity is dose dependent that may protect the plant

from the attack of weed and other predators. Therefore, it is concluded that the other parts of the edible plant could be safe when it is used in pharmaceutical manufacturing in future after further research in this regard.

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## Physicochemical and Phytochemical Standardisation of *Khulanjan* (Rhizome of *Alpinia galanga* L. Willd.) – An Important Unani Drug

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### Abstract

*Khulanjan*, a rhizome of *Alpinia galanga* (L) Willd. (family; Zingiberaceae) is an important Unani drug found throughout India and has been recognized in many Traditional Systems of Medicine due to its medicinal and cosmetic values in various ailments and beauty enhancement. Keeping in view of the authentication and quality assurance of medicinal plants the physicochemical and phytochemical studies of *Khulanjan*, was conducted to meet Pharmacopoeia standard and quality control of Unani drugs before using them for health care. The physicochemical analysis includes parameters like ash values, bulk density, moisture content, loss on drying at 105°C, pH values and successive extractive values and fluorescence analysis of powder and extracts of test drug whereas the phytochemical analysis consists of thin layer chromatography, qualitative determination of different phytochemicals present in its rhizome.

The data can be used as markers in the identification and standardization of *Khulanjan* that should be utilized in all the pharmaceutical industries as raw material.

### Keywords

*Alpinia galanga* (L) Willd., Physicochemical and phytochemical analysis, Standardization.

### 1. INTRODUCTION

The drug *Khulanjan* consists of dried rhizomes of *Alpinia galanga* (L) Willd. Syn. *Alpinia calcarata* Rosc. of family Zingiberaceae (Anonymous, 2007). The plant is found in the Eastern Himalayas and South Western Ghats. It is also cultivated throughout India especially in Bengal, South India and Assam. The plant is cultivated during late summer or early. Flowering takes places during April-May (Anonymous, 1987; Anonymous, 2003; Anonymous, 2007).

It has a pungent, hot and spicy taste with an aromatic ginger like odour. According to Unani Physicians, the *Mizaj* (temperament) of *Khulanjan* (rhizome of *Alpinia galanga* [L.] Willd.) is of hot and dry (Ibn Hubl, 2005), but there is controversy with regard to its degree of temperament. According to some authors it is hot and dry to the second degree (Attar, 1888; Harvi, 1895; Ibn Sina, 2007; Ghani, 2010; Khan, 2013), whereas Ibn Baitar, 1987; states that it is hot and dry to the third degree. It is an official drug mentioned in various Pharmacopoeias as well as reported to possess several therapeutic activities. Rhizome is cylindrical, branched, 2 to 8 cm in diameter, scaly leaves arranged circularly; externally reddish brown, internally orange Figs. 1 and 2 yellow, fracture hard and fibrous; fractured surface rough (Anonymous, 2003). It is used in Unani



Fig. 1

Plant of *Alpinia galanga* (L) Willd



Fig. 2

Rhizome of *Alpinia galanga* (L) Willd (market sample)

Medicine having aromatic, bitter, stimulant, aphrodisiac, carminative, expectorant, diuretic, stomachic, semen viscositive, renal tonic, anti-inflammatory and cardio tonic (Husain, 1875; Harvi, 1895; Ibn Baitar, 1987; Ghani, 2010; Haleem, 2009; Khan, 2013). The drug is useful in headache, lumbago, rheumatic pain, sore throat, chest pain and renal diseases. Unani Physicians used the rhizome in impotence, bronchitis and dyspepsia; it is used as disinfectant to destroy bad smells in the mouth and any other parts of the body. It is also advocated in diabetes mellitus, (Nadkarni, 1954; Anonymous, 1970; Kirtikar and Basu, 1987; Khory, 1985). In Ayurvedic Medicine, the drug is used as a depressant to the cardiovascular system, and also improves appetite, taste and voice; useful in “vata”, and cardiovascular diseases (Battacharjee, 2004; Chopra *et al.*, 1958).

*Alpinia galanga* (L) Willd. contains volatile oil (0.5-1%), resin (20%), kaempferol, galangin, alpinin dihydroflavanol, galangol, phlobepine, tannins and starch in abundance. Kaempferol, galangin and alpinin are the flavonoids (Chopra *et al.*, 1958; Lubhaya, 1982; Rastogi *et al.*, 2001; Anonymous, 2003;

Anonymous, 2004; Bhattachrjee, 2004; Farooqi, 2013). Essential oil from rhizome exhibited antimicrobial activity against Gram-positive bacteria (Rastogi *et al.*, 1995).

The drug *Khulanjan* was already mentioned in *Unani Pharmacopoeia of India*, Part I, Vol. II, published in 2007 by Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy, Ministry of Health & Family Welfare, Government of India, New Delhi wherein only few parameters for the identity and purity of the drug along with TLC behaviour of petroleum extract were adopted. Apart from this work the drug *Khulanjan* was still unexplored with regards to its detailed standardization, therefore present study was undertaken to carry out its physicochemical and phytochemical analysis that could be considered as standard tools for Unani pharmaceutical industries and to include this parameter in Unani Pharmacopoeia as well.

## 2. MATERIAL AND METHODS

The study was conducted in the Department of Ilmu Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University Aligarh during the year 2015-2016. The test drug,

*Khulanjan* (rhizome of *Alpinia galanga* [L] Willd.) was procured from Dawakhana Tibbiya College Aligarh Muslim University, Aligarh and identified in the pharmacognosy section of department of Ilmu Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University Aligarh, authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi with the reference: (NISCAIR/RHMD/Consult/2015/2843/36), a herbarium sample of drug was prepared and submitted to Mawalid Salasa Museum of Department of Ilmu Advia for future reference with the voucher no of SC-0171/15.

### 2.1. Organoleptic Characters

The macroscopical examination of test drug was done using the organoleptic characters i.e. appearance, colour, odour, taste.

### 2.2. Physicochemical Analysis

The physicochemical analysis of *Khulanjan* was carried out using the following parameters.

**2.2.1. Ash Values:** it includes total ash, water soluble ash, and acid insoluble ash (Anonymous, 1968; Jenkins *et al.*, 1967; Afaq *et al.*, 1994).

**2.2.2. Solubility:** Solubility test of powdered drug in water and alcohol was carried out according to the method described in Anonymous, 1968.

**2.2.3. Moisture Content:** Moisture content of drug was determined by toluene distillation method described by Jenkins *et al.*, 1967 and Afaq *et al.*, 1994.

**2.2.4. Loss on drying at 105°C:** it was conducted as per the parameter mentioned in Jenkins *et al.*, 1967, Anonymous, 1987 and Afaq *et al.*, 1994

**2.2.5. Bulk Density:** Bulk density was performed using tapped density (Compacted state) with the help of digital bulk density apparatus.

**2.2.6. pH values:** pH value of 1% and 10% aqueous solution was determined as per the procedures mentioned in Jenkins *et al.*, 1967 and Anonymous, 1987.

**2.2.7. Successive Extractive Values:** The Successive extractive values of *Khulanjan* in different solvents viz. Petroleum ether, Diethyl ether, Chloroform, Acetone, Ethanol and Distilled water were determined with the help of Soxhlet's apparatus (Successive method) according to the methods described in Anonymous, 1968; Anonymous, 1987; Anonymous, 2003.

### 2.2.8. Fluorescence Analysis:

**(a) Fluorescence Analysis of the successive extracts of the test drug:** Fluorescence analysis of Successive extracts of drug sample viz. petroleum ether, diethyl ether, chloroform, acetone, ethanol and aqueous were done in day light and UV lights (Anonymous, 1992). The changes in colour were noted which was summarized in Table 5.

**(b) Fluorescence Analysis of powdered drugs:** Fluorescence analysis of the powder of test drug was done. The powdered drug was treated with different chemicals and observed in daylight and under ultra violet lights (Anonymous, 1992). The changes in colour were noted and summarized in Table 6.

### 2.3. Thin Layer Chromatographic Analysis

The thin layer chromatographic patterns of aqueous extract of test drug (*Khulanjan*) were studied in various mobile phases i.e. n-Butanol, acetic acid and water in the ratio of 4:1:5. The plates were later sprayed with different reagents to detect the spots representing various constituents followed by determining the Retardation Factor ( $R_f$  value).

### 2.4. Qualitative Analysis of *Khulanjan*

The Qualitative analysis of different

chemical constituents i.e. phenols, alkaloids sterols, terpenes, flavonoids, saponins, glycosides, amino acids, protein, carbohydrates and resin present in various extract of test drug was carried according to the scheme proposed by Bhattacharjee, 1969.

### 3. RESULTS AND DISCUSSION

#### *Organoleptic Characters*

On macroscopic examination of *Khulanjan* (Rhizome of *Alpinia galanga* (L) Willd.) it was found to be brownish in colour with aromatic odour and pungent in taste and hard in appearance as shown in Table 1.

**Table 1: Organoleptic Characters of *Khulanjan* (Rhizome of *Alpinia galanga* (L.) Willd.)**

S.No.	Organoleptic characters	Results
1.	Appearance	Hard
2.	Colour	Brown
3.	Odour	Aromatic
4.	Taste	Pungent

#### *Physicochemical Analysis*

Different physicochemical parameters were analysed and the mean values along with SEM were recorded in Table 2.

#### *Fluorescence Analysis of Successive Extracts of Khulanjan*

Fluorescence analysis of extract of rhizome of *Alpinia galanga* was done for identification and treated with different chemicals and observed which was summarized in Table 3.

#### *Fluorescence Analysis of Powdered Drug of Khulanjan*

Fluorescence analysis of powdered of rhizome of *Alpinia galanga* Willd. was done for identification and treated with different chemicals and observed under different lights and summarized in Table 4.

#### *Qualitative Analysis of Khulanjan (Rhizome of *Alpinia galanga* (L) Willd.*

The Qualitative analysis of different chemical constituents present in the drug was carried out according to the scheme proposed by Bhattacharjee (1969) and mentioned in Table 5.

#### *Thin Layer Chromatography (T.L.C.)*

The thin layer chromatographic patterns of aqueous extract of test drug (*KHULANJAN*) were studied, when the plates were examined under day light, UV light and Iodine chamber, three (03) spots were detected in each treatment. The  $R_f$  values were calculated and the details are mentioned in Table 6.

The safe and stable herbal drug may be marketed if its therapeutic use is well documented in indigenous System of Medicine and viewed by World Health Organization (Jani *et al.*, 2010). The WHO has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards (Ekka *et al.*, 2008).

Standardization of Unani drug *Khulanjan* (Rhizome of *Alpinia galanga* (L) Willd. is considered necessary as it helps in understanding the significance of physical and chemical properties of the test drug being analyzed in term of their observed activities and specially to the determination of the purity and quality of the test drugs and chemical official to it as in Unani Pharmacopoeia.

The present physicochemical and phytochemical studies of the test drug *Khulanjan*

**Table 2: Physicochemical Study of *Khulanjan* (*Alpinia galanga* Willd.)**

S.No.		Parameters	Percentage (w/w)
1.		<b>Ash values</b>	
	A	Total Ash	4.53±0.20
	B	Water soluble ash	2.74±0.066
	C	Acid insoluble ash	1.566±0.03
2.		<b>Solubility</b>	
	A	Alcohol soluble	1.92±1.06
	B	Aqueous soluble	1.91±0.30
3.		<b>Moisture content</b>	1.33±0.03
4.		<b>Loss on drying at 105°C</b>	1.66±0.42
5.		<b>Bulk density (g/ml)</b>	6.1±0.00
6.		<b>pH values</b>	
	A	1% pH value (water solution)	5.77±0.11
	B	10% ph value (water solution)	5.09±0.043
7.		<b>Successive Extractive values</b>	
		Pet. Ether	0.53±0.20
		Di. Ether	0.32±0.12
		Chloroform	0.39±0.12
		Acetone	0.42±0.11
		Alcohol	0.35±0.15
		Aqueous	0.33±0.19

Note: Values are average of three times experiments with Standard Errors

**Table 3: Fluorescence Analysis of *Khulanjan* (Rhizome of *Alpinia glanga* [L] Willd. Extract)**

S.No.	Extract	Day Light	UV Short	UV Long
1.	Petroleum ether	Transparent	Transparent	Transparent
2.	Diethyl ether	Transparent	Transparent	Transparent
3.	Chloroform	Light Brown	Light Green	Black
4.	Acetone	Light Brown	Light Green	Light Black
5.	Alcohol	Brown	Green	Black
6.	Aqueous	Dark Brown	Dark Green	Dark Black

**Table 4: Florescence Analysis of Powder of *Khulanjan*  
(Rhizome of *Alpinia galanga* [L] Willd. with Different Reagents)**

S.No.	Powdered drug + chemical reagents	Day Light	UV Short	UV Long
1.	Powdered drug + Conc. HNO <sub>3</sub>	Light yellow	Green	Dark brown
2.	Powdered drug + Conc. H <sub>2</sub> SO <sub>4</sub>	Dark brown	Light green	Black
3.	Powdered drug + Conc. HCl	Brown	Light green	Blackish brown
4.	Powdered drug + 2% Iodine Solutions	Brown	Green	Black
5.	Powdered drug + Glacial Acetic Acid + HNO <sub>3</sub>	Light brown	Light green	Dark brown
6.	Powdered drug + Glacial Acetic Acid	Brown	Green	Black
7.	Powdered drug + NaOH (10%)	Light brown	Green	Black
8.	Powdered drug + Dil. HNO <sub>3</sub>	Brown	Light brown	Blackish brown
9.	Powdered drug + Dil. H <sub>2</sub> SO <sub>4</sub>	Brown	Light green	Black
10.	Powdered drug + Dil. HCl	Brown	Green	Black
11.	Powdered drug + Benedict's Reagent	Orange	Dark green	Black
12.	Powdered drug + Fehling Reagent	Blue	Light green	Black
13.	Powdered drug + KOH (10%) Methanol	Brown	Green	Black

**Table 5: Major Phytochemical Screening of *Khulanjan*  
(Rhizome of *Alpinia galanga* [L] Willd.)**

S.No.	Chemical constituents	Test reagents	Inference
1.	Alkaloids	Dragendrrf's reagent Mayer's reagent	-ve -ve
2.	Amino acid	Ninhydrin Solutions	-ve
3.	Carbohydrates	Molish test Benedict's test	-ve -ve
4.	Flavonoids	Mg ribbon and dil, HCL	+ve
5.	Glycosides	NaOH	+ve
6.	Phenols/Tannins	Ferric chloride Lead acetate	+ve +ve
7.	Proteins	Xanthoproteic test	-ve
8.	Saponins	Frothing with NaHCO <sup>3</sup>	+ve
9.	Sterols/terpenes	Salkowiski's reaction	+ve
10.	Resins	Acetic anhydride test	-ve
11.	Starch	Iodine test	+ve

**Table 6: TLC of Aqueous Extract of Rhizome of *Khulanjan*  
(Rhizome of *Alpinia glanga* [L] Willd.)**

Treatment	No. of spots	Colour spots	R <sub>f</sub> values
Day light	1	Yellow	0.86
UV Short	1	Dark green	0.86
Iodine Vapour	1	Brown	0.86

n-Butanol: Acetic acid: water (4:1:5)



TLC Profile of Aqueous extract of *Khulanjan* (Rhizome of *Alpinia galanga* [L] Willd.)

(Rhizome of *Alpinia galanga* [L] Willd.) have set the standard quality and purity of the test drug. It is also important because it helps in characterization of constituents or groups of constituents that frequently lead to establish the structural activity relationship and the likely mechanism of action of the drug. Physicochemical constituents present in the drug vary, not only from plant to plant but also among different samples of same species depending upon various atmospheric factors, storage and drying conditions. A little deviation from the normal in terms of quality and quantity of the constituents may alter the effects of the drug.

The above mentioned parameters could be considered as standard tools for the establishment of standardization of *Khulanjan* in Unani Pharmacopoeia. Further pharmacological screening of the test drug may

be carried out to validate the claims of Unani Physicians on scientific basis.

#### 4. CONCLUSION

*Alpine glanga* L. Willd. (Zingiberaceae) is abundly found in India it is a valuable medicinal and cosmetic Unani drug. Although further validation is also required but through the establishment of its standards according WHO guidelines such research findings could be proved that *Kulanjan* is a good raw material drug for pharmaceutical and cosmetic industries to manufacture valuable products.

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## **Knowledge and Perceptions of Parents of Children Below 2 Years of Age Towards Immunization in Twin Cities of Pakistan**

### **A Challenge for Achieving Millennium Development Goals**

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#### **Abstract**

Immunization is one of the most cost-effective public health interventions which have a large impact on public health. The main objective of the study is to assess knowledge and perceptions of parents regarding immunization of children less than two years of age in twin cities of Pakistan. A descriptive cross-sectional study design was used to evaluate the knowledge and perception of parents regarding immunization practices. The sample size was calculated to be 382. Convenient sampling technique was used to select the respondents and semi-structured questionnaire was used in the region. After data collection, data was cleaned, coded and entered in SPSS version. Descriptive statistics of frequency and percentage was calculated. Kruskal-Wallis and Mann-Whitney tests ( $p \leq 0.05$ ) were performed to find out the differences among variables. Parents with Master's Degree and having monthly income between Rs20,000-30,000 had significantly better knowledge. While no significant differences ( $p \leq 0.05$ ) were found between the genders, age, occupation, city and number of children. The results of the present study concluded that knowledge of parents regarding immunization of children less than two years of age was not

adequate but they had positive perceptions toward immunization. Besides the availability of EPI immunization guidelines for parents in the form of immunization card they miss schedules of immunization because they are unaware of the schedule, immunization timing of children, and knowledge about vaccine preventive diseases. There is a dire need to educate the people about its importance and to enhance their knowledge regarding diseases against which they must immunize their children less than two years of age.

#### **Keywords**

Immunization, EPI, Knowledge, Parents, Children, Public health, Pakistan.

#### **1. INTRODUCTION**

Prevention is primarily the most effective public health strategy in controlling the rate of infectious diseases in childhood and reducing infant mortality rate worldwide (Nations, 2000). Immunization is the one of way to prevent children from infectious childhood diseases. Access to such services and up to date immunization coverage are essential for protecting every age group from debilitating and potentially life threatening effects of infectious diseases (WHO, 2015). For this purpose, World

Health Organization initiated the Expanded Program on Immunization in 1974, through which less than 5% children were vaccinated against six commonly existed diseases (polio, pertussis, diphtheria, measles, tetanus and tuberculosis), during its first year. In 1998 nearly 74% of children were immunized against infectious diseases around the world. In 2000-2008 immunization rate was 78% around the world. But still in a few countries, including Afghanistan, Nigeria, India and Pakistan an increased number of cases of polio are being reported and the effort to eradicate polio across the world wide countries is stressfully continued (WHO, 2015; WHO, 2009).

In the case of Pakistan, the Expanded Programme on Immunization (EPI) was launched in 1978 to protect children by immunizing them against infectious diseases occurring in childhood. According to the Pakistan Social and Living Standards Measurement and Pakistan Demographic and Health Survey report 2013, the total coverage for fully immunized children in Pakistan ranges between 56%-88% (UNICEF, 2013). Various efforts have been done to improve immunization coverage in Pakistan but still immunization is not whole heartedly accepted yet in Pakistan. Interventions like public awareness and health education activities are running in the country to improve the immunization coverage as well as adequate budget is provided for such purpose, the EPI coverage rate is still very low (Naeem *et al.*, 2011).

There are various factors identified as barriers to the acceptance of immunization which include lack of awareness, lack of access to such facilities, poor quality of services, unavailability of vaccines, immunization disparities between rural and urban areas, misconceptions and rumors related to vaccine safety, increasing poverty and ignorance, social

cultural backwardness and unavailability of authenticated data on it. A major barrier observed in developing countries is deficiency in knowledge and poor perception of parents regarding immunization (Taylor *et al.*, 2002). Due to this reason, the service is not utilized properly even after efforts of the government to make these services available free of cost (Burns and Zimmerman, 2005). It is the responsibility of the health care professionals and physicians to keep record of all the vaccination visits by parents and educate them and keep them up to date about new vaccination plans and keep themselves up to date about the risks and contraindications of vaccines (Asim *et al.*, 2012). Therefore, present study has been designed to assess knowledge and perceptions of parents regarding immunization of children less than two year of age specially in twin cities of Pakistan.

## 2. MATERIALS AND METHODS

### 2.1. Study Design

A descriptive cross-sectional study design was used to assess knowledge and perceptions of parents regarding immunization of children less than two year of age in twin cities of Pakistan. National bioethical committee is present for this type of research and it states that only institutional head approval is required for this type of study. Informed and verbal consent for participation was also taken from the respondents. Respondents were ensured for the confidentiality of information verbally as well as confidentiality under taking was signed by the principal investigator.

### 2.2. Study Population, Sample Size and Sampling of Respondents

Parents having children less than two years of age residing in the vicinity of Rawalpindi and Islamabad constituted the

population of study. Calculations of sample size were performed using Raosoft® sample size calculator to determine the size of sample that represents the population of parents. Considering the total respondent who visited to the selected OPD departments of the hospitals and immunization centers, having at least one child under two years of age, the sample size was calculated to be 382 to achieve ninety five percent confidence level with five percent margin of error. Convenient sampling technique was used to select the respondents.

### **2.3. Study Tool**

The data was collected by using semi structured questionnaire developed through extensive review of literature and by using standard immunization guidelines for the parents. The Pakistan EPI childhood immunization guidelines were chosen as reference for the development of the questionnaire which comprised of four sections. The first section included information regarding respondent demographic characteristics such as gender, age, occupation, qualification and total number of children. In the second section, perceptions of parents regarding the importance of immunization, religious beliefs, vaccination timing, factors effecting immunization practices, awareness campaign and its activeness in Pakistan were explored. Five point likertscale was used to assess responses where 1 indicated strongly disagree and 5 indicated strongly agree. Section three of the questionnaire included a set of statements in which respondents were asked questions related to knowledge of parents regarding Pakistan EPI childhood immunization schedule of children under two years of age in terms of name of vaccine given at birth, at six weeks of the age of the child, at ten weeks of the age of child, at fourteen weeks of the age

of child, at ninth months of the age of child, during 12-23 months of the age of the child was assessed. Responses were assigned as 1 = yes/correct and 2 = no/incorrect. The composite score range was 14-28 and a lower score indicated better knowledge. In the last section, the satisfaction of parents regarding immunization service in Pakistan was explored. Two focused group discussions were carried out at different time intervals with four different groups of experts including clinicians, specialists, physicians and doctors from academia. Each group comprised 3-4 participants for the development, finalization, face and content validity of the data collection tool. Pilot testing was carried out on 38 respondents (10%) of the total sample size before execution of the final study. A Cronbach alpha value of 0.76 confirmed the reliability and internal consistency of the questionnaire.

### **2.4. Data Collection and Analysis**

Data was collected by the principal investigator trained by the supervisor from June to August 2015. The questionnaire was hand-delivered to the parents by the data collectors. After data collection, data was cleaned, coded and entered in SPSS version 16. Skewness tests were performed and histograms with normal curves were used to check the normal distribution of data. Descriptive statistics of frequency and percentage was calculated. Kruskal-Wallis and Mann-Whitney tests ( $p \leq 0.05$ ) were performed to find out differences among variables.

## **3. RESULTS AND DISCUSSION**

### **3.1. Demographic Characteristics**

Out of 382 respondents, 55.76% ( $n=213$ ) were male; while. 44.24% ( $n=169$ ) were female. Nearly two percent ( $n=7$ ) of respondents were having no formal education.

About 3.6% (n=14) of the respondents had education up to the primary level, 15.44% (n=59) had education up to the secondary and 5.23% (n=20) had completed their higher secondary education. About one third of the respondents, 37.95 % (n=145) were graduates, and 35.86% (n=137) of the respondents were having education up to the Masters level in different fields. Among the respondents, 27.48% (n=105) had a single child, 33.25% (n=127) had two children and 21.46% (n=82) had three kids. The rest of 17.80 % (n=68) respondents were having more than three kids. A detailed description given (Table 1).

### **3.2. Perceptions of Parents Regarding Immunization of Children Under Two Years of Age**

The result highlighted that most of the respondents 96.85% (n =370) were thinking that child immunization is important. Majority of respondents, 91.1% (n=348) were agreed that immunization is safe for children. Considering the religious point of view 25.65% (n=98) of the respondents were agree on the point that immunization is prohibited in religion. A detailed description is given (Table 2).

### **3.3. Knowledge of Parents Regarding Immunization Schedule for Children Under Two Years of Age**

The correct Knowledge of the respondents regarding its schedule for children under two year of the age were vaccines given at birth (n=42, 10.98%), vaccines given in the sixth week of birth (n=40 , 10.46%), vaccines given at the age of tenth weeks (n=31, 8.37%), vaccines given at fourteen weeks of age (n=32 , 8.37%), vaccines given at nine months of age (n=46, 12%), vaccines given during twelve to twenty three months of age (n=53, 13.87%). A detailed description is given (Table 3).

### **3.4. Comparison of Parents'**

#### ***Knowledge Scores about Immunization of Children Under Two Years of Age by Demographic Characteristics***

The composite scores for knowledge were taken into account when assessing the knowledge of prescribers for the whole therapeutic regimen. Significant differences ( $p \leq 0.05$ ) were found among the knowledge scores of parents with different qualifications and monthly incomes. Parents with masters degree and having monthly income between Rs.20,000-30,000 had significantly better knowledge. While no significant differences ( $p \leq 0.05$ ) were found between the genders, age, occupation, city and number of children. A detailed description is given (Table 4).

Parents play a key role in the immunization of their children. Their perception, knowledge and satisfaction towards immunization of their children influence the immunization coverage rate. Despite the important role of parents in such programs, parents do not have sufficient knowledge regarding its schedule, although they receive immunization cards from the hospitals/ immunization centers. The results of the present study showed that most of the respondents were aware of its importance. Similar findings were reported from other studies conducted in UK and Pakistan (Zagminas *et al.*, 2006; Singh *et al.*, 2010).

The result of the present study showed that many respondents agreed on prohibition of immunization in religion and most of them were Muslims. Similarly Muslims were reported the highest in number among non-vaccinated families and their household also had lesser complete coverage of vaccination than the other religious communities by different studies (Singh *et al.*, 2010). Timing and side effects of immunization are considered as important factors towards changing the attitude of

**Table 1: Demographic Characteristics**

<b>Indicators</b>		<b>Rawalpindi n (%)</b>	<b>Islamabad n (%)</b>	<b>Composite n (%)</b>
<b>Gender</b>	Male	71 (18.58)	142 (37.17)	213 (55.76)
	Female	76 (19.89)	93 (24.34)	169 (44.24)
<b>Age (years)</b>	< 20	2 (0.5)	2 (0.5)	4 (1.047)
	21-30	56 (14.65)	110 (28.79)	166 (43.45)
	31-40	73 (19.10)	88 (23.03)	161 (42.14)
	40	13 (3.4)	38 (9.94)	51 (13.35)
<b>Education/ Qualification</b>	No formal education	2 (0.5)	5 (1.3)	7 (1.83)
	Primary Education	8 (2.09)	6 (1.57)	14 (3.66)
	Secondary Education	28 (7.32)	31 (8.11)	59 (15.44)
	Higher Secondary	4 (1.05)	16 (4.18)	20 (5.23)
	Graduation	51 (13.35)	94 (24.60)	145 (37.95)
	Masters	51 (13.35)	86 (22.51)	137 (35.86)
<b>Monthly income in Rupees</b>	< Rs.10,000	5 (1.30)	8 (2.09)	13 (3.40)
	Rs.10,000-20,000	24 (6.28)	36 (9.42)	60 (15.70)
	Rs.20,000-30,000	9 (2.35)	27 (7.06)	36 (9.42)
	Rs.30,000-40,000	17 (4.45)	36 (9.42)	53 (13.87)
	Rs.40,000-50,000	14 (3.66)	30 (7.85)	44 (11.51)
	> Rs 50,000	27 (7.06)	63 (16.49)	90 (23.56)
	No income source	48 (12.56)	38 (9.94)	86 (22.51)
<b>Occupation</b>	Government Job	11 (2.87)	32 (8.37)	43 (11.25)
	Healthcare professionals	10 (2.6)	20 (5.23)	30 (7.85)
	Private employee	24 (6.28)	50 (13.08)	74 (19.37)
	Business	11 (2.8)	34 (8.9)	45 (11.78)
	Engineers	8 (2.09)	7 (1.83)	15 (3.93)
	Academia	20 (5.23)	47 (12.30)	67 (17.54)
	Lower staffs	6 (1.57)	6 (1.57)	12 (3.14)
	Housewives	48 (12.56)	38 (9.94)	86 (22.51)
	Bankers/other professionals	4 (1.04)	4 (1.04)	8 (2.10)
<b>Number of Children</b>	1	40 (10.47)	65 (17.01)	105 (27.48)
	2	46 (12.04)	81 (21.20)	127 (33.25)
	3	29 (7.59)	53 (13.87)	82 (21.46)
	> 3	29 (7.59)	39 (10.200)	68 (17.80)

Values within paranthesis are percentage.

Table 2: Perceptions of Parents Regarding Immunization of Children Under Two Years of Age

S.No.	Indicator	Islamabad			Rawalpindi			Composite		
		Strongly agree/ Agree	Neutral	Strongly disagree/ Disagree	Strongly agree/ Agree	Neutral	Strongly disagree/ Disagree	Strongly agree/ Agree	Neutral	Strongly disagree/ Disagree
1.	Immunization is important	228 (59.68)	10 (2.62)	0	142 (37.17)	2 (0.52)	0	370 (96.85)	12 (3.14)	0
2.	Immunization is beneficial	219 (57.33)	14 (3.66)	5 (1.30)	129 (33.77)	13 (3.40)	2 (0.52)	366 (95.81)	27 (7.06)	7 (1.83)
3.	Immunization is safe for children	226 (59.16)	12 (3.14)	0	140 (36.65)	3 (0.78)	1 (0.26)	348 (91.1)	15 (3.92)	1 (0.26)
4.	Immunization is prohibited in religion?	26 (6.81)	30 (7.85)	182 (47.64)	26 (6.81)	16 (4.18)	102 (26.70)	98 (25.65)	56 (14.65)	284 (74.34)
5.	The timing of vaccination matters	185 (48.42)	33 (8.63)	20 (5.24)	121 (31.67)	14 (3.66)	9 (2.35)	306 (80.09)	47 (12.29)	29 (7.59)

Values are number of individual.

Values within parenthesis represents corresponding percentages.

Table 3: Knowledge of Parents Regarding Immunization Schedule for Children Under Two Years of Age

Immunization schedule (Vaccines)	Islamabad		Rawalpindi		Composite	
	Correct	Incorrect	Correct	Incorrect	Correct	Incorrect
At birth	26 (6.80)	210 (54.97)	16 (4.18)	128 (33.50)	42 (10.98)	338 (88.46)
At 6 weeks	22 (5.75)	216 (56.54)	18 (4.71)	126 (32.98)	40 (10.46)	342 (89.5)
At 10 weeks	21 (5.49)	217 (56.80)	11 (2.88)	133 (34.81)	31 (8.37)	350 (91.6)
At 14 weeks	17 (4.45)	221 (57.85)	15 (3.92)	129 (33.77)	32 (8.37)	350 (91.6)
At 9 months	27 (7.08)	210 (54.97)	19 (4.97)	125 (32.72)	46 (12)	335 (87.67)
During 12-23 months	28 (7.33)	210 (54.97)	25 (6.54)	119 (31.15)	53 (13.87)	329 (86.1)

Values are number of individual.

Values within parenthesis represents corresponding percentages.

**Table 4: Comparison of Parents' Knowledge Scores About Immunization of Children Under Two Years of Age by Demographic Characteristics**

Variable	n	Knowledge Score Median knowledge score (14-28)	Test Statistics	P-value
<b>Gender</b>				
Male	231	195.56	16312.000 <sup>a</sup>	0.201
Female	169	181.77		
<b>City</b>				
Islamabad	144	189.35	16827.000 <sup>a</sup>	0.811
Rawalpindi	238	192.80		
<b>Occupation</b>				
Government employee	53	146	8.713	0.121
Healthcare professionals	49	110		
Private employee	74	140		
Business	95	155.80		
Engineers	25	141.80		
Academia	86	125.95		
<b>Age (years)</b>				
>20	4	160.62	3.913	0.418
20-30	166	196.58		
30-40	161	181.61		
>40	51	202.97		
<b>Qualification</b>				
No formal education	7	304.50	18.784	0.002
Primary	14	240.49		
Secondary	59	215.84		
Higher secondary	20	209.52		
Graduate level	145	181.21		
Masters level	137	177.28		
<b>Monthly income in Rs.</b>				
< Rs.10,000	53	182.77	13.26	0.021
Rs.10,000-20,000	60	175.77		
Rs.20,000-30,000	42	131.97		
Rs.30,000-40,000	73	145.42		
Rs.40,000-50,000	64	134.90		
>Rs.50000	90	138.49		
<b>Number of children</b>				
1	105	182.99	1.363	0.851
2	127	191.29		
3	82	192.85		
>3	68	199.71		

<sup>a</sup>Kruskal Wallis, <sup>b</sup>Mann Whitney

people towards child immunization. The results of the present study showed that nearly half of the participants agreed that short term side effects after vaccination can effect in adoption of it. They also believed that the vaccination timing matters and they would like to follow the schedule. Similar findings were reported from another study from Pakistan which revealed occurrence of side effects like rashes, pain, fever and swelling after immunization and some people also had practiced the reversion of the communicable diseases even though vaccination course was completed (Zagminas *et al.*, 2006).

Affordability and unavailability of the vaccines at the time of vaccination in immigrating centres, long waiting time, non-cooperative attitude of staff and lack of awareness regarding immunization timings as the main factors contributing toward delays in immunization of children were observed. Similar logistic problems, attitude of the healthcare staff during the campaign of immunization, long waiting time were reported as barriers towards delayed immunization in other countries (Siddqi *et al.*, 2010).

As a result of present study inadequate knowledge of parents regarding vaccination schedule of BCG, DPT, Polio and Measles vaccine were also highlighted. More than half of the respondents reported their satisfaction with the services of immunization team and more than seventy percent of the respondents were satisfied with the services of immunization provided in hospitals. Similar inadequate knowledge of parents regarding immunization was also reported from other studies conducted in Pakistan and India (Raithatha *et al.*, 2003). The present study also reported that although majority of the parents were not aware of the proper immunization schedule but they were satisfied with immunization services offered by government in Pakistan. The results were

contradictory to the study conducted in the united kingdom which revealed that majority of the parent were in grave concern about the practice offered by government and vaccine safety (Raithatha *et al.*, 2003).

It is depicted in this study that more than half of the respondents agreed that the immunization services provided to children less than two years of age are free of cost in Pakistan. Furthermore, it was highlighted that mostly the immunization activities are limited to urban areas only, whereas little importance is given to rural areas. Similarly significant difference was reported between urban and rural mothers regarding the importance of vaccination as well as the age of initiation and completion of vaccination schedule in India (Mahalingam *et al.*, 2014).

#### 4. CONCLUSION

The results of this study led us to conclude that knowledge of parents regarding immunization of children under 2 years of age was not adequate but they had positive perceptions toward immunization of children. Besides availability of Expanded Programme on Immunization (EPI) guidelines for parents in the form of immunization cards, they miss schedules of immunization because they are unaware of the schedule, immunization timing of children, and knowledge about vaccine preventive diseases.

There is a dire need to educate people about the importance of immunization and to enhance their knowledge regarding diseases against which they must immunize their children under two years of age. Collaborative working of the government as well as healthcare professionals is of deem importance. Awareness Programs should be launched focusing on people with less or no formal education. Strengthening the service of mobile immunization team by

providing better incentives to them as well as their appropriate training must be conducted. Training sessions for parents regarding immunization should also be arranged. Above all, the vaccines of immunization which are giving to the age of two years old child should have quality control standards regarding their efficacy and safety.

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## Diagnosis of Hypertension on the Basis of Four Temperament Theory of Avicenna in *Al-Qanoon fi al-Tibb*

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### Abstract

According to WHO in Pakistan above about 150,000 people suffer from cardiac diseases, in 2002 and hypertension is top of the list. Developing country like Pakistan faces severe social, economical and psychological burden as shown in Table 1. Human gets affected by its psychology, diet and environment which become source of his health or disease.

The *Canon of Medicine* (Arabic: *Al-Qanun fi al-Tibb*) is an encyclopedia of medicine in five books compiled by Ibn Sina (Avicenna) and completed in 1025. It presents a clear and organized summary of all the medical knowledge of the time. *The Canon of Medicine* is based upon the Four Humors of Hippocratic medicine, but refined in various ways.

There are four major groups of causes which are responsible in hypertension increase in dryness, hotness, moistness and coldness. When we apply the laws of nature to inhibit the cause like nature does we can hypertension and get cure. Nature always treats the dryness (Autumn/Spring) with hotness (summer), hotness with moistness (Rain), moistness with coldness (winter) and coldness with dryness in the form of seasons. There are specific characters of each temperament.

In present study patients (n=40) randomly

selected in Outpatient Department of Shifa-ul-Mulk Memorial Hospital at Hamdard University, Karachi, Pakistan. These patients according to their temperaments classified according to their temperament. The most common temperament in hypertensive patients was atrabilious (Dry) followed by phlegmatic (Cold and Moist).

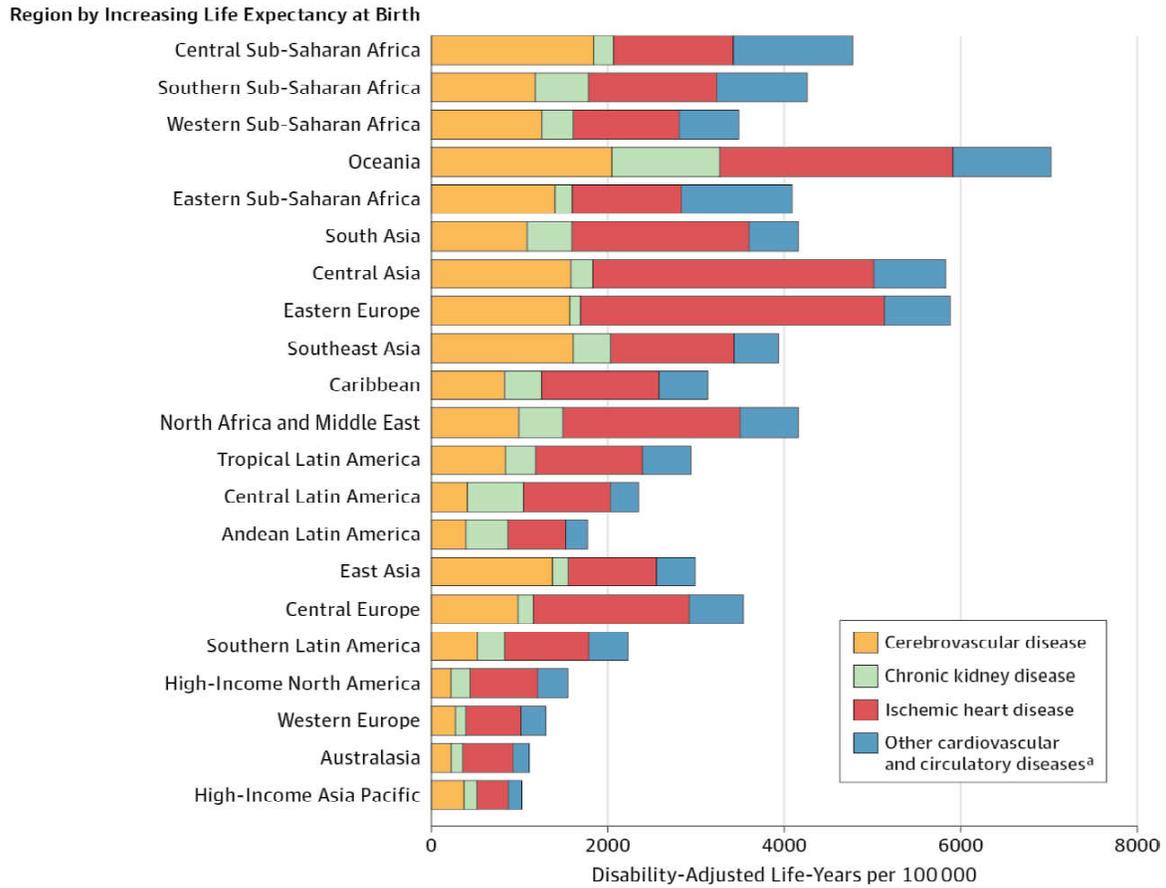
### Keywords

Hypertension, Temperament, Diagnosis, Avicenna.

### 1. INTRODUCTION

An element of such belief is apparent in the chapter of 'al-Qawa' which relates "the manifestations to an interruption of vital life essence to the brain." He combined his own view with that of the Four Humors (*Ihklat*) to establish a new doctrine to explain the mechanisms of various diseases in another work he wrote, *Treatise on Pulse*.

"From mixture of the four humors in different weights, [Allah the most high] created different organs; one with more blood like muscle, one with more black bile (*Saooda*) like bone, one with more phlegm (*Bulgham*) like brain, and one with more yellow bile (*Safra*) like liver and lung. Allah the most high created the souls from the softness of humors; each



**Table-1:** Global Prevalence of Hypertension

(Mohammad *et al.*, 2106)

**Table 1a: Pathological Cause Hypertension**

Systems	Diseases
Renal	Glomerulonephritis, Renal artery stenosis
Endocrinal	Cushing’s syndrome, Pheochromocytoma, Renin secreting adenomas, Thyrotoxicosis
Vascular	Vasculitis
Neurologic	Intracranial mass, Central nervous system trauma
Pregnancy related	Preeclampsia, Eclampsia
Autoimmune	Scleroderma renal crisis
Pharmacologic	Sympathomimetic, Clonidine withdrawal, Beta blocker withdrawal, Cocaine, Amphetamines

**Table 1b: Fate of Hypertension**

<b>Cause</b>	<b>Effect</b>
Decrease blood pressure from left ventricle, i.e. <100 mmhg	Renin and aldosterone system activation
Sodium and water retention	Hypervolemia
Activation of Angiotensin I – convert to Angiotensin II	Vasoconstriction
<b>Raising the Blood Pressure</b>	
Atherosclerosis	Lumen narrowing causing Hypertension
Neurotransmitters (Catecholamine's)	Raise the systolic pressure

soul has its own weight and amalgamation. The generation and nourishment of proper soul takes place in the heart; it resides in the heart and arteries, and is transmitted from the heart to the organs through the arteries. At first, the proper soul enters the master organs such as the brain, liver and reproductive organs; from there it goes to other organs while the nature of the soul is being modified in each of the organ. As long as the soul is in the heart, it is quite warm, with the nature of fire, and the softness of bile is dominant. Then, that part which goes to the brain to keep it vital and functioning, becomes colder and water, and in its composition the serous softness and phlegm vapor dominate. That part, which enters the liver to keep its vitality and functions, becomes softer, warmer and sensibly wet, and in its composition the softness of air and vapor of blood dominate. In general, there are four types of proper spirit one is brutal spirit residing in the heart and it is the origin of all spirits. Another as physicians refer to it is sensual spirit residing in the brain. The third – as physicians refer to it is natural

spirit residing in the liver. The fourth is generative i.e. procreative spirits residing in the gonads. These four spirits go between the soul of absolute purity and the body of absolute impurity” (Amber Haque, 2004).

### **1.1. Prevalence**

According to WHO in only Pakistan 150,000 people suffering from cardiac diseases, in 2002 and hypertension is top of the list. The prevalence of hypertension is increasing day by day in Pakistan since last decade. Hypertension has increased morbidity and mortality. Developing country like Pakistan faces severe social, economic and psychological burden. Human gets affected by its psychology, diet and environment which become source of his health or disease (Table 1).

### **1.2. Fate of Hypertension**

When heart is unable to circulate oxygenated blood properly in the body is called heart failure that affects all systems of body. It

**Table 2: Avicenna's Four Primary Temperaments**

<b>Contents</b>	<b>Dry (Yabis)</b>	<b>Hot (Haar)</b>	<b>Moist (Ratab)</b>	<b>Cold (Barid)</b>
Relation to weather	Autumn	Summer	Rain	Winter
Element (Annasir)	Air	Fire	Soil	Water
Humors ( <i>Ikhlal</i> )	Black bile ( <i>Saooda</i> )	Yellow bile ( <i>Safera</i> )	Blood ( <i>Al-Dam</i> )	Phlegm ( <i>Bulgham</i> )
Organs ( <i>Aaza</i> )	Heart (Muscles)	Liver	Spleen	Brain
Subjective sensations ( <i>Infialat Nasania</i> )	Insomnia, wakefulness, hot intolerance	Bitter taste, excessive thirst, burning at cardiac end of stomach	Mucoid salivation sleepiness	Lack of desire for fluids
Physical signs ( <i>Allamat-e-Zahiria</i> )	Rough skin, acquired habit	High pulse rate, lassitude	Diarrhea, swollen yelids, rough skin, acquired habit	Flaccid joints

(Lutz, 2002)

is a chronic disease but diagnosed incidentally due to variety of heart problems. Heart failure may be of right, left or of both sides. Systolic and diastolic heart failure may also found. In case of congestive heart failure blood retain in its pathway that may be lungs, liver, GIT, and extremities. Common causes of heart failure are coronary artery disease and uncontrolled high blood pressure. Other is congenital heart disease, heart valve abnormality, arrhythmia, heart attack, amyloidosis, hypo or hyperthyroidism, severe anemia, excessive iron, sarcoidosis, emphysema etc as shown in Table 1a.

Left sided failure appear as increase rate of breathing, crackles, pulmonary edema and

sever hypoxemia represent as cyanosis and other are Dyspnea, orthopnea, paroxysmal nocturnal Dyspnea, fatigability, cardiac asthma and dizziness. Right sided appear as pitting peripheral edema, ascities, Hepatomegaly, raised jugger venous pressure (JVP), parasternal heave, impaired liver function and coagulopathy. Biventricular failure develop pleural effusion. Preventions for heart failure are to avoid alcohol, smoking, practice healthy active lifestyle along with proper mental and physical rest (Osler, 2004).

By generating pressure heart forces blood to pump through arteries to our muscles and organs. Blood pressure depends on forceful contraction of heart (left ventricle) and arterial

passage. When blood is forced with increased pressure by heart or narrow passage of arteries leads to hypertension. Arterial system reduces its elasticity along with increasing age and increase tendency of hypertension. Further causes and risk factors are mentioned in Table 1b.

### **1.3. Co-Relation of Pathophysiology with Laws of Nature**

The *Canon* also adopted the ancient theory of four temperaments and extended it to encompass “*emotional aspects, mental capacity, moral attitudes, self-awareness, movements and dreams.*” It summarized Avicenna’s own theory of four temperaments presented in a Table 3 (Mona *et al.*, 2009).

### **1.4. Objectives**

To make the temperamental classification of hypertensive patients, according to Avicenna’s theory.

To evaluate prevalence of the most common temperament in hypertensive patients and risk factors

### **1.5. Study Design**

This is a descriptive observational /cross sectional interview based study.

## **2. MATERIALS AND METHODS (n=40)**

The patients included in the trial were those reporting to the Medical Outpatient Department at Shifa-ul-Mulk Memorial Hospital for Eastern Medicine, Hamdard University (SMMH). They were thoroughly examined for clinical signs and symptoms according to the temperamental history. Their blood pressure and pulse rates were recorded.

### **2.1. Inclusion Criteria**

Patients with all grades of hypertension,

that was either newly detected or resistant to previous drug therapy were informed about the trial and were enrolled after signing a consent form. Patients with satisfactory results for these studies were considered for assessment.

### **2.2. Exclusion Criteria**

Patients with recent history of myocardial ischemia, congestive cardiac failure, left ventricular failure, renal failure, Diabetes Mellitus and cerebrovascular accidents were excluded from the study.

Forty patients (aged 35-70 years) were randomized selected to receive Blood pressure and pulse rate were recorded in the supine position by the same doctor at the same time of the day at weekly intervals, using the same sphygmomanometer. The mean of three readings was noted to confirm the diagnosis.

At first we classify the patient’s complain in temperamental order according to data sheet. Educate the patient about the Temperamental causes of the disease. Give the restriction and diet plan to prevent the causes of disease. Record the level of complain on a data sheet.

## **3. RESULT AND DISCUSSION**

There is highly significant variation have been noted in temperamental assessment. Twenty seven (27) patients were atrabilious in nature (as atherosclerosis is common in atrabilious temperament), while thirteen patients were phlegmatic (Cold and moist) as hypervolemia is major cause of hypertension.

## **4. CONCLUSION**

The conclusion is variety of temperament is noted in hypertensive patients This temperamental therapeutic system should be harnessed and practiced. This study prove the significance and importance of Avicenna’s theory of temperamental classification as



**Table 3: Evaluation of Treatment of Hypertention Through Four Temperamental Theory of *Al-Qanoon***

Name	Age	Cell	NIC
<b>Temperamental Group</b>			
<b>Clinical Complain</b>	<b>Dry</b>	<b>Hot</b>	<b>Moist</b>
Heart Burn	4	2	2
N/V	1	1	4
Flatulence	4	1	2
Dry Mouth	4	1	1
Sore Throat	4	1	1
Constipation	4	1	2
Thirst	4	1	1
Br. Palm N Sole	4	1	1
Br. Micturation	4	1	1
Hot Flushes	4	1	1
Bittertaste	3	1	1
Tylishm	1	1	4
Excessive Sleep	1	2	4
FATIGUE	4	2	3
INSOMNIA	4	1	1
Headache	4	2	3 frontal
Occipital heaviness	4	1	1
Angers	4	3	1
Urine frequency	2	2	4
Cold intolerance	2	1	4
Hot intolerance	4	2	1
Palpitation	4	2	3
Chest Heayness	1	1	4
Exertional Dyspnea	4	1	1
Nocturnal Dyspnea	2	1	4
<b>Others</b>	Itching 4		Post nasal dripping

1= Absent; 2 = Less common and Mild; 3 = Common or Moderate; 4 = Most common and Severe

discussed the Laws of Nature and Laws of nature are Universal Truth. There is need to study and understand these laws to apply in our Clinical practice. We should gathered the data and compile it scientifically. Most of the diseases became a public diseases like Diabetes, Hypertension, Asthma, Arthritis and Hepatitis etc. High diagnostic Skill and specific modifying drugs giving early symptomatic relief but no cure but by applying the laws of nature we can get cure very easily and cost effectively.

#### **Author's Contribution and Declaration**

Asif Iqbal: Conducted and design the study. Abdul Bari: Data collection.

#### **Conflict of Interest**

The authors declare that there is no conflict of interest.

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## Sociodemographic Study of Ocular Morbidity in School Children of Aligarh

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### Abstract

Ocular Morbidities with its economic and social consequences represents an important Public Health Problem in various part of the world. This was a cross sectional study. The present study was planned to know the prevalence of ocular morbidity in school Children and their correlation with socio demographic profile if any. School children of age 5-12 years of selected AMU schools in the field practice area were the study population. The sample size calculated was 1050 but 1129 were covered in this study. In present study prevalence of ocular morbidity in students were found 32.3%. The prevalence was more among female candidates. So to conclude present study highlights important problem of ocular morbidity in school children. Refractory error was commonest form of ocular morbidity amongst them. Significant association was found between ocular morbidity and socio economic status and diet.

### Keywords

Ocular morbidity, school children, religion, education, socio economic status.

### 1. INTRODUCTION

Blindness is one of the significant social problems in India (Desai *et al.*, 1989). The

pattern of ocular diseases varies in different part of the world and is influenced by racial, geographic, socioeconomic and cultural factors (Sharma *et al.*, 2009). Next to Egypt, India has the highest incidence of blindness in the world, particularly in younger age group and for prevention of blindness school is the best centre for implementing comprehensive eye health care (Sharma *et al.*, 1984). The rate of infection and complication are influenced by a number of socio-economic and socio cultural factors and reason (Sethi and Kathra, 2000). Children do not complain of defective vision and may not even be aware of their problem. They adjust to the poor eyesight by sitting near the blackboard, holding the books closer to their eyes, squeezing the eyes and even avoiding work requiring visual concentration. This warrants early detection and treatment to prevent permanent disability. School-going children of age group (6-16 years) represent 25% of the population in the developing countries. They offer significantly representative material for these studies as they fall best in the preventable blindness age group, are a controlled population i.e. they belong to a certain age group and are easily accessible and schools are the best forum for imparting health education to the children. Schools are also one of the best centers for implementing the comprehensive eye healthcare programmes effectively (Gupta *et al.*, 2009).

**2. METHODS**

School surveys were conducted in various Public Primary classes schools (five) of Aligarh Muslim University, Aligarh. Valid consent (informed consent) was taken from the parents of students in writing after explaining the procedure to the subject prior to inclusion in the study. Ocular Examination of school children between age group 8 to 12 years was performed by Dr. Naeem after taking one year training with one private ophthalmologist clinic. In this study, total of 1129 children were examined.

The data from the Performa were converted into tabulated form and recorded in Microsoft Excel 2007 sheets. Finally unpaired student 't' tests and ANOVA test were applied by using a Computerized Software G pad for the purpose of statistical analysis of the data.

***Informed Consent***

Informed consent from the parent of students obtained with the permission from school head through their class teachers. 20

**3. RESULTS AND DISCUSSION**

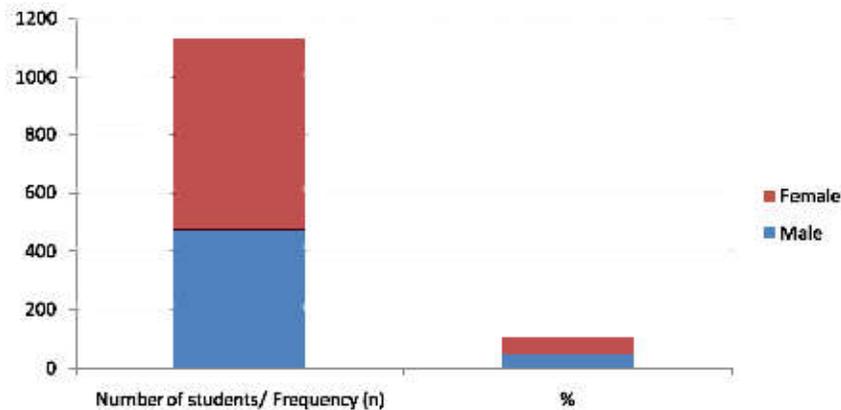
**Table 1: Age Wise Distribution of Population**

Age (Years)	Frequency	Percent occurrence
8	189	16.8
9	271	24.0
10	340	30.1
11	173	15.3
12	156	13.8
<b>Total</b>	<b>1129</b>	<b>100.0</b>

As shown in Table 1 maximum number of children were from 10 years age (30.1 %), and least number of children found in 12 years age (13.8 %). While 9 years' ages were 24 %, 16.8 % in 8 years age and 15.3 % were from 11 years age group.

The female students among the studied group were more about 58.1% as compare to male students that were 41.9% and the ratio was 1:1.39 (M:F).

Most of the candidates were Muslim



**Fig. 1:** Gender wise distribution of students

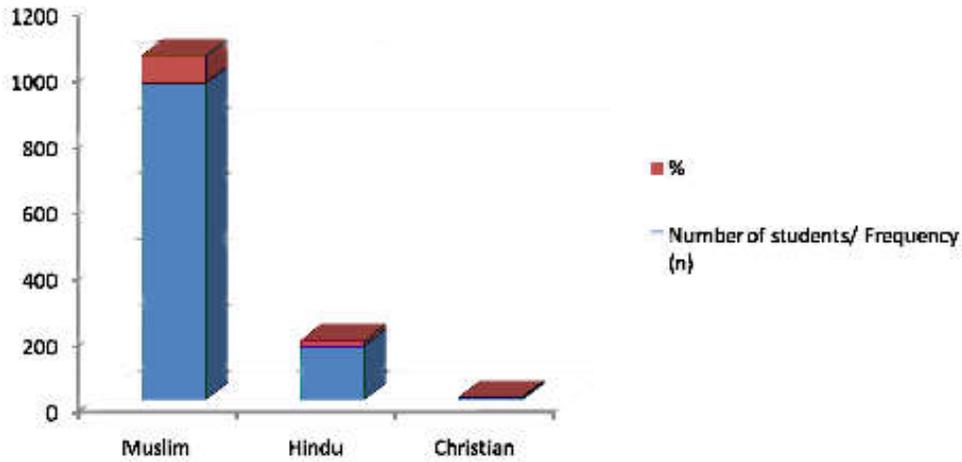


Fig. 2: Distribution of students according to Religion

(85.5%). Subjects in the category of Hindu were 14.5% and least number of students were from Christian category and were about 0.5%.

**Table 2: Distribution of Studied Group on the Basis of Father’s Education**

Education	Frequency	%
Illiterate	187	16.6
Just literate	230	20.4
High school	302	26.7
Intermediate	87	7.7
Graduate and above	323	28.6
<b>Total</b>	<b>1129</b>	<b>100.0</b>

It is observed from table 2 that majority of fathers were Graduate and above (Post graduate) i.e., 28.6%. Percentage of Education of father qualified as High school and Intermediate were 26.7% and 7.7% respectively. Just literate (up to class V) educated category was 20.4%. In Illiterate category they were about 16.6%.

**Table 3: Distribution of Subjects on the Basis of Mother’s Education**

Education	Frequency	%
Illiterate	377	33.4
Just literate	282	25.0
High school	185	16.4
Inter mediate	102	9.0
Graduate and above	183	16.2
<b>Total</b>	<b>1129</b>	<b>100.0</b>

Table 3 represent that majority of mothers were illiterate and this group comprises of 33.4%. One quarter (25%) were in the literate category. Intermediate were least in number and they were about only 9%. High School passed was 16.4% and Graduate and above were 16.2%.

**Table 4: Distribution of Students According to Socio Economic Status**

Socio Economic Status	Frequency	%
I (Upper class)	73	6.5
II (Upper middle class)	130	11.5
III (Middle class)	178	15.7
IV (Lower middle class)	425	37.7
V (Lower class)	323	28.6
<b>Total</b>	<b>1129</b>	<b>100.0</b>

Illustrate ratio of which the subjects were categorized on the basis of the criteria of Socio Economic Class set by Gururaj and Prasad (2014) and it was found that students belonging to lower SES classes (SES class IV and V) were more (37.7% and 28.6% respectively) than upper classes. About 6.5% were from upper SES class I and 11.5% were from SES class II, while in middle SES classes III, there were only 15.7%.

**Table 5: Diet Wise Distribution of Studied Group**

Types of Diet	Frequency	%
Vegetarian	189	16.7
Mixed	940	83.3
<b>Total</b>	<b>1129</b>	<b>100.0</b>

Majority of the students (83.3%) were consuming mixed type of diet where as vegetarian were least in percentage (16.7%) as shown from Table 5.

**Table 6: Prevalence and Pattern of Ocular Morbidities Among the Students**

Number of ocular morbidity	Number of affected children	% of total ocular morbidity
Refractive error	310	84.9
Squint	12	3.4
Conjunctivitis	29	7.9
Stye, Blepharitis	14	3.8
<b>Total</b>	<b>365</b>	<b>100.0</b>

The overall ocular morbidity was found 32.3% (365 out of 1129 subjects).

The detail of all ocular morbidity among the affected group is as follows:

- 1) Refractive error was the highest i.e., 84.9% among all affected group (365 students)
- 2) Squint cases were 3.4%
- 3) Percentage of red eye (conjunctivitis) was 7.9%.

Eye lid problems like Stye and Blepharitis were found 3.8% students.

In Fig. 3, the percentage of ocular morbidity was highest in 10 years age (28.2%), and next in 9 years of age group (23.3%). No significant association of ocular morbidity was found as the age advanced. Ocular morbidity in 8 years of age was 18.4%, in 11 years this was 15.3% and in 12 years it was 14.8%.

In Fig. 4, the percentage of overall ocular morbidity in female students was 62.7%, while in male students the percentage of ocular morbidity was 37.3% lower than female students. The percentage of refractive error and Conjunctivitis in female students was 30.5% and 2.6% respectively while in male students were 23.3 % and 2.5 % respectively which is lower than female candidates.

### Socio Demographic Factors Corelation with Ocular Morbidity

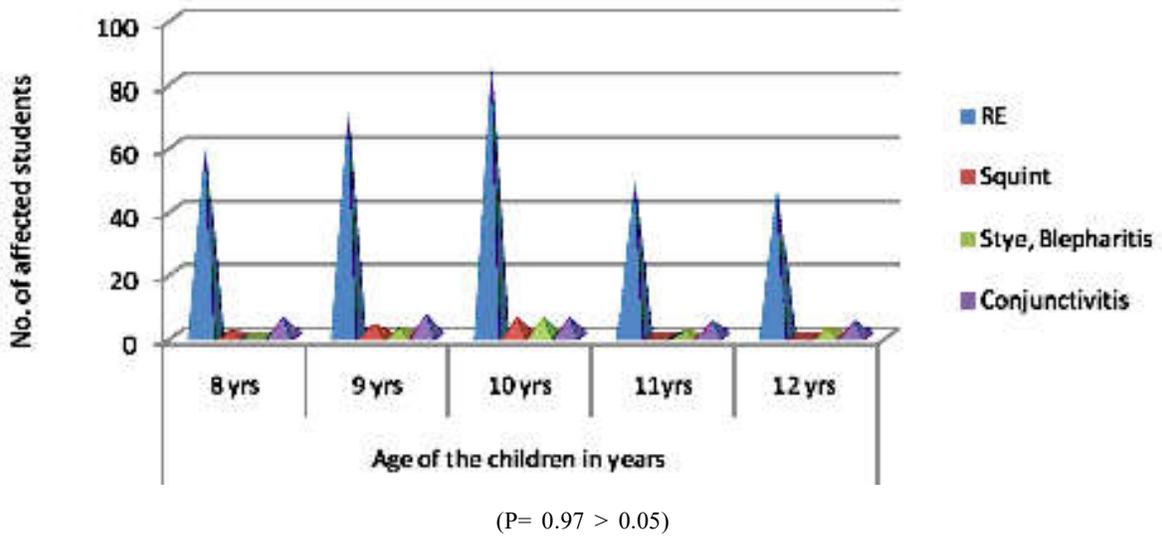


Fig. 3: Relation between age of the children and Ocular Morbidity

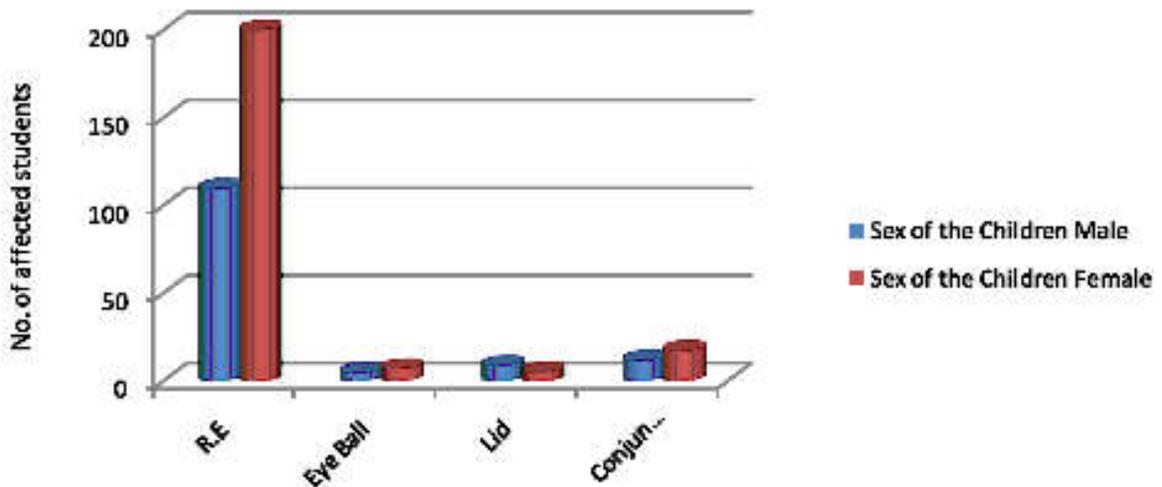


Fig. 4: Relation between sex of the children and Ocular Morbidity

**Table 7: Shows Relation Between Religion and Ocular Morbidity**

Religion	R.E.	Eye ball	Lid	Conjunctiva	Total
Muslim (959)	269 (73.7%)	12 (3.3%)	13 (3.6%)	22 (6.0%)	316 (86.6%)
Hindu (164)	40 (10.9%)	0 (0.0%)	1 (0.3%)	7 (1.9%)	48 (13.1%)
Christian (6)	1 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)
<b>Others</b>	0	0	0	0	0

(P = 0.31&gt;0.05)

**Table 8: Relation Between Father's Education and Ocular Morbidity**

Ocular morbidity	Father's Education				
	Illiterate (187)	Just literate (230)	High school (302)	Intermediate (87)	Graduate and above (323)
Refractive Error	54	80	72	26	78
Squint	4	5	1	0	2
Stye/Blepharitis	3	4	4	0	3
Conjunctivitis	6	9	7	2	5
<b>Total</b>	<b>67</b> <b>(18.4%)</b>	<b>98</b> <b>(26.8%)</b>	<b>84</b> <b>(23.0%)</b>	<b>28</b> <b>(7.7%)</b>	<b>88</b> <b>(24.1%)</b>

(P = 0.93&gt;0.05)

**Table 9: Relation Between Mother's Education and Ocular Morbidity**

Ocular morbidity	Mother's Education				
	Illiterate (377)	Just literate (282)	High school (185)	Intermediate (102)	Graduate and above (183)
Refractive Error	117	79	50	27	37
Squint	5	5	1	0	1
Stye/Blepharitis	3	3	3	3	2
Conjunctivitis	10	5	7	1	6
<b>Total</b>	<b>135</b> <b>(37.0 %)</b>	<b>92</b> <b>(25.2 %)</b>	<b>61</b> <b>(16.7 %)</b>	<b>31</b> <b>(8.5 %)</b>	<b>46</b> <b>(12.6 %)</b>

(P = 0.81&gt;0.05)

As shown from Table 7 ocular morbidity was found highest among Muslims in studied group i.e., 86.6%. Least ocular morbidity was found in Christian's students and it was 0.3%, while in Hindu category the ocular morbidity was 13.1%.

Ocular morbidity was found highest (26.8%) among the children whose father come in the category of just literate education while least (7.7%) ocular morbidity was found in intermediate education (Table 8). Ocular morbidity in the children of illiterate father was 18.4%, high school passed was 23.0% and those

students whose father were graduated and above the ocular morbidity was 24.1%. But the result was non significant.

As depicted in Table 9 ocular morbidity was highest (37.0%) among the children whose mother were illiterate while least (8.5%) was found evident in intermediate education. Among the children just in literate mother it was found about 25.2%, high school passed was 16.7% and those students whose mother were graduated and above the ocular morbidity was 12.6%. However, the result was non significant.

**Table 10: Relationships Between Socio Economic Status and Ocular Morbidity**

Ocular morbidity	Socio Economik Status				
	I (73)	II (130)	III (178)	IV (425)	V (323)
RE	17	31	57	105	100
	4.6%	8.4%	15.6%	28.7%	27.4%
Squint	0	1	2	6	3
	0.0%	0.3%	0.5%	1.6%	0.8%
Stye, Blepharitis	3	1	1	5	4
	0.8%	0.3%	0.3%	1.4%	1.1%
Conjunctivitis	1	4	4	11	9
	0.3%	1.1%	1.1%	3.0%	2.5%
<b>Total</b>	<b>21</b>	<b>37</b>	<b>64</b>	<b>127</b>	<b>116</b>
	<b>5.8%</b>	<b>10.1%</b>	<b>17.5%</b>	<b>34.8%</b>	<b>31.8%</b>

The Ocular morbidity in the students belonging to SES I and SES II was almost equal in percentage i.e., 28.8% and 28.5% respectively while it in the students of Socio

Economic Status III and V was also equal i.e., 35.9% in each group as shown in Table 10.

Ocular morbidity in studied group of Socio Economic Status IV was found 29.9%.

**Table 11: Relation Between Diet pattern and Ocular Morbidity**

Ocular Morbidity	Diet Pattern	
	Vegetarian (189)	Mixed (940)
RE	51	259
	13.9%	70.9%
Squint	1	11
	0.3%	3.0%
Stye, Blepharitis	2	12
	0.5%	3.3%
Conjunctivitis	9	20
	2.5%	5.5%
<b>Total</b>	<b>63</b>	<b>302</b>
	<b>17.3%</b>	<b>82.7%</b>

It was found from Table 11 that ocular morbidity was approximately equal in the students of vegetarian group and those who were taking mixed type of diet. In vegetarian ocular morbidity was 17.3% while in group who were taking mixed type of diet it was 82.7%.

Vision is essentially very important to people of all age group, but it is more important in children and adolescents as it plays a key role in their mental, physical and psychological development. Most of the childhood blindness is easily treatable and preventable; however, if it is not detected and prevented on time it may lead to permanent disability.

The study undertaken was a cross-sectional study conducted on 1129 of 8-12 year of age group school going children at AMU Aligarh.

#### ***Age Distribution and Ocular Morbidity***

The mean age of children in the study was  $8.8 \pm 1.3$  years. Out of 1129 students from different school of AMU, Aligarh, there were 16.8 % students in the age group of 8 years, 24% children in the age group of 9 years, 30.1% participants in the age group of 10 years, 15.3% in the age group of 11 years and 13.8% students in the age group of 12 years. Majority of the students were from 10 years of age and least number of the students was from 12 years of age.

The pattern of ocular morbidity with regards to the age of the children of ocular morbidity was highest in 10 years age (28.2%), and least in 12 years of age group (14.8%) as depicted in Table 6. No significant association of ocular morbidity was found as the age advances. ( $p=0.97 > 0.05$ ).

Prasanna *et al.*, (2012) in their study found an increase in the prevalence of ocular morbidity with age, which was similar to that in the study of Niti *et al.*; Harpal (2011) in his study observed the most common age group affected being 8-12 years, followed by 13-16 years.

#### ***Sex wise Distribution of Students and Ocular Morbidity***

Out of total 1129 students from schools, 473 were male students and 656 were female students, with a sex ratio 1:1.39 (M:F).

In this cross sectional study, among 365 (out of 1129) affected children, 139 were males (37.16%) and 235 were females (62.83%). The increase prevalence among female students may be due to the higher number of female participants (656) than male participants (473). While, when prevalence was compared in terms of respective population of females and males, the overall prevalence was also more in females as compare to males i.e., 35.8% and 29.4% respectively.

Similarly, ocular morbidity in terms of refractive error and Conjunctivitis was also found to be more in female students (30.5% and 2.6% respectively) than male students (23.3% and 2.5% respectively).

There are studies which also showed a higher prevalence of ocular morbidity (OM) among female children. Mohammad *et al.*, (2014), in their study found ocular morbidity in the form of refractive error to be more prevalent in girls compared to boys.

But in the study done by Harpal (2011) in Bhopal, Madhya Pradesh, he found that out of the total 3016 affected students' ocular morbidity was more prevalent in the male children (7.7%) i.e. 1617 compared to female children (6.72%) i.e. 1399. Perhaps it may be due to more exposure and movement of male children.

Rajesh *et al.*, (2007), in their study found

no sex wise significant association between ocular morbidity in children.

#### ***Relation Between Religion and Ocular Morbidity***

From Table 7, it is shown that ocular morbidity was found highest among Muslims in studied group i.e., 86.6%. Least ocular morbidity was found in Christian's students and it was 0.3%, while in Hindu category the ocular morbidity was 13.1%. But the result statistically is non significant (P value is  $0.31 > 0.05$ ). It might be due to very small number of subjects from other religions like Hindu and mostly Christian.

Similar results were found in study done by Pankahj *et al.*, (2013).

In the study done by Rajesh *et al.*, (2007) in Delhi it was found that Compared to Hindu subjects, prevalence of ocular morbidity was almost two times higher in subjects of other religions (Muslim). But this difference might be due to very small number of subjects from other religions.

An earlier study also reported higher prevalence of ocular morbidity among Muslims (Goel, 1994).

#### ***Relation Between Father's Education and Ocular Morbidity***

It is shown from Table 8 ocular morbidity was found highest (26.8%) among the children whose father come in the category of just literate education while least (7.7%) was found in intermediate education. The children of illiterate father it was 18.4%, high school passed was 23.0% and those students whose father were graduated and above the OM was 24.1%. But the result was non significant statistically. (P =  $0.93 > 0.05$ ). It might due to lack of knowledge regarding ocular health.

Similarly in the study done by Rajesh *et al.*, (2007) in Delhi there was no significant

association found between ocular morbidity and literacy status of the subjects' parents.

But the Studies conducted in Ahmadabad and Maharashtra (Deshpande and Malathi, 2011; Prajapati *et al.*, 2010) noted that with increase in education level of parent's, prevalence of ocular diseases in children decreases. Dandona *et al.*, (2002) found a significant association between father's education and prevalence of refractive error Suggesting that education plays a very important role in increasing awareness regarding the ocular diseases.

#### ***Relation Between Mother's Education and Ocular Morbidity***

It was found highest (37.0 %) among the children of illiterate mothers while least (8.5 %) ocular morbidity was found in intermediate education. Whereas in the children of just literate mother was 25.2 %, high school passed was 16.7 % and those students whose mother were graduated and above the ocular morbidity was 12.6 %. But the result was non significant. ( $P = 0.81 > 0.05$ ) as mentioned in Table 9

Similar results were found in the study done by Rajesh *et al.*, (2007) in Delhi.

But in the the study done by Deshpande and Malathi (2011) in Maharashtra and Prajapati *et al.*, (2010) in Gujrat, found significant association between mothers literacy and ocular morbidity. It might be because of illiteracy there may be ignorance, poverty and leading to various disease conditions including ocular morbidity.

#### ***Relationships between Socio Economic Status and Ocular Morbidity***

In the current study, significant difference was observed between lower social class IV ( $P = 0.01 < 0.05$ ) and SES class V ( $p$  value  $0.01 < 0.05$ ) in regard with ocular morbidity. No significant difference was found among upper

socioeconomic class IV Class V and middle social class III.

There are studies which also showed same pattern of ocular morbidity among different socioeconomic groups. Similar results were reported by Deshpande and Malathi (2011) in North Maharashtra.

Similarly, Parajapati *et al.*, (2010) in their study found significant difference between lower social class and ocular morbidity. This may be due to better economic stability of medium and higher classes which ultimately leads to improved nutrition and hygiene of the adolescents. A highly significant association was reported between Socio-economic status and ocular morbidity (Parajapati *et al.*, 2010).

As socio economic status affects nutritional status, health care services availability, an association between socioeconomic status and ocular morbidity may be established.

#### ***Relation Between Diet Pattern and Ocular Morbidity***

The overall pattern of ocular morbidity in vegetarian group was 17.3 %, while in mixed type of diet group, the ocular morbidity was 82.7 %. Statistically result was found significant ( $p$  value  $0.02 < 0.05$ ) as shown in Table 11.

Similar finding were reported by Viswa *et al.*, (2011) in their study from urban areas of Visakhapatnam, India that 87.9% of the ocular morbidity was found in students having mixed diet and ocular morbidity 12.1% was found in vegetarian (Viswa *et al.*, 2011).

From the study of Ankita *et al.*, (2013) it was seen that the children with vegetarian diet showed the prevalence of 82.41% as compared to children who had mixed diet (17.59%) and the difference was statistically significant ( $p < 0.05$ ).

Dietary deficiency of vitamin A leads to development of xerophthalmia in those children

taking insufficient green leafy vegetables coloured fruits and food of both origins as reported by other studies (Ahmed *et al.*, 2006).

### 3. CONCLUSION

In our study an overall prevalence of ocular morbidities was 32.3% (n=365 out of 1129) was seen in our study.

Refractive error was most common of it with a prevalence of 84.9%, followed by conjunctivitis 7.9%, Stye and Blepharitis 3.8% and Squint 3.4%.

No significant association of ocular morbidity was found as the age advances ( $P = 0.97 > 0.05$ ).

The ocular morbidity was more in females 62.8% compared to males i.e., 37.2%. While, when prevalence was compared in terms of respective population of females and males, the overall prevalence was also more in females as compare to males i.e., 35.8% and 29.4% respectively.

No significant association was found in religion and ocular morbidity ( $P$  value is  $0.31 > 0.05$ ) probably due to very small number of subjects from other religions.

No significant relation was found between parents literacy status and ocular morbidity. This might be due lack of knowledge regarding ocular health.

Significant difference was observed between ocular morbidity and lower social class IV and SES class V while no significant difference was found between ocular morbidity and upper socioeconomic class IV and V and middle social class III. This may be due to high standard of living with good personal hygiene, balanced, nutritious and healthy diet intake, better facilities for study etc.

There is significant difference between vegetarian and ocular morbidity. The overall pattern of ocular morbidity in vegetarian group

of students was found 17.3 %. While mixed type of diet group, the ocular morbidity was 82.7 %.

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