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Hakim Mohammed Said

Did you have life easy?
No. But you made it your mission
To make it a little easier for others.

Orphaned at two,
You opened your heart to all children,
Giving them respect, education, care,
Making sure their little voices didn't go unheard.

As an adult, you had to start life twice:
Once in your birthplace, once in your land of choice.
You wore holes in your shoes,
But your spirit of service stayed intact.

Two rented rooms, pieces of rented furniture:
You started small, but your heart was big.
Your God-given gifts, you used in God's name,
And He blessed you hundredfold.

Many men's pride is swelled with success;
Yours increased your humility.
So you turned your institution over to Allah –
Its profits to profit the poor.

All men have dreams – a thing apart from life.
You said Why not? and worked to make them real.
Free clinics and dispensaries on wheels,
Hospitals, schools, colleges, even a university!

You married modern science to ancient medicine,
And bred respect for *hikmat* in doubting hearts.
Journals, books, societies, and conferences,
Assemblies, think-tank – all bear witness to your drive.

You spoke the truth, unwavering, unafraid.
One morning a gun spoke, to silence you forever.
O, vain attempt! Your words, your works live on.
The gun but gave you a martyr's immortality!

– A tribute from Khaula Yasmin Qureshi.

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INFORMATION FOR CONTRIBUTORS

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Title Page Should Contain: Title of the manuscript, Short title, Authors names with superscripts representing their affiliation clearly marked *corresponding author with Telephone, Fax and E-mail address.

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The Text For Articles Should Contain: Main headings in caps and bold as:
1. INTRODUCTION, **2. MATERIALS AND METHODS** (2.1 sub-headings [in ***bold italics***] describing animals, chemicals, preparation of extract, experiments and statistical analysis), **3. RESULTS AND DISCUSSION, 4. CONCLUSION** and **ACKNOWLEDGEMENTS** (if needed) before References, **5. REFERENCES.**.
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(Books) 1. Gaill, W., Jon, A.W. (1995). *Manual of Clinical Microbiology*. ASM Press, Washington, DC. 6th Edn., pp. 1327-1332.
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Antimicrobial Efficacy of *Hibiscus schizopetalus* (Mast) Hook

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Abstract

Plants are an important source of antimicrobial products, most of them are efficacious against diverse organisms including fungi, yeasts and bacteria, insects and nematodes. The present study was aimed at measuring the antimicrobial effects of methanolic extract of flower and leaves of *Hibiscus schizopetalus* (Mast) Hook. Antibacterial and antifungal activities of *H. schizopetalus* extracts (HFE and HLE) were evaluated by the agar well diffusion and agar dilution methods, respectively . The flower extract of *H. schizopetalus* (HFE) revealed antibacterial activity against *Streptococcus pyogenes, S. faecalis, Enterobacter aerogenes* and *Proteus vulgaris* with zone of inhibition of 5 mm, 9 mm, 3 mm, 6 mm, respectively. The extracts (HFE and HLE) showed prominent activity against human pathogens *Aspergillus flavus, A. niger* and specially for *Candida albicans* with zone of inhibition of 5 mm, 4 mm, 10 mm, 6 mm, 3 mm and 14 mm, respectively. The microbial inhibitory effects of the extracts of flower and leaves of *H. schizopetalus* are favorable to introduce this plant as potential candidate for new drug development regarding treatment of diseases caused by these pathogens.

Keywords

Antimicrobial, *Hibiscus schizopetalus* (Mast) Hook, methanolic extract, agar well diffusion and dilution methods.

1. INTRODUCTION

Resistance to antibiotics is one of the major problem facing public health world-wide (Byarugaba, 2004; Okeke *et al*., 2005). It is a natural consequence of the adaption of infectious pathogens to antimicrobials used in several areas, including medicine, food animals, crop production and disinfectants in farms, hospital and households (Bloomfield, 2002; McEwen and Fedorka-Cray; 2002; Vidaver, 2002; Wise and Soulsby 2002). Bacteria have developed resistance to all known antibiotics and, as so, the economic burden associated with these multidrug-resistant bacteria is high. In order to find novel antimicrobial agents with new modes of action, plants have been explored as sources for the identification of new and effective antimicrobials. Plants are an important source of antimicrobial products, most of them with efficacy against diverse organisms including bacteria, fungi and yeasts, insects and nematodes (Abreu *et al*., 2013). Phytochemicals present in the plant are able to inhibit peptidoglycan synthesis, damage microbial membrane

structures, modify bacterial membrane surface hydrophobicity and also modulate quorum-sensing (QS) (Rasooli *et al*., 2008). Natural extracts were in frequent use both in developing functional foods and treating illnesses.

The present study was aimed at measuring the antimicrobial effects of methanolic extract of flower and leaves of *H. schizopetalus* (Mast) Hook. The plant belongs to the family Malvaceae. This plant is a common ornamental shrub cultivated in Pakistan. The plant also found in various region of the world (Yasin, 1979). *H. schizopetalus* is the allied specie of *H. rosa – sinensis* L. sharing the same genera *Hibiscus* but the difference in both plant is the direction of flower (Fig. 1). Leaves of plant are alternate, ovate to lanceolate, often with a toothed or lobed margin; resemble those of *H. rosa – sinensis* leaves. Various parts of the plant are indicated to be used in cold, cough and fever (Anonymous, 2010; Rahmatullah *et al.*, 2010; Jalan, 2002). According to the current literature methanolic extract of flower and leaves showed significant analgesic, antipyretic and antidiabetic potentials (Zahid *et al*., 2012; Zahid *et al*., 2014).

2. MATERIALS AND METHODS

2.1. *Plant Material*

H. schizopetalus (Mast) Hook leaves and flower were collected from the premises of University of Karachi, Pakistan. The plant materials were identified and authenticated by Prof. Dr. Suriya Khatoon, Department of Botany, University of Karachi, Pakistan. A voucher specimen No. 082 was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.

2.2. *Preparation of Extract*

Flower and leaves of *H. schizopetalus* (1 kg) were air dried in shade separately and pulverized coarsely. The plant material was soaked in methanol at room temperature for 7 to 10 days followed by filteration through

(A) **(B)**

Fig. 1: *Hibiscus schizopetalus* (Mast) Hook (A) and *Hibiscus rosa – sinenesis* (B)

Whatman filter paper No. 1. The residue was evaporated under vacuum using rotary evaporator (Buchi Rotavapor R-200) at 40±2°C. The dried extracts (HFE and HLE) 73.8 g and 50.1 g, respectively were stored at 4°C until further analysis.

2.3. *Test Organisms*

Micro-organisms (Gram positive and negative bacteria and various fungal strains) were obtained from the Laboratory of Microbiology, Department of Microbiology, University of Karachi, Pakistan.

a) Gram positive bacteria: *Staphylococcus aureus, S. aureus* AB188, *S. capitis, S. epidermis, Streptococcus pyogenes, S. faecalis, Bacillus subtilis, B. vulgates, Micrococcus luteus, Corynebacterium xerosis*

b) Gram negative bacteria: *Salmonella typhimurium, Salmonella paratyphi A, Salmonella paratyphi B, Bdellovibrio bacteriovorus* HD 100, *Proteus mirabilis, P. vulgaris, Serratia marcescens, Escherichia coli, Enterobacter aerogenes* and *Pseudomonas aerogenosa.*

Three different types of fungal pathogens were used for assessment of antifungal activity:

a) Human pathogens: *Aspergillus flavus, A. niger, Candida albicans, Micosporum canis, Trichophyton tonsurans.*

b) Animal pathogens: *Microsporum gypseum, Trichophyton mentagrophyte*.

c) Plant pathogens: *Fusarium solani, Rhizopus stolonifer, Saccharomyces cerevisiae* and *Pencilllium chrysogenum* ATCC.

2.4. *Testing for Antibacterial Activity*

Antibacterial activity of *H. schizopetalus* extracts (HFE and HLE) was evaluated by the agar well diffusion method (Shareef *et al.*, 2012). The plates were swabbed (sterile cotton swabs) with respective bacterial organisms. Wells were made in each of these plates using sterile cork borer. Stock solution of extracts (HFE and HLE) was prepared at a concentration of 1 mg/ml in DMSO. About 100 µl concentrations of extracts were added with sterile syringe into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 18-24 h. The results were recorded by measuring zone of inhibition using ampicillin as standard drug. All data of antimicrobial activity was the average of triplicate analyses.

2.5. *Testing for Antifungal Activity*

Antifungal activity of extracts (HFE and HLE) was carried by agar dilution method. The culture of organisms was grown on Sabouraud dextrose agar (SDA). The broth was incubated at 37°C for 24 hours. Inoculums were prepared by dilution of 24 hours old culture in saline. Miconazole, amphotericin B and benlate were used as standard drugs. The zone of inhibition of fungal growth was measured and compared with standard drugs (Irshad *et al.*, 2012).

3. RESULTS AND DISCUSSION

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Lutterodt *et al.*, 1999). This may therefore explain the demonstration of antimicrobial activity shown by plant extracts. Gram-negative bacteria are generally less susceptible to plant extracts than Gram-positive due to their outer membrane of lipopolysaccharide and lipoprotein, which is resistant towards antibacterial substances (Alzoreky and Nakahara, 2003; Chopra and Greenwood, 2001).

The antibacterial profile of flower extract of *H. schizopetalus* (HFE) revealed the activity

against 3/10 Gram positive bacteria tested i.e. *Streptococcus pyogenes, S. faecalis* and *S. captis* with respective zone of inhibition of 5 mm, 9 mm and 16 mm. The extract HFE were also effective against 50% of Gram negative bacteria used i.e. *Bdellovibrio bacteriovorus* HD 100, *Enterobacter aerogenes Escherichia coli, P. mirabilis* and *Proteus vulgaris* with respective zone of inhibition of 7 mm, 3 mm, 11 mm, 8 mm and 6 mm. While leaves extract of *H. schizopetalus* (HLE) showed significant activity against all tested Gram positive and Gram negative bacterial strains when compared to the standard drug ampicillin (Table 1).

Extracts of *H. schizopetalus* (HFE and HLE) showed prominent activity against human pathogens *Aspergillus flavus, A. niger* and specially in *Candida albicans* with zone of inhibition 5 mm, 4 mm, 10 mm, 6 mm, 3 mm

Table 1: Antibacterial Profile of *H. schizopetalus* Flower and Leaves Extracts

Classification of bacteria	Bacterial strains	Zone of inhibition (mm)		
		HFE	HLE	Ampicillin
Gram positive	*Bacillus subtilis*	–	22	17
	Bacillus vulgatus	–	4	13
	Corynebacterium xerosis	–	15	15
	Micrococcus luteus	–	12	12
	Staphylococcus aureus	–	9	12
	Staphylococcus aureus AB 188	–	12	14
	Staphylococcus epidermis	–	19	18
	Staphylococcus capitis	16	17	12
	Streptococcus faecalis	9	12	16
	Streptococcus pyogenes	5	8	13
Gram negative	*Bdellovibrio bacteriovorus* HD 100	7	16	14
	Enterobacter aerogenes	3	4	16
	Escherichia coli	11	15	12
	Proteus mirabilis	8	15	17
	Proteus vulgaris	6	14	11
	Pseudomonas aerogenosa	–	10	11
	Salmonella typhimurium	–	9	12
	Salmonella paratyphi A	–	8	13
	Salmonella paratyphi B	–	8	12
	Serratia marcescens	–	11	12

Concentrations of extracts and standard drug =100 µg/ml.

H. Schizopetalus: Flowers (HEF) and Leaves (HLE) extracts.

and 14 mm, respectively. Fungal pathogens (*Aspergillus* species etc.) have created serious problems worldwide causing a number of human, plants and animal diseases. *Aspergillus flavus* is pathogenic specie which produces variety of aflatoxins which contaminate food commodities. *C. albicans* is a yeast-like fungus classified as an opportunistic pathogen, changing physiology causing candidiasis, the most common form of mycotic infection. Risk factors that increased the incidence of *Candida* infection include compromised immunity, hormonal imbalances use of broad spectrum antibiotics, metabolic and nutritional disorders, and poor oral hygiene (Harman, 1998). No activity was observed against animal pathogens (*Micosporillum gypsiccus, Trichophyton mentagrophyte)*. In case of plant pathogens activity of extracts (HFE and HLE) was observed only against *penicillium* specie with inhibition zone of 3 mm, 6 mm, respectively antifungal activity is summarized in Table 2.

The inhibitory effect of the extracts of flower and leaves of *H. schizopetalus* against human pathogenic and fungal strains could introduce this plant as potential candidate for new drug development for the treatment of disease caused by these pathogens.

5. Conflict of Interest

The authors declare that they have no conflict of interest.

Table 2: Antifungal Profile of *H. schizopetalus* Flower and Leaves Extracts

Types of Pathogens	Fungal strains	Zone of inhibition (mm)	
		HFE	HLE
Human	*Aspergillus flavus*	5	6
	Aspergillus niger 18	4	3
	Candida albicans	10	14
	Micosporum canis	–	–
	Trichophyton tonsurans	–	–
Animal	*Microsporum gypsum*	–	–
	Trichophyton mentagrophyte	–	–
Plant	*Fusarium solani*	–	–
	Rhizopus stolonifer	–	–
	Saccharomyces cerevisiae	–	–
	Penicillium chrysogenum	3	6

Conentration. of extracts and standard drug =100 µg/ml.

H. Schizopetalus: Flowers (HEF) and Leaves (HLE) extracts.

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Comparative Clinical Evaluation of Herbal and Allopathic Medicine in Acute Tonsillitis

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Abstract

Tonsillitis is usually caused by a viral infection or less commonly, a bacterial infection. Symptoms may include sore throat, fever, enlargement of the tonsils, pain in swallowing, and enlarged lymph nodes around the neck. Herbal remedies are popularly used due to its potential action to relieve early tonsillitis, cost-effectiveness and with fewer adverse effects. This research aimed to determine the efficacy of herbal medicine for the management of acute tonsillitis. Study was conducted at Shifa-ul-Mulk Memorial Hospital for Eastern Medicine, Madinat-ul-Hikmah, Hamdard University. Patients (n=78) were randomly assigned to receive test drug lozin (500 mg) thrice a day and control drug cephradine (250 mg) thrice a day or 500 mg twice a day for 5-12 days with a follow-up visit of 2-12 days. Both medications after a 10-day treatment period caused a significant improve-ment in all the signs and symptoms. The herbal formulation lozin demonstrated similar efficacy compared to allopathic medicine cephradine for the treatment of acute tonsillitis.

Keywords

Acute tonsillitis; Lozin herbal formulation; Cephradine.

1. INTRODUCTION

Tonsils are the two lymph nodes located on each side of the back of throat. They are involved in defense mechanism and hence prevent various infections. Upon bacterial and/ or viral infection followed by inflammation the condition is referred to as tonsillitis (Windfuhr *et al,* 2016). Most cases of tonsillitis are caused by viral infections that also causes other diseases for example, rhinoviruses (common cold), influenza virus and para influenza virus (laryngitis) and croup entero viruses (hand, foot and mouth disease), adenovirus (diarrhoea) and rubella virus (measles).

Bacterial tonsillitis are caused by a number of different types of bacterias, mainly *Streptococcus* group. In the past, serious bacterial infections, such as diphtheria and rheumatic fever, have also been linked with tonsillitis. However, due to vaccination against them and availability of other treatments these are rarely reported.

Herbal remedies are most commonly used medication for tonsillitis due to its potential action to relieve its sign and symptoms. Literature search using NAPALERT data (Natural Product Alert Database, University of Illinois at Chicago) revealed that many plant drugs are used for the treatment of tonsillitis such as,

Glycyrrhiza glabra L., *Trigonella foenum-graecum* L., *Solanum nigrum* L., *Alpinia galangal* L., *Cordia dichotoma* L. and *Morus nigra* L. and hence were selected to produce a coded herbal formulation referred to as Lozin. Among them, *Glycyrrhiza glabra* L. has a long history of usage to treat illnesses such as peptic ulcer, colds and other viral infections (stimulate interferon production and expectorant/cough suppressant properties); microbial and parasitic infections (stimulate the immune system) and cancers (Nutrasanus, 2004). The essence of *Trigonella foenum-graecum* L. used as an effective herbal drug for tonsillitis (Usmanghani *et al.,* 1997). *Solanum nigrum* L. is prescribed for the treatment of hepatomegaly, splenomegaly, gastritis, sore throat and glossitis (Zakaria *et al.,* 2006). *Alpinia galangal* L. contains enzymes, polysaccharides and nutrients exhibiting antibacterial effects against *Streptococci, Staphylococci* and coliform bacteria. It is a useful treatment for sore throat and catarrhal infections (Awan, 1956).The dried fruit of *Cordia dichotoma* L. is valued on account of its mucilaginous nature and demulcent properties for the treatment of coughs and chest infections (Vohora, 1986). Extracts of the plant *Morus nigra* L. possesses antibacterial and fungicidal activities (Butt *et al.*, 2008).

Cephradine, is a semisynthetic cephalosporin antibiotic clinically used as a short-term treatment against tonsillitis by inhibiting bacterial cell-wall synthesis, however, is ineffective for long-term therapy due to its multiple side effects.

2. MATERIALS AND METHODS

2.1. *Study Design*

A monocentric, prospective, randomized, open-label and active-controlled study was undertaken. Patients were randomly assigned to one of two treatment groups: those receiving the coded herbal formulation lozin (Test group) and those given cephradine sodium (Control group). This study was conducted according to the principles of good clinical practice i.e., informed consent was obtained from the patients before enrollment, a proper history was taken and a clinical examination was carried out at each follow up visit. The study period was 3 years from November 2003 to February 2007. Patients of both genders (>15 years and <70 years) with a clinical diagnosis of acute tonsillitis were selected. The total sample size of this study was 78 subjects and comprised lozin group (n=45) and cephradine group (n=33).

The clinical protocol was approved by the appropriate Independent Ethics Committee of the Faculty of Eastern Medicine, according to principles based on the Declaration of Helsinki. The objective was to prove that herbal medicine is effective in acute tonsilitis and has fewer side effects.

2.2. *Setting*

On the basis of a preliminary clinical examination, the patients suffering from sore throat were selected from the out-patients enrolled at Shifa-ul-Mulk Memorial Hospital. They were referred to the project physician and included in the study based on the inclusion and exclusion criteria as described below:

Inclusion Criteria

The consent for clinical trial using either herbal or allopathic medicine was undertaken from all the patients. The clinical diagnosis of acute tonsillitis was based on: A sore and scratchy throat and/or pain on swallowing (odynophagia) accompanied by at least two of the clinical signs: a) Pharyngeal erythema and/ or exudate, cervical adenopathy and b) Uvular edema and fever.

Exclusion Criteria

The major exclusion criteria for this trial were: *a*) Patients with hepatic impairment, *b*) Patients non-resident of Karachi because of the inherent difficulty in conducting follow-up visit, *c*) Having a history of adverse reactions to any of the study drugs or contraindications for their use, *d*) Suffering from complicated and serious conditions like coma, meningitis, and encephalitis or head injury, *e*) Hospitalized for serious diseases, *f*) Symptoms that collectively suggest pharyngitis (laryngitis, coryza, conjunctivitis, diarrhea and cough), *g*) Infection of the deep tissues of the upper respiratory tract (epiglottitis, retropharyngeal or buccal cellulitis) or of the suprapharyngeal respiratory tract and its connecting structures (sinusitis, otitis media, or orbital/periorbital cellulitis), *h*) History of rheumatic heart disease, *i*) Known impaired renal function, as shown by a creatinine clearance $\leq$ 25 ml/min, *j*) Myasthenia gravis, patients currently being treated with systemic anti-bacterials or treated with systemic anti-bacterials within 14 days prior to enrollment. Moreover, patients were excluded from the study if they had: *a*) temperature >38.5°C (oral temperature) or >39.0°C (axillary temperature), *b*) Pharyngitis was exudative and/or ulcerative, *c*) Active gastrointestinal ulcers, *d*) Gastro-intestinal disease, or *e*) Presumed infectious mononucleosis.

2.3. *Clinical Assessment*

The main outcome measures were:

1. Clinical response of signs and symptoms of tonsillitis determined at the end of therapy.

2. Number of cases in which a change in medicine was needed (herbal or allopathic)

3. Microbiological eradication.

4. Physician global evaluation of the patient condition (using a 5-point arbitrary scale): Complete = 1, Marked = 2, Moderate = 3, Mild = 4 and None = 5 were employed.

Clinical outcome was defined as: Clinical cure (Complete resolution of signs and symptoms); Improved (Clinical signs and symptoms are reduced but not completely resolved) and failure (Signs and symptoms worsened, persisted or reappeared).

2.4. *Statistical Analysis*

Statistical analysis were performed with SPSS, using excel software, the Chi Squared Test. All differences were considered statistically significant if the '*p*-value' was less than 0.05. A two-sided *t* test for two independent samples was used to compare the mean ages of males and females, and both sexes combined of the two treatment groups.

3. RESULTS

The therapeutic effectiveness of formulated herbal medicine lozin was compared with the allopathic medicine cephradine sodium as a treatment for acute tonsillitis.

3.1. *Patient Demographic Characteristics*

There were 45 subjects in the test group and 33 in the control group were selected that complied with the inclusion/exclusion criteria. The mean age of the subjects participating in the test group (group 1) was 32 years (range 15-68), compared with the control group (group 2) which had a mean age of 33 years (range 18-50); the gender ratio is shown in Table 1.

3.2. *Baseline Complaint History*

The patients (84%) suffering from infections were categorized into mild, moderate and severe. The demographic and baseline characteristics of the patients included in the groups evaluable for efficacy were similar in

Table 1: Age Intervals for Lozin and Cephradine Groups

Age (Years)	Lozin (Test group)	Cephradine (Control group)	Number of patients (n)
15-20	7	5	12
21-26	11	8	19
27-32	15	9	24
33-38	5	6	11
39-44	5	4	9
45-50	2	1	3
Total	45	33	78

both lozin and cephradine groups (P>0.05). The disease-related demographic and baseline characteristics of the patients enlisted (Tables 2 and 3) were age, presenting symptoms, local examination of throat and oral cavity.

The patients with acute tonsillitis were diagnosed and grouped into classes with a) Throat pain, b) Difficulty in swallowing and c) Rhinitis representing baseline complaints, Test group (Lozin) and Control (Cephradine sulphate).

The test *versus* control populations in each category were equally balanced as shown in Tables 2 and 3 (P>0.05). All patients had one or more pretreatment symptoms of tonsillitis which were almost identical in both treatment groups (P>0.05) except for difficulty in swallowing. The most frequent symptom was throat pain (100%) in both treatment groups.

3.3. *Overall Clinical Response*

The total duration of treatment (test and control) were similar; the mean duration of treatment was 13.4±5.4 days and 13.4±6.0 days in the test and control groups, respectively. Among the clinically evaluable patients, the total durations of treatment in both groups were also similar, with a mean duration of 14.3±4.6 days and 14.1± 4.6 days in the test and control groups, respectively.

Both medications caused a significant improvement in all the signs and symptoms noted at baseline after a 10-day post-treatment period. There was also a significant decline in systemic symptoms like cough, difficulty in swallowing and fever. Clinically, 96.6% *vs* 94.2% (Test *vs* Control group) patients were cured, 2.4% showed improvement while 1% *vs* 3% of the patients did not respond to the therapy and needed rescue medication.

3.4. *Microbiological Response*

Clinically relevant pathogens isolated at baseline were similar in both groups (P>0.05) as listed in Table 4. *Streptococcus pyogenes* was the most commonly isolated bacterial pathogen in both treatment groups isolated from 78 evaluable patients. The microbiological success rate at the final visit was similar for the two groups, with a diagnosis of acute tonsillitis (P>0.05). Sub-group analysis of the microbiological outcome by gender, age, and

Table 2: Clinical Symptoms at Baseline for Acute Tonsillitis

Presenting complaint		Lozin (Test group)	Cephradine (Control group)	Total (n)	*p* value
Throat Pain	Yes	45	33	78	
	No	0	0	0	
	Total	45	33	78	
Difficulty in swallowing	Yes	44	29	73	
	No	1	4	5	0.078
	Total	45	33	78	
Rhinitis	Yes	4	4	8	0.716
	No	41	29	70	
	Total	45	33	78	

Table 3: Local Examination of Throat and Oral Cavity at Baseline in Acute Tonsilitis

Presenting complaint		Lozin (Test group)	Cephradine (Control group)	Total (n)	*p* value
Soft palate	Normal	13	6	19	
	Congested	32	27	59	0.276
	Total	45	33	78	
Uvula	Inflamed	30	19	49	
	Normal	15	14	29	0.412
	Total	45	33	78	
Post tonsillar pillar	Congested	29	31	60	
	Normal	15	2	17	0.009
	Nodular	1	0	1	
	Total	45	33	78	
Anti tonsillar pillar	Congested	33	27	60	
	Normal	12	6	18	0.38
	Total	45	33	78	
Post pharyngeal wall	Inflamed	27	19	46	
	Normal	18	14	32	0.83
	Total	45	33	78	
Regional lymph nodes	Palpable	15	13	28	
	Not Palpable	30	20	50	0.581
	Total	45	33	78	

race demonstrated comparable results between the groups. There were also some co-pathogens, like *Chlamydia pneumoniae* and *Mycoplasma pneumonia,* in acute tonsillitis, whereas *Staph* spp., *H. influenzae, Streptococcus pneumoniae, Bacteroides fragilis* and *Corynebacterium diphtheria* were the co- pathogens as presented in Table 4.

After therapy, pathogens decreased dramatically in both treatment groups (Table 4). The rates of complete elimination of throat pathogens were equal in both treatment group at all times (P>0.05). In the microbiologically rates of the selected baseline pathogens (*S. aureus*, *S. pyogenes*, *Corynebacterium diphtheria, Bacteroides fragilis* and others) at the final visit are summarized for the microbiologically evaluable patients in Table 4. Eradication rates generally were similar between the two treatment groups for these pathogens. The cure rates in both treatment groups decreased with advanced age. However,

Table 4: Status of Throat Culture Before and After Treatment in Acute Tonsilitis

Treatment	Micro-organisms	Lozin (Test group)	Cephradine (Control group)	Total (n)	*p* value
Before	*Streptococcus pyogenes*	37	24	61	0.955
	Staph aureus	3	4	7	
	Haemophilus influenza	1	1	2	
	Streptococcus pneumoniae	2	2	4	
	Cornybacterium diphtheria	1	1	2	
	Bacteroides fragilis	1	1	2	
	Streptococcus pyogenes	3	2	5	
After	*Staph aureus*	1	2	3	0.709
	Haemophilus influenza	0	0	0	
	Streptococcus pneumoniae	1	0	1	
	Cornybacterium diphtheria	1	1	2	
	Bacteroides fragilis	0	1	1	
Micro-organism growth status	No growth	39	27	27	

the overall clinical success or efficacy of the test medication was similar in acute tonsillitis (P>0.709).

4. DISCUSSION

The pesent study demonstrates that lozin is as effective as cepharadine in the management of patients with acute tonsillitis. The results were similar in microbiologically evaluable patients, for which, again, the test treatment eradication response was higher than that of the control. However, both the clinical success rate and microbiological eradication rate were higher in the lozin group.

Treatment of patients with tonsillitis is often empirical. Therefore, selection of an appropriate antibacterial agent should be, to a great extent, based on its anti-microbiological activity. In this study, the most frequently isolated pyogenic throat pathogens were *Streptococcus pyogenes* and *S. pneumoniae* while *H. influenzae; H. parainfluenzae, Staphylococcus aureus, Moraxella catarrhalis* were less common. Epidemiological data from Western countries on sore throat in general and specifically Group A beta-hemolytic streptococcal infections (GABHS), both community and hospital-based, are more readily available. However, there is considerable variation in the prevalence of GABHS sore throats from one country to another. For example, in Dhaka 22% of 601 children had a positive culture but only 2.2% is due to GABHS (Faruq *et al.*, 1995). Recurrent tonsillitis was reported in 11.7% of Norwegian children in one study and estimated in another study to affect 12.1% of Turkish children (Kvestad *et al.*, 2005).

This study demonstrates that both medications have predictable activity against both typical and atypical throat tract pathogens. In the microbiologically evaluable patients, test therapy provided an overall eradication rate by pathogen which was higher in comparison with that observed in the cephardine group because of higher antimicrobial effect of the lozin.

The broad-spectrum coverage of the lozin offers a potential advantage over both cepharadine and newer macrolide therapies. All isolates from microbiologically evaluable patients were successfully eradicated by its use in acute tonsillitis and the eradication rate was higher compared to cepharadine. In selecting antimicrobial agents for the treatment of tonsillitis the practitioner must consider not only the documented efficacy but also the adverse-event profile of the agent and its cost.

This important trial is the first to provide data demonstrating that lozin therapy is an effective and safe polyherbal therapy for the empirical treatment of patients with tonsillitis. The herbs present in it has a documented clinical efficacy against all common typical and atypical throat pathogens. If bacteria are not the cause of infection, then the treatment is usually directed more at cause. Antibiotics will not help to treat viral sore throat. The cephalosporins are also resistant to beta-lactamase enzymes although this is generation-dependent. The inappropriate use of antibiotics can have a significant negative impact both on individual patients and public health in general.

Lozin has antimicrobial, anti-inflammatory, immunomodulatory, and antioxidant actions due to the presence of active phytoconstituents. For example: Glycyrrhizin from *Glycyrrhiza glabra* exhibits potent antimicrobial activity and also potentiates the reticulo endothelial system and enhances immunostimulation. It acts on macrophage function, leading to stimulation of macrophages de-novo, and beta-glycyrrhetinic acid from *G. glabra* which is a potent inhibitor of the classical complement pathway (Nose

et al., 1998). Trigonelline from *Trignella foenum-graecum* effective in treating fever, cough (Srinivasan, 2006). Alpha-solanine from *Solanum nigrum* used for the treatment of hepatomegaly, splenomegaly, inflammation of the stomach and sore throat (Keeler *et al.,* 1983). Galangin from *Alpinia galangal* show antibacterial effects against *streptococci, staphylococci* and coliform bacteria also useful for treatment of sore throat, bronchitis and catarrhal infections (Usmanghani *et al.,* 1997). Salicyl aldehyde from *Cordia dichtoma* posses demulcent properties for the treatment of coughs and chest infections (Rastogi *et al,* 1993). Anthocyanin from *Morus nigra* have antibacterial activity, used in treatment of cold, influenza, cough. (Dharrmananda, 2003).

5. CONCLUSION

The herbal formulation lozin based on a complex mixture of chemical compounds present in different herbs represent a compound formulation. It emerged as on effective pharmacological agent similar to cepharadine against tonsillitis. There were no untoward manifestations associated with the use of this medication and it was acceptable by all treated patient and found to be safe.

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Apis mellifica — An Effective Insect Drug

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Abstract

Apis mellifica (Syn: *Apis mellifera*) belonging to family Apidae. Its extract contains melittin, glycosidase, hyaluronidase, phosphatase α-glucosidase, phospholipase A2, phospholipase B, protease inhibitor, apamine, adolapine, secapine, minimine, histamine, tertiapin, dopamine, mast cell degranulating peptide, pamine, procamine A, noradrenalin, γ-aminobutyric acid, α-amino acids, glucose, fructose, complex ethers, potassium, calcium, magnesium. *A. mellifica* extract was made from whole insect. It is used in bites, stings, sore throat, urine retention, pain and headaches. There are no documented side effects associated with it.

Keywords

Honey bee, Homoeopathy, Anti-inflammatory.

1. INTRODUCTION

Apis mellifica is approximately 15 mm long, shiny black, covered with orange-red and grey hair insect with broad spineless tibiae. The posterior margins of the segments and legs are brown, with gradual transition to yellowish-red. Maxillary palps are single-membered and claws are two membered, while hind limb has bristles. There are three complete cubital cells in the wing along with the radial cells. A duct connects the barbed sting with the poison sac (Raes *et al*., 1985; Tsujiuchi *et al*., 2007; Farah-Saeed and Ahmad, 2016a).

Historical Background and Medicinal Uses

Bee products medicinal uses have been described in ancient literature by Hippocrates, Aristotle and Galen. Bee venom was used in middle ages as a medical remedy to relieve pain and to treat inflammatory diseases such as arthritis and rheumatism. The use of *A. mellifica*, a whole honeybee was discovered by Rev. Brauns in 1835 as a homeopathic remedy, in Thuringia, Germany. Dr. Constantine Hering, in 1853, published the evidences of *A. mellifica* efficacy in his American Provings. The safety and effectiveness of *A. mellifica* has made it a popular homeopathic remedy (Dunglison 1856; Urtubey 2003; Hellnerm *et al*., 2008; Hering 1879; Simics, 2003).

Preparation of A. mellifica Extract/Tincture

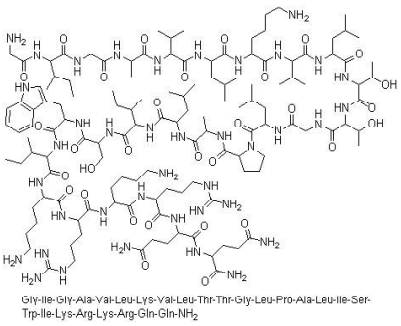
Live honey bees are placed in a clean, wide-mouthed suitable container, preferably of glass. After mincing and shaking, the menstrum is poured in, and macerated for fourteen days, swirling three times daily. The mother tincture obtained is filtered. It is important that the bees should not be pressed. It is repeatedly diluted

Table 1a: Chemical Constituents Activities of *A. mellifica* L.

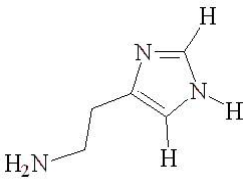
S.No.	Chemical constituents	References
1.	Melittin	Banks and Shipolini, 1986; Dotimas and Hider, 1987; Shkenderov and Ivanov, 1983; Han *et al.*, 2010; Muli and Maingi, 2007; Urtubey, 2005
2.	Hyaluronidase	
3.	Phosphatase α-glucosidase	
4.	Phospholipase A2 (PLA2)	
5.	Phospholipase B	
6.	Apamine	
7.	Adolapine	
8.	Secapine	
9.	Minimine	
10.	Glycosidase Tertiapin	
11.	Mast cell degranulating peptide	
12.	Pamine	
13.	Procamine A	
14.	Protease inhibitor	
15.	Noradrenalin	
16.	Histamine	
17.	Dopamine	
18.	γ-aminobutyric acid	
19.	α-amino acids	
20.	Glucose	
21.	Fructose	
22.	Complex ethers	
23.	Potassium	
24.	Calcium	
25.	Magnesium	

Table 1b: Pharmacological Activities of *A. mellifica* L.

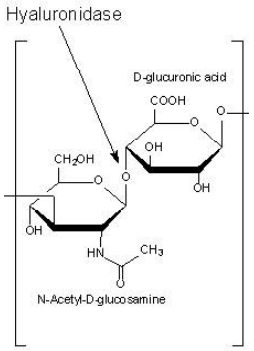
Pharmacological actions	References
Rheumatism, Cancerous tumors, Multiple sclerosis, Dementia, Post stroke paralysis, Polyneuritis, Ganglion nerve inflammation, Neurodynia, Cerebellar ataxy, Syringomyelia, Inflammation of facial nerve, Myopathy, Trigeminal neuralgia, Post-traumatic inflammation of plexus nerve, Inflammation of arachnoid CNS membrane, Parkinson, Arteriosclerosis, Endarteritis, Angina pectoris, Arrhythmia, Eczema, Dermatitis, Psoriasis, Furunculosis, Healing of cicatrices, Baldness, Colitis, Ulcers, Asthma, Bronchitis, Pharyngitis, Tonsillitis, Ear nerve neuritis, Polyarthritis, Osteochondrosis, Hypertension, Urinary retention, Edema, and dropsy. In homeopathy for treatment of: inflammatory diseases of eyes, ears, respiratory organs, Diseases of digestive system α-bladder, kidney; Skin diseases, allergies, acne, abscesses; Scarlet and German measles, diphtheria, Glandular and genital diseases, Heart and blood circulation diseases; Psychiatric diseases, Kidney inflammation, Urine suppression and general edema.	Ellingwood, 1919; Kokot and Matysiak, 2009; Krylov, 1995; Krylov *et al*., 2007 Ludyanskii, 1994; Pinto, 2009; Putz *et al*., 2006; Russell *et al*., 2004; Savilov, 2010; Schraner, 2007; Shkenderov and Ivanov, 1983; Son *et al*., 2007; Urtubey, 2005.



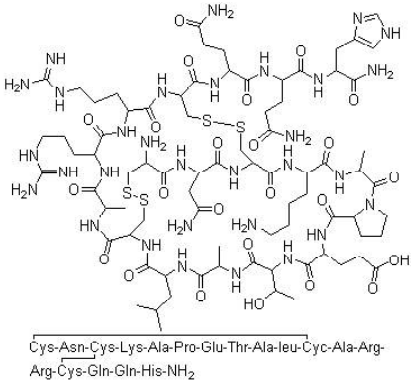
Melittin



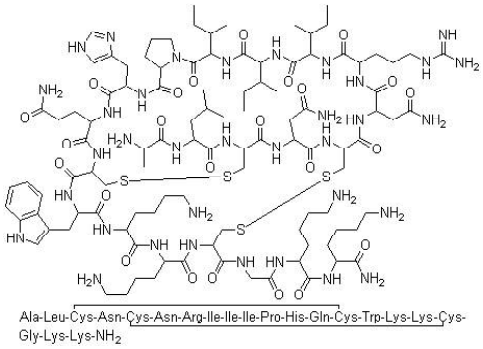
Histamine



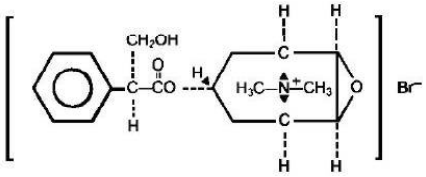
Apamine



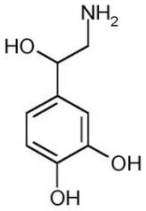
Hyaluronidase



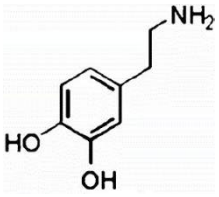
Tertiapin



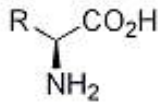
Noradrenaline



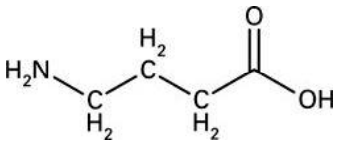
α-amino acid



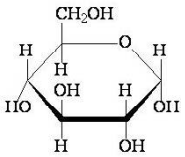
Dopamine



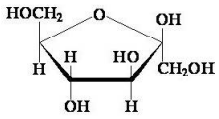
Pamine



γ-amino butyric acid



Glucose



Fructose

Fig. 1
Active chemical constituents of *Apis mellifica*

Table 2: Active Constituents in *A. mellifica* L. with their Therapeutic Efficacy

S.No.	Active constituents	Therapeutic efficacy	References
1.	Melittin	Increases capillary permeability, lowering the blood pressure, decrease total cholesterol and triglyceride but increased HDL in serum, lowers blood coagulation. Immuno-stimulatory and immuno-suppressive, radioprotective, influences the central nervous system, improved motor function and inhibit neuronal death in the spinal cord, anti-cancer, increase cell viability and decrease apoptosis, anti-bacterial, anti-fungal and anti-viral. Reduce swelling and granulomatous responses. Anti-arthritic, anti-inflammatory effect for liver effects	Shkenderov and Ivanov, 1983; Urtubey, 2005; Son *et al*., 2007; Asafova *et al*., 2000; Jeong *et al*., 2011; Ludyanskii, 1994; Krylov, 1995; 2007; Savilov, 2010, Lee *et al*., 2014; Han *et al*., 2014; Kim *et al*., 2011; Park *et al*., 2012; 2014
2.	Phospholipase A	Destroys phospholipids and dissolves the cell membrane of blood cells; lowers the blood coagulation and blood pressure, prevents neuronal cell death caused by prion peptides	
3.	Phospholipase B	Detoxicating activity	
4.	Hyaluronidase	Catalyses the hydrolysis of protein, dilates blood vessels, increases their permeability, causing increased blood circulation	
5.	Apamine	Anti-inflammatory, increases the defense capability, immune-supressor and stimulates the CNS	
6.	MCD (Mast cell degranulating peptide)	Anti-inflammatory effect, stimulates CNS, lyses mast cells, releasing histamine, serotonin and heparin, melittin-like effect increasing capillary permeability	
7.	Adolapin	Inhibits the specific brain enzymes, cyclo-oxygenase and lipooxygenases, anti-rheumatic, decreases pain, anti-pyretic, inhibits the aggregation of erythrocytes	
8.	Protease inhibitor	Inhibits the activity of different proteases, thereby reducing inflammation and act as anti-rheumatic	
9.	Cardiopep	Radioprotective	
10.	Minimin		
11.	Procamine		
12.	Histamine	Dilates blood vessels, increasing the permeability of blood capillaries and increase blood circulation, stimulates smooth muscles	

till the virulent aspect of the bee venom is removed, leaving only the curative agent. As a consequence it becomes an effective potent medicine for an actual honeybee sting and for ailments that have similar symptoms (*British Pharmacopeia,* 2015).

A) Physico-chemical Examination

Relative Density

According to *British Pharmacopoeia* (Vol. IV) the relative density of *A. mellifica* extract is 0.890-0.910 (*British Pharmacopeia,* 2015).

Dry Residue

According to *British Pharmacopoeia* (Vol. IV) the dry residue is not less than 1.25 and not more than 1.60% (*British Pharmacopeia,* 2015).

Chemical Identification

In reaction with chemical reagents the extract of *A. mellifica* extract showed the presence of tannins, saponin glycosides, carbohydrates, and steroids (Farah-Saeed, 2014).

Spectroscopy (FTIR)

A. mellifica revealed significant peaks at 3235.87 (OH), 2937.57 (C-H), 1621.79 and 1519.64 (aromatic ring), 1050 (C-O-C) cm^{-1} (Farah-Saeed and Ahmad, 2016a; Kokot and Matysiak, 2009).

B) Pharmacological Activities

Insecticidal

Paralysis effect and mortality was seen with the increase in dose. Exposure to 100 mg of *A. mellifica*, mean mortality time was 10.6±2.60 hours (Farah-Saeed, 2014).

Anthelmintic

A. mellifica administered in the snails at 1 mg, 25 mg, 50 mg, 75 mg and 100 mg respectively, did not reduce its activity even after 72 hours. Howeever, at 500 mg of the drug, time dependent decrease of activity with shell discoloration was observed. At 1000 mg, time dependent decrease in activity was also observed, indicating that at the end of 72 hours out of 6 snails, 2 (33%) had paralytic effect with shell discoloration (40±5.54), whereas 4 were found to be dead with 60% mortality (Farah-Saeed, 2014).

Anti-bacterial

A. mellifica did not exhibit zone of inhibition against bacterial or fungal pathogens. No minimum inhibitory concentrations (MIC) against bacterial pathogen was observed against the tested pathogens (Farah-Saeed, 2014).

Al-Ani *et al*., (2015) investigated the antimicrobial activity of bee venom and its main component, melittin, single or in double-drug and triple-drug combinations with antibiotics (vancomycin, oxacillin, and amikacin) or antimicrobial plant secondary metabolites (carvacrol, benzyl isothiocyanate, the alkaloids sanguinarine and berberine) against drug-sensitive and antibiotic-resistant microbial pathogens. The secondary metabolites were selected corresponding to the molecular targets to which they were directed, being different from those of melittin and the antibiotics (Issam *et al*., 2015).

Anti-oxidant

A. mellifica (1 mg) exhibited 88.93% DPPH scavenging activity and 82.98% total anti-oxidant activity by phospho molybdate method (Farah-Saeed, 2014).

Neuro-pharmacological

The anxiolytic activity was assessed using open field, head dip, light and dark, cage cross and swimming apparatus. The CNS depressive effects were observed at 100 mg/kg of *A. mellifica* extract as follows; in open field

Table 3: Identification of Chemical Constituents of Drug *A. mellifica* by Chemical Reagents using Thin-layer Chromatography

Chemical constituents	Chemical reagents	Reaction
Tannins	Lead acetate	Very slight precipitation
Saponins		Froth formation
Alkaloids	Dragendorff's reagent	No orange precipitates
Carbohydrates	Molish test	Color changes to purple
Proteins	Ethanol	Soluble
Sterols	Lielarmann Burchardt reagent	No Purple ring formation
Steroids	Napthol-H_2SO_4	Purple color which on addition of water remains purple
	H_2SO_4	Golden brown

TLC of *A. mellifica* extract in chloroform – methanol – water (80:20:2) solvent system displayed the presence of several spots of different chemical classes. The R_f values of the spots were: 0.02, 0.10, 0.15, 0.23, 0.35, 0.45, 0.66 at 254 nm and 0.03, 0.09, 0.21, 0.43, 0.50, 0.0.73, 0.78, 0.87 at 366 nm. In ethyl acetate – methanol – water (100:16.5:13.5), the R_f values of the spots were: 0.01, 0.09, 0.23, 0.38, 0.46, 0.53, 0.59 at 254 nm and 0.03, 0.05, 0.11, 0.16, 0.22, 0.26, 0.39, 0.46, 0.52 at 366 nm. (Farah-Saeed and Ahmad, 2016a; Kokot and Matysiak, 2009).

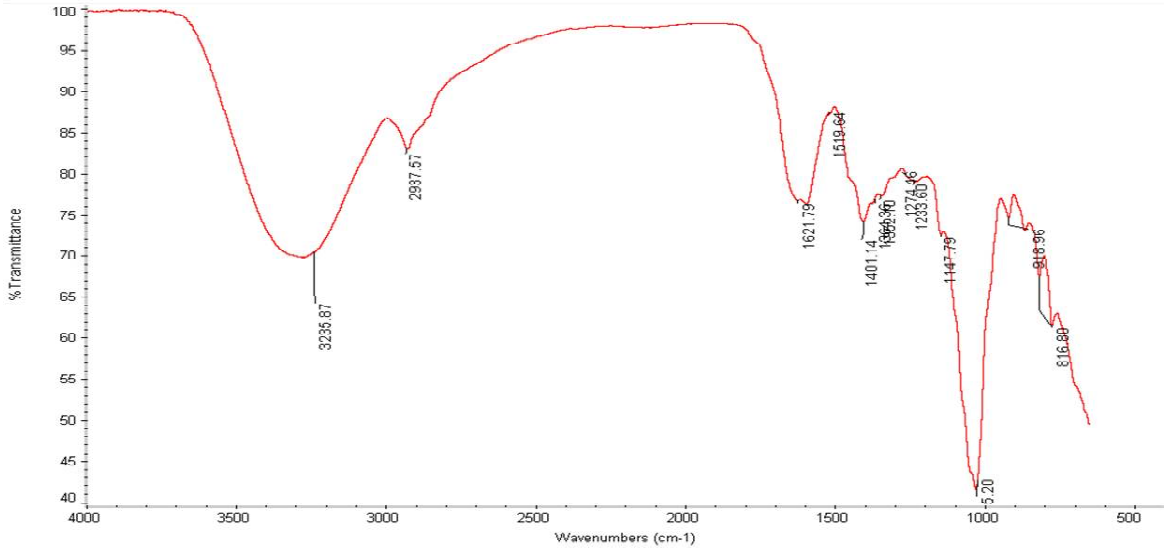


Fig. 2
FT-IR spectra of *A. mellifica*

activity (28±2.84) counts in 30 minutes were observed, while in head dip test, the mice dipped head (13.33±2.61) times. At the dose of 300 mg/kg the pronounced depressed effects were observed in case of light and dark, cage cross and swimming activities. Number of entries in light compartment is 9.33±2.93 times. The readings of cage cross is (23.33±2.44) times. In forced swimming test (FST) the Mean forced mobility time was (1.25±0.04) seconds. Locomotor and exploratory activity was observed to be substantially reduced in comparison to control and standard Diazepam (2 mg/kg^{-1}) (Farah-Saeed, 2014; Farah-Saeed *et al*., 2015a).

Analgesic

The writhes were counted for three phases, each of 10 minutes, respectively. The inhibition of acetic acid induced writhes by aspirin was observed in all three phases: First (62%), second (21.71%) and third phase; (22.24%). *A. mellifica* at the dose of 300 mg/kg also exhibited inhibition in first (35.36%), second (21.71%) and third phases (32.36%) (Farah-Saeed 2014; Farah-Saeed *et al*. 2015b).

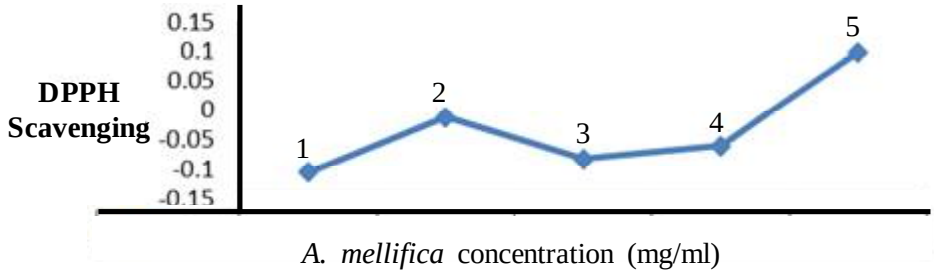


Fig. 3a
DPPH scavenging activity of *A. mellifica*

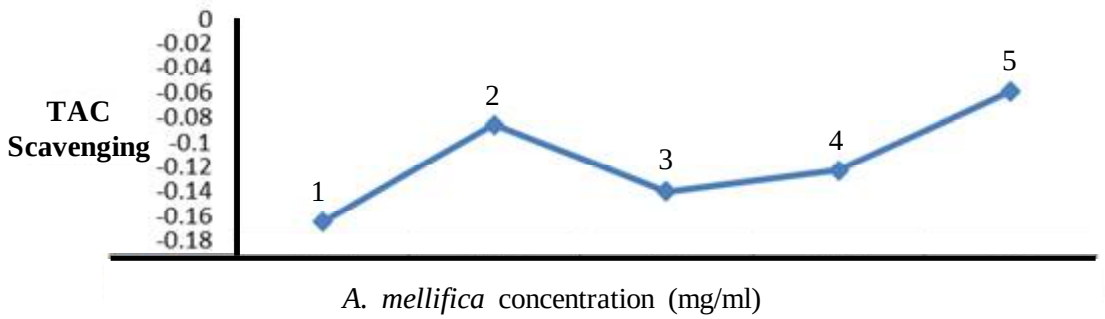


Fig. 3b
TAC activity of *A. mellifica*

Various concentration of *A. mellifica* used (mg/kg) represented as: 1, 5, 10, 50 and 100, sequentially (1-5) 2,2-diphenyl-1-picrylhydrazyl (DPPH); Total Antioxidant Capacity (TAC)

A. mellifica (500 mg/kg) showed maximum inhibition of the licking and biting response in first phase (56.60%) second phase (6.94%) and third phase (25.89%) as induced by formaldehyde (Farah-Saeed, 2014; Issam *et al*., 2015).

Anti-inflammatory

A. mellifica (300 mg/kg) at 1.5 hours exhibited maximum paw volume inhibition 11.76%. However, *A. mellifica* (500 mg/kg) showed 25.64% maximum paw volume inhibition at 3.5 hours. Aspirin at 1.5 hours revealed maximum paw volume inhibition 22.22% (Farah-Saeed, 2014; Farah-Saeed and Ahmad, 2016b).

Kung-Woo *et al*. (2003) reported the anti-inflammatory activity of the n-hexane, ethyl acetate, and aqueous partitions from bee venom (*A. mellifera*) using cyclooxygenase (COX) activity and pro-inflammatory cytokines (TNF-α and IL-1β) production, *in vitro*. COX-2 is involved in the production of prostaglandins that mediate pain and support the inflammatory process. The aqueous partition of bee venom showed strong dose-dependent inhibitory effects on COX-2 activity (IC_{50} = 13.1 µg/mL), but did not inhibit COX-1 activity (Kung-Woo *et al*., 2003).

Anti-arthritic

Recently therapeutic efficacy of bee venom therapy in China was observed in knee osteoarthritis. In combination with conventional drugs, bee venom therapy was also found to relieve the symptoms and prevent relapse of rheumatoid arthritis (Kwon *et al*., 2001; Liu *et al*., 2008).

Diuretic

The mice given oral dose of 300 mg/kg of *A. mellifica* extract exhibited pronounce diuretic activity 1.90±0.0024 at the end of 4 hours as compared to the control 0.93±0.0036. Furosemide 10 mg/kg showed diuretic activity 2.52±0.0033 (Farah-Saeed, 2014; Farah-Saeed *et al*., 2015c).

Anti-urolithic

A. mellifica extracts (25%, 50%, 75% and 100%) had no inhibitory effect on calcium oxalate crystallization (Farah-Saeed, 2014; Farah-Saeed *et al*., 2015c).

Carbon Tetrachloride Treated Rabbits

The rabbits treated with *A. mellifica* extract for three months were administered carbon tetrachloride 1.5 ml before taking out blood via cardiac puncture for LFT (Liver function test). Total bilirubin (0.033±0.0046), direct bilirubin (0.023±0.0046) and gamma GT (9±0.632) levels were found lowered. SGPT (240±0.632) and alkaline phosphatase (126.83±0.658) levels were found elevated as compared to the control group (Farah-Saeed, 2014; Mehjabeen *et al*., 2015b).

C) Effects of *A. mellifica* on Various Parameters

Hematology

Test group (male) treated with *A. mellifica* extract, slight elevation in hemoglobin (11.75±0.0836), red blood cells count (6.0±0.01308), hematocrit (40.85±0.0836), Mean corpuscular volume (MCV) (68.05±0.0836), Mean corpuscular hemoglobin (MCH) (19.35±0.0836) and total leucocyte count (7.25±0.0836) was observed, while platelet count (471.5±0.836) was significantly raised in test group as compared to the control group.

Test group (female) treated with *A. mellifica* extract led to a decline in hemoglobin (10.416±0.0658), (RBC) Red blood

cells count (5.425±0.00836), hematocrit (36.25±0.0836) MCV (67.13±0.1254), MCH (19.31±0.0658), while an increase in white blood cells count (10.835±0.0739) and platelet count (323.167±0.658) was observed (Farah-Saeed, 2014; Mehjabeen *et al*., 2015a).

Kidney Function

Urea (30.5±0.83), creatinine (0.925±0.008), serum calcium (14.45±0.014), albumin (5.035±0.0083), albumin/globulin ratio (A/G ratio) (2.205±0.012) levels were elevated, whereas, phosphorus (4.275±0.008), uric acid (0.045±0.0083), total proteins (7.32±0.01) and globulin (2.28±0.012) were reduced in male test group treated with *A. mellifica* extract as compared to respective male control group.

Urea (40.5±0.83), creatinine (0.718±0.01), phosphorus (3.695±0.139), total protein (7.53±0.0096), albumin (4.86±0.013) and A/G ratio (1.82±0.009) levels were reduced, whereas, serum calcium (14.765±0.0083), uric acid (0.0450.0083) and globulin (2.671±0.01) levels were raised in female group treated with *A. mellifica* extract in comparison to its female control group (Farah-Saeed 2014; Mehjabeen *et al*., 2015a).

Cardiac Enzymes

LDH (Lactate dehydrogenase) (205.5± 0.83) level was lowered, CPK (Creatin phosphokinase) (1758.83±1.036) and CK-MB (Iso-enzymes CKM and CKB) (851.16±1.036) enzymes were raised in male control group treated with *A. mellifica* extract in comparison with its respective male control group.

The cardiac enzymes; LDH (312.5±0.83), CPK (842.16±1.036) and CK-MB (888.67±0.96) were found raised in female test group treated with *A. mellifica* extract as compare to its female control group (Farah-Saeed, 2014; Mehjabeen *et al*., 2015a).

Lipid Profile

Triglycerides (287.16±1.18) and Very low density lipo-protein (VLDL) (57.83±1.11) were raised, however, cholesterol (40.5±1.009), High density lipo-protein (HDL) (5.5±0.83) and Low density lipo-protein (LDL) (3±0.63) levels were lowered in male test group treated with *A. mellifica* extract as compare to its respective male control group.

Cholesterol (161.16±1.036), triglycerides (45.50.83), HDL (32.33±0.96), LDL (123.5±0.83), VLDL (9.42±1.034) levels were raised in female test group treated with *A. mellifica* extract in comparison to its respective control group (Farah-Saeed, 2014; Mehjabeen *et al*., 2015a).

Liver Enzymes

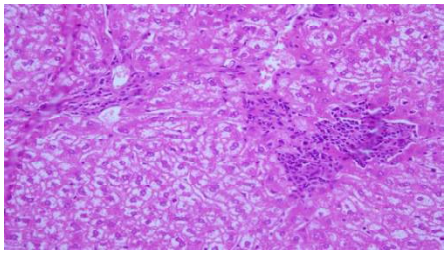
Direct bilirubin (0.12±0.017) was raised, while the rest of the liver enzymes, that is, Serum glutamic-oxaloacetic transaminase (SGOT) (38.83±1.036), total bilirubin (0.255±0.00836), Serum glutamic-pyruvic transaminase (SGPT) (28.33±1.15), alkaline phosphatase (88.5±0.836) and gamma Gamma glutamyl transferase (GT) (12.5±0.836) were reduced in male test group treated with *A. mellifica* extract as compared to the male control group.

Total bilirubin (0.245±0.0083) was slightly reduced, while the other liver enzymes; direct bilirubin (0.065±0.0083), SGOT (51.67±1.154), SGPT (110.67±0.96), alkaline phosphatase (101.83±1.036) and gamma GT (9.5±0.836) were found raised in female test group treated with *A. mellifica* extract in comparison to the respective female control group (Farah-Saeed, 2014; Mehjabeen *et al*., 2015a).

Urine Analysis of A. mellifica Treated Rabbits

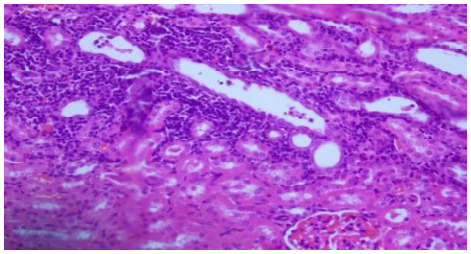
The urine of the male group treated with

Liver



8a

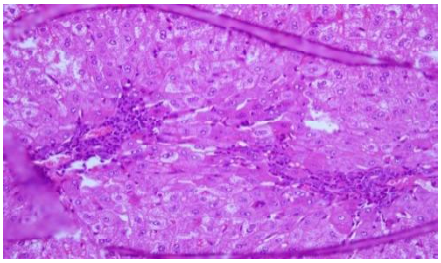
Kidney



8b

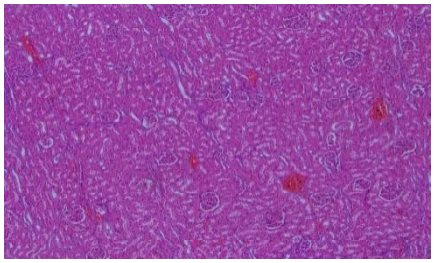
Figs. 8a and b
Histopathology of liver and kidney tissues of male rabbits treated with *A. mellifica*

Liver



9a

Kidney



9b

Figs. 9a and b
Histopathology of liver and kidney tissues of female rabbits treated with *A. mellifica*

A. mellifica extract was yellow in colour and turbid, like that of its respective male control group. The pH was 9.05, slightly raised as compare to that of its respective control group. While the rest of the urine parameters of the test group were alike that of control group.

The urine parameters of the female test group were parallel to that of its respective female control group (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

Histo-pathological Studies

Old healed myocardial infarction in the wall of left ventricle and inter-ventricular septum was found in the cardiac tissues of the male group treated with *A. mellifica* extract. No significant pathology was seen in gastric tissues. In liver tissues, mild portal inflammation and peri-portal fibrosis with foci of lobulitis was observed. Chronic nonspecific pyelonephritis was found in renal tissues (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

In cardiac tissues focal myocytolysis of right ventricular wall was seen, while in liver tissues, mild portal inflammation and peri-portal fibrosis with centrilobular hepatocytic degeneration was observed. No significant pathology was seen in gastric and renal tissues (Farah-Saeed 2014; Mehjabeen *et al.* 2015a).

2. CONCLUSION

A. mellifica is a very effective medicine. It is safe and effectively being used in homoeopathic system of medicine for the different organs pathologies associated with inflammation, especially of kidneys and skin.

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Pain Management: An Overview

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Abstract

Pain is an unpleasant sensory and emotional experience caused by actual or potential tissue damage. This damage is nature's warning that something is not well within the body. This condition causes loss of workforce and also affects the patients family members in socio-economical and psychological terms. There is an increase in knowledge regarding pain management in recent years. These developments in pain management may provide different opportunities to the patient and their families to lead a more comfortable and productive life. Managing pain is not about making it disappear rather it is about keeping it under control. The aim is not to stop pain in its stride, but to avert the damage caused by it. Prolonged pain is demoralising and debilitating and should be controlled as fast as possible and with all possible means. For this reason in addition to pharmacological treatment now a days non pharmacological treatment options are on rise.

Keywords

Pain, Tissue damage, Pharmacological interventions, Non-pharmacological interventions.

1. INTRODUCTION

According to International Association for the study of pain (IASP), pain is " an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP, 1979).

The word pain comes from the Latin word 'Poena', meaning punishment. From times immemorial, pain was considered as form of punishment meted out by God for the sins committed by man. The word pain brings to one's mind misery and suffering.

The physician Albert Schweitzer, proclaimed in 1931 that, "pain is a more terrible lord of mankind than even death itself" (Ranjani, 2016).

Although there is an increase of knowledge and developments in technological resources regarding the pain, many patients still experiences it. This situation causes for reduction in living quality and functional situations of the patients, increase in the fatigue levels and impairment in daily life activities in working capacity and social interactions (Nash *et al.*, 1999; McMillan, 2000).

There are various remedies for pain management, either pharmacological or non pharmacological interventions, but they all do not necessarily work for everyone. As people respond differently to the same pain stimuli according to their respective thresholds, an

analgesia which can produce dramatic pain relief in one person, may be perceived as relatively ineffective by another.

Pain is known as *Waja'* in Unani system of medicine, it is an Arabic word and described as disturbed perception of body. According to Jalinoos (Galen), the main cause of pain is breach in continuity only, but Ibn Sina (Avicenna) stated that any changes in the temperament (*Sue Mizaj Mukhtalif)* or/and breach in continuity (*Tafarruqe Ittesal*) as well is the cause of pain.

The *Sue Mizaj Mukhtalif* may be *sada* (simple) or *maddi* (humoral). *Sue Mizaj Maddi* which is associated with the substance or matter may be *Sue Mizaj har Maddi* (abnormal hot temperament with humoural involvement) and *Sue Mizaj barid Maddi* (abnormal cold temperament with humoural involvement).

According to Jalinoos (Galen) breach in the continuity may be developed by some external and internal causes. The external causes which directly produce *tafarruqe ittesal* may be stretching force, tear, burn, crush, and prick. While the internal causes are *khilt laazeh* (irritant humour), *khilt akkal* (corrosive humours), *ghaleez riyah* (viscous pneuma), *ghaleez khilt* (viscous humours) (Sina, 1993; Rushd, 1987; Mirza, 2014).

1.1. *Classification of Pain*

Physiological Classification of Pain

Nociceptive Pain: (nocere – injure, Latin). Nociception is the activity of peripheral nervous system that transmits or processes the information about noxious events associated with tissue damage to central nervous system. Nociceptive pain is of following types:

i) Somatic pain: Irritation or damage to musculoskeletal system, originating from bone, muscle, connective tissue etc. This type of pain can be described as aching, sharp, stabbing, throbbing and is well localized.

ii) Visceral pain: Originating from body organs such as pancreas, liver, gastro-intestinal tract etc. This type of pain is described as cramping, dull, colicky and squeezing. It is diffused, poorly localized, and may be referred to other areas.

Neuropathic pain: It is caused by an injury or dysfunction of the peripheral or central nervous system. It is often described as: burning, shooting, stabbing, numbness or tingling. It has the following types:

i) Central neuropathic pain as in post stroke pain, spinal cord injury, multiple sclerosis and syringomyelia

ii) Peripheral neuropathic pain may be focal, multi focal, symmetrical for example trigeminal neuralgia, carpal tunnel syndrome, nerve root fibrosis, post-herpetic neuralgia, vasculitis etc. Other sensations of neuropathic pain – Dysesthesia (bugs crawling on the skin, pins and needles). Allodynia (pain to a non painful stimulus). Hyperalgesia (increased pain sensation to a normally painful stimulus).

Mixed pain: Neuropathic pain may coexist with nociceptive pain. In some disease conditions, patients may have mixed pain consisting of somatic visceral and neuropathic pain all at the same time or each separately at different times. Examples include trauma that damages tissue and nerves, burns (that affect skin as well as nerve endings), and cancer that causes external nerve compression as well as damaging nerves by infiltration.

Classification Based on Duration of Pain

***Acute pain*:** Is of sudden onset, is felt immediately following injury, is severe in

intensity, but is usually for short time period. It arises as a result of tissue injury stimulating nociceptors and generally disappears when the injury heals.

Chronic pain: Is continuous or recurrent pain that persists beyond the expected normal time of healing. Chronic pain may begin as acute pain and persist for long periods or may recur due to persistence of noxious stimuli or repeated exacerbation of an injury. Chronic pain may also arise and persist in the absence of identifiable pathophysiology or medical illness. Chronic pain can negatively affect all aspects of daily life, including physical activities, school attendance, sleep patterns, family interactions and social relationships and can lead to distress, anxiety, depression, insomnia, fatigue or mood changes, such as irritability and negative coping behaviour (Ranjani, 2016; PMG, 2012; Arthur *et al.,* 2005).

1.2. *Classification of Pain According to Unani System of Medicine*

Jalinoos (Galen) and Ibn Sina (Avicenna) categorised pain into different types as shown Table 1.

Table 1: Classification of Pain with Body Sites Affected

Unani classification (*Waja'*)	Nature of pain	Body sites affected
Ayayi	Fatigue	Whole body
Hakkak	Pruritic	Skin
Khadri	Neuropathic	Nerves
Khashin	Rough	Skin (Psoriasis)
Laazeh	Irritant	Stomach (Heart burn)
Misalli	Stabbing	Large Intestine
Mufassikh	Incisive	Muscles
Mukassir	Bony	Skeletal
Mumaddid	Distension	Stomach
Nakhis	Pricking	Lungs (Pleurisy)
Rikhu	Dull ache	Soft tissue muscles
Saqeel	Heavy	Liver and spleen
Saquib	Perforating	Colon
Zaghit	Compression	Heart
Zarbani	Throbbing	Acute inflammation, (Migraine)

Sina (1993).

Analgesics

There are three groups of agents which alleviate pain: 1) Some contrary to the cause of pain which removes the cause e.g. anethum, linseed made into a poultice and applied over the painful area. 2) Any agent which counteracts the acrimony of the humours, induces sleep or dullness or soothes the sensitive faculties and decrease activity e.g. inebriants milk, oil, aqua dulcis etc. 3) An agent which infrigidates and dulls the sensation in the painful part e.g. all narcotics and somniferous drugs (Sina, 1993).

1.3. *Pain Pathway: (Nociception)*

There are four main components of the pain mechanism: i) Transduction, ii) Transmission, iii) Modulation and v) Perception.

Transduction: During transduction, which occurs in the periphery, the damage to human tissue causes nociceptive stimulations which activate nerve endings i.e. primary afferent nociceptors.

***Transmission*:** During the transmission the information is conveyed from the peripheral nervous system to the dorsal horn of the spinal cord where nerve cells activate and the information is then processed to higher centres i.e. to the brainstem. It is believed that the thalamus forwards the message to the frontal cortex which assigns meaning to the pain. The information is then conveyed to the somatosensory cortex, which identifies and localises the pain and finally to the limbic system where the information is interpreted as pain.

Modulation: Modulation is interaction between pain-transmitting and non-pain transmitting neurons i.e. pain control in the nervous system. The activation of non-pain transmitting neurons at the spinal cord can interfere with signals from pain fibres and inhibit or modulate an individual's experience of pain. Modulatory inter-neurons in the spinal cord are either inhibitory or excitatory.

***Perception*:** Perception of pain is a neurophysiologic phenomenon comparable to the sense of heat or touch when the neurons transmit pain and evoke a subjective response to pain. The emotional response to the perception (e.g., depression, fear, anxiety, suffering), and the pain behaviour in response to those emotions and perceptions guide the observer to believe the individual is suffering from pain i.e. talking about pain, grimacing or moaning (Ylinen *et al.*, 2009; Ylinen *et al.*, 2007).

1.4. *Pain Assessment*

The Visual Analogue Scale (VAS)

It is presented as a 10 cm line, anchored by verbal descriptors, usually 'no pain' and 'worst imaginable pain'. The patient is asked to mark a 100 mm line to indicate pain intensity. The score is measured from the zero anchors to the patient's mark. Using a millimetre scale to measure the patient's score will provide 101 levels of pain intensity.

The Numerical Rating Scale (NRS)

It is a 11, 21 or 101 point scale where the end points are the extremes of no pain and pain as bad as it could be, or worst pain. The NRS can be graphically or verbally delivered. When presented graphically the numbers are often enclosed in boxes and the scale is referred to as an 11 or 21 point box scale depending on the number of levels of discrimination offered to the patient.

The Verbal Rating Scale (VRS)

The VRS comprises a list of adjectives

used to denote increasing pain intensities. The most common words used being: no pain; mild; moderate; and severe or intense pain (Williamson *et al.*, 2005).[1]

Pain Meter (PAULA)

Pain meter uses 5 coloured emoticon faces on the front of a ruler and corresponding VAS score on the back. Patients are allowed to move a slider to mark the pain which they are experiencing. Use of this pain meter resulted in less variance than pain scores obtained from standard VAS (Machata *et al.*, 2009).

1.5. *Pharmacotherapy of Pain*

Pharmacological Pain Management in Unani System of Medicine

In Unani system of medicine drugs that are used for analgesia are presented in Tables 2 and 3.

Table 2: Unani Analgesics with Botanical Name and Dose

Classical name	Botanical name	Dosage
Afyoon	*Papaver somniferum* L.	25-50 mg
Bazrul Banj	*Hyoscyamus niger*	15-60 mg
Dhatura	*Datura stramonium*	500 mg
Shokran	*Conium maculatum* Linn.	1 g
Suranjan	*Colchicum luteum*	125-375 mg
Tukhme kahu	*Lactuca scariola* Linn.	3-5 g

Ghani (2008) and Rafiquddin (1985).

Table 3: Adjuvant Unani Analgesics with Botanical Name and Dosage

Classical name	Botanical name	Dosage (g)
Baboona (Muhallilat)	*Maticaria chamomilla*	2-3
Katan (Moaddelat)	*Linum usitatissimum*	2-4
Nakhoona (Muhallilat)	*Trigonella uncata*	5
Shibt(Moaddelat)	*Anethum graveolens*	3

Ghani (2008) and Rafiquddin (1985).

1.6. *Pharmacological Pain Management in Conventional System of Medicine*

WHO – Step Analgesics Ladder

The WHO 3 step guidelines were first published in 1986 and are considered to be the gold standard for managing pain in advanced cancer. This analgesia ladder has been modified for acute pain, chronic non cancer pain and cancer pain. This revised ladder integrates a fourth step which includes nerve blocks, epidurals and neurolysis (phenolisation, alcoholisation) (Vargas, 2010).

Step 1: Non opioids ± adjuvants
Step 2: Non opioids + Weak opioids ± adjuvants
Step 3: Non opioids + Strong opioids ± adjuvants
Step 4: Nerve Blocks, Epidurals

Adjuvant analgesia tend to be drugs that are licensed for indications other than pain. Hence, they are not primarily classified as analgesia even though they may relieve pain that is usually not responsive to standard analgesia which include antidepressants, anticonvulsants, antispasmodics and steroids etc (Vargas, 2010; WHO, 1986).

Non-steroidal Anti-inflammatory Drugs (NSAIDs)

These drugs are the corner stone of the treatment for mild to moderate pain. All NSAIDs are analgesic, anti inflammatory and antipyretic effects. They modify the nociceptive responses induced by the polypeptide bradykinin, and also inhibit the enzymatic synthesis of prostaglandins from long chain fatty acids (Tables 4 and 5).

Opioid Analgesics

The term opium is derived from the Greek term for juice, the drug being derived from the juice of poppy plant, *Papaver somniferum*. Drugs such as morphine mimic endogenous opioid chemicals known as enkephalins, endorphinin, and dynorphins. Enkephalin in the dorsal horn of the spinal cord and endorphin in the periaqueductal gray region of the brain are neurotransmitters involved in "closing the pain gate, "thereby, inhibiting the pain transmission.

These are drugs conventionally used for indications other than analgesia, but may be analgesic in specific circumstances. These drugs include antidepressants, anticonvulsants, benzodiazepines, corticosteroids, etc. (Table 6).

1.7. *Non-pharmacological Pain Management*

Cognitive behavioural therapies

Relaxation – respiration techniques – It is provided to focus on the respiration and avoid disturbing thoughts by taking a deep breath slowly through the nose and giving it back in a long time through the mouth

Distraction**:** Getting the attention away from the pain reduces its severity. The aim in using that technique is to increase the tolerance for pain and decrease the sensitivity towards pain. This method includes listening to music, watching television, reading books and dreaming.

Meditation and Praying: In the traditional meaning, meditation is generally focusing on the moment. This act is realized with an individual focusing on respiration, a word or picture. Sometimes arbitrary connection with God while praying may also works wonder in pain relief.

Hypnosis: It is the state of conscious change similar to sleep. Hypnosis requires the body to relax and the patient to focus on an object, a stimulant or memory. Hypnosis is "the deep physical relaxation state during which subconscious can be reached and important abilities are suspended".

Aromatherapy: It is using the essential oils that are obtained from flowers, herbs and trees to improve health and well being. These

Table 5: Non-steroidal Anti-inflammatory Drugs Dosage for Adults

Generic name	Dosage (mg)	Half life (hours)
Aspirin	75-325 QID	2-20
Celecoxib	100-200 BID	10
Diclofenac	50 BID	2
Ibuprofen	200-800 TID	2
Indomethacin	25-50 TID	5
Ketoprofen	50-75 TID	3
Ketorolac	15-30 QID	2
Naproxen	250-500 BID	13
Piroxicam	20 QID	50

Twice a day (BID); Thrice a day (TID); and Four times a day (QID)
Gehdoo (2013).

Table 4: Opioid Analgesics Dosage for Adults

Drug	Route	Dose	Schedule
Codeine (weak opioid)	Oral	30-60 mg	4-6 hourly
Fentanyl	IV	1-2 µg/kg	30-40 min.
Morphine	Oral/IV	10 mg	4 hourly
Pentazocine	Oral/IM	50-100/30-60 mg	4-6 hourly
Pethidine	Oral/IM	50-100 mg	4 hourly
Tramadol	Oral/IV/IM	1-2 mg/kg	4-6 hourly

Gehdoo (2013) and Jain (2013).

Table 6: Adjuvant Drugs Used in Conventional System of Medicine for Neuropathy

Drug	Dosage per day	Time to be effective
Amitriptyline (Antidepressants)	10-75 mg	6-8 week
Nortriptyline (Antidepressants)	70-100 mg	6 week
Pregabalin (Anticonvulsant)	75-600 mg	4-6 week
Gabapentin (Anticonvulsant)	100-3600 mg	4 week
Diphenhydramine (Sedative)	200 mg	–
Promethazine (Sedative / Antipsychotic)	100 mg	–
Prednisone (Steroid)	5 mg	–

Tripathi (2013).

oils are applied by being respired through oily gauze that is placed under the nostrils of the patient or as massage oils being applied on skin (Williams *et al.,* 2009; Richardson *et al.,* 2006; Jackson *et al.,* 2008).

1.8. *Peripheral Therapies (Physical Agents/Skin Stimulation)*

Transcutaneous Electrical Nerve Stimulation (TENS)

Pain is reduced when the area is rubbed or stimulated due to activation of non nociceptive fibers inhibiting the nociceptive response in the dorsal horn of the spinal cord. TENS uses electric current produced by a portable device to stimulate the nerves for therapeutic purposes In TENS, non nociceptive fibers are selectively stimulated with electrodes in order to produce this effect and thereby inhibiting pain. People using cardiac pacemaker should be excluded for this therapy (Sluka *et al.,* 2003).

Acupuncture and Acupressure

Acupuncture, one of the important components of Traditional Chinese Medicine (TCM) is acceptable worldwide as a scientific treatment method. It provides the body to restore balance by means of stimulating some special points on the body with needles. Acupoint stimulation such as manual acupuncture involves the penetration and manipulation of a fine needle through the skin into specified points on the body to evoke a sensation. More than 360 acupoints are located along 14 meridian channels that cover the body in a web like interconnecting matrix (Lin *et al.,* 2009; Chen *et al.,* 2014).

Cryotherapy

The pain-relieving benefits of snow and ice were first documented by the Greek physician Hippocrates thousands of years ago. It is a relatively new form of treatment in which the body is briefly exposed to very cold temperatures in order to promote healing and other therapeutic results. Cytotherapy has been shown to decrease inflammation of the body's tissues, muscles and joints. It can also help improve the body's circulation and healing, and also slow down cellular metabolism and reproduction. Cryotherapy can help to reduce pain and muscle spasms in the body as well as reduce the swelling of injuries. It promote and accelerate healing in joint, muscle and tendon injuries. The most common type of cryotherapy is an ice pack and most effective in crushed form because it conforms comfortably to the contours of the injured area (Saini, 2015).

Hammam (Turkish Bath)

Hammam is well known regimental therapy in Unani system of medicines. It consists of various rooms where facilities are available for a bath followed by shower and massage. It reduces pain and improves general health. It reduces the viscosity of the humours and improves health of the debilitated individuals. It improve metabolism, increase innate heat of the body and excrete waste products through skin (Hamdani, 2010).

Dalk (Massage Therapy)

Dalk is also an integral part of Unani system of medicine. By this diversion of morbid matter (*Imalae mawad*, *Tanqiya mawad*). Evacuation of morbid matters from the site of affected organ.It also induces sedation, analgesia and increase blood circulation. Unani medicine describes detailed types of massage e.g. Rough massage (*Dalk khishan*), smooth massage (*Dalk amlas*), prolonged massage (*Dalk kaseer*) or short duration massage (*Dalk qaleel*) (Lone *et al.,* 2011).

Hijamah (Cupping Therapy)

It a well known Unani regimental therapy

meaning "to suck". *Hijamah* refers to a Unani regimental mode of treatment. It is an ancient method which was practically used among the Chinese, Babylonians, Egyptians, Greeks, Romans, Arabs and Indians. It is also used to relieve severe pain in any part of the body. This purpose is achieved either due to diversion of materials away from the site of pain or by dissolution of morbid material (Sheikh *et al.*, 2014).

2. CONCLUSION

Pain can be managed effectively with the combination of pharmacological and non-pharmacological therapies. Understanding of the pharmacology of all potentially useful agents, available therapeutic goals, patient conditions and adverse effects of the drugs used will greatly help to optimize outcomes. Unani drugs and regimens may play a vital role to mitigate pain due to its safety and efficacy inspite of controversial scientific status, insufficient facilities for clinical research and lack of updated literature. Unani System of medicine has tremendous potential in pain management. The role of non-pharmacological approaches to pain management is evolving, and some non-pharmacological and complementary therapies have an important contribution towards patient care however, some approaches have not been shown to be of benefit, so it is essential that for management of severe pain appropriate analgesics and adjuvant should be considered along with complenentary therapies using evidence based approach.

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Invitro Anticancer Activity of *Vitis vinifera*

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Abstract

Vitis vinifera was evaluated for *in vitro* anticancer activity using Dalton's lymphoma ascites cells. Its dried seeds aqueous extract (10-200 µg/ml) induced dose dependent cytotoxicity with an IC_{50} value of ~150 µg /ml favoring its anti-cancer action.

Keywords

Vitis vinifera, Anticancer activity.

1. INTRODUCTION

Vitis vinifera is a woody perennial vine belonging to the family *Vitaceae* and is commonly known as vine or grape (English), angoor (Hindi, Urdu) and *munthiri* (Tamil). This plant is indegenous to subtropical, hot tropical and mild climatic region in India and cultivated for its edible fruit belonging to eustard grape family (Bombardelli *et al.,* 1995). The top five producers of grapes in the world are Italy, France, United States of America, Spain and China (Shikhamany, 2001). Its trunk length can reach 35 m, although in the pruning stage it only measures 3 m in height. Its leaves are 5-23 cm in diameter, circular or oval, thin, toothed or slightly dentated on the edges, glabrous with a dull green colour on the upper surface and stromy gray tone on the lower surface between 4 and 5 lobes. The flowers are numerous and are arranged opposite to the leaves and grouped in clusters. The fruit, grape is pulpy and small 6-12 mm in diameter with circular or oblong shape (Alonso, 2004). The seeds of *V. vinifera* is used against breast, gastrointestinal tract and other human cancers (Kaur, 2006). Its skin and seeds are used in natural medicine and considered to be a good source of natural anti-oxidants (Torres *et al.,* 2002; Yinaz and Toledo, 2004). It possesses various pharmacological activities such as anti-oxidant (Castillo *et al.,* 2000; Yinaz and Toledo, 2004), anti-cancer (Li *et al.,* 2008; Kaur *et al.,* 2009), anti-microbial (Palma *et al.,* 1999; Nilgun *et al.,* 2004), anti-inflammatory (Li *et al.,* 2001), hypertensive activities and also associated with reduced risk of cardio vascular diseases (Clifton, 2004; Peng *et al.,* 2005).

2. MATERIALS AND METHODS

Seeds of *V. vinifera* were collected from a local shop near to Cherraan's College of Pharmacy, Coimbatore. They were separated from the fruits and dried under the sunlight. Seeds (65 g) were powdered and extracted using water and to subjected preliminary phytochemical screening according to the standard procedures.

3. *IN VITRO* CYTOTOXICITY STUDY

The dried seeds aqueous extract of *V. vinifera* was subjected to short term *in vitro* toxicity using Dalton's lymphoma ascites

cells (DLA). These tumor cells aspirated from the peritoneal cavity of the tumor bearing mice, washed thrice with phosphate buffered saline (PBS) or normal saline (0.9% NaCl). The cell viability was determined by trypan blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1 ml) was added to tubes containing various concentration (10, 20, 50, 100 and 200 µg /ml) of aqueous extract and the volume was made up to 1 ml using PBS. Control tubes contained only cell suspension. These assay mixture were incubated for 3 h at 37°C. Further cell suspension was mixed with 0.1 ml of trypan blue 1% and kept for 2-3 min and loaded on a haemocytometer. Dead cells take up the blue color of stain while live cells appear colourless. The number of stained and unstained cells were counted and percent cell death was calculated as shown below:

$$\text{Percent cell death: } \frac{\text{Dead cells (Blue)}}{\text{Live cells (transparent)+Dead cells}} \times 100$$

Table 1

Aqueous extract (µg)	Dalton's lymphoma ascites cell death (%)
10	12
20	24
50	30
100	42
200	57

Effect of *V. vinifera* on viability of Dalton's lymphoma ascites cells.

4. RESULTS AND DISCUSSION

The seeds of *V. vinifera* have been traditionally claimed for a large number of pharmacological actions and medicinal uses. The seeds (aqueous extract) of *V. vinifera* demonstrated concentration dependent increase in the percentage of Dalton's lymphoma ascites cells death with 57% cytotoxicity at 200 µg/ml (Table 1). The study showed aqueous extract of *V. vinifera* seed have anticancer activity. It offered a scientific justification of its traditional use against cancer (Ye *et al.*, 1999). Phytoconstituents mainly: carotenoids, flavonoids, glucosinolates, lignans, phytoestrogens, proanthocyanidins, tannins, terpenoids etc. (Kaur, 2009) may be responsible for the cytotoxicity. However, further phytochemical studies are needed to isolate the active compounds responsible for this action.

It will be helpful to develop a novel plant based anticancer drug.

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Phytochemical Screening and Standardization Profile of *Adansonia digitata* L. – A Universal Remedial Plant

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Abstract

Globally, the significance of medicinal plant in the cure of variety of illnesses has been increasing the expansion of science of phytopharmaceuticals and desire for treatment in chronic and acute diseases which initiates new enthusiasm to develop herbal medicine. The present study was conducted on mature leaves of *Adansonia digitata* L. used in traditional system of medicine for curative and preventive treatment of asthma, dysentery, fever, hemorrhoids, insect bites, malaria, measles, sores trypanosomiasis, ulcers, etc. This plant is used as a staple food because it has nutritional value too. The present investigation describes phytochemical screening and standardization profile of mature leaves of *A. digitata*. Various extracts of leaves were prepared and subjected to phytochemical screening using different solvents and detailed microscopic and fluorescence studies were also carried out.

Keywords:

Adansonia digitata L., Phytochemical screening, Microscopic, fluorescence studies

1. INTRODUCTION

Medicinal plants are natural treasure of herbal remedies as they contain thousands of valuable bioactive compounds which give enormous therapeutic effect. These plants play a vital role in evolution of modern medicine and for health of mankind (Pooja, 2012). Many conventional curative agents however, transformed into new drugs although there is continuous expansion in the field of research and possible utilization of potential herbal treatments (El-Kamalia and El-Khalifab, 1999; Scheuring *et al.,* 1999; Ganiyat *et al.,* 2010).

The medicinal shrub *Adansonia digitata* L. (Bombacaceae) has 25 genera and about 250 species. It spreads widely in the savannas of tropical and southern Africa (Sugandha *et al.,* 2014). It is mostly found in the lands of Benin, India, Mali, Pakistan, Senegal, and Sudan etc (Hey *et al.,* 2007). This plant is also cultivated in Karachi and some parts of Interior Sindh (Fig. 1). In local language, it is called as "Gorakh Imli" while in English it is known as "Baobab" (Muok *et al.,* 2000). Different parts of plant are effective to treat different disease like its fruit pulp is use to cure fever, measles and dysentery. Mash prepared from dried powdered root is given to malarial patients as a tonic. A semi-fluid gum obtained from Baobab bark is used to treat sores (Atawodi *et al.,* 2002; Cassius *et al.,* 2000). Its bark is also recommended for infants to gain weight as it contain high level of calcium, zinc, copper and

Fig. 1
Plant of *Adansonia digitata* L.

rich fat content. Different parts of plant have renowned antioxidant and anti-inflammatory properties (Emmy *et al.,* 2010; Caluwe *et al.,* 2009; Masola *et al.,* 2009). While crude extract of stem and root bark have significant antimicrobial and antifungal activity (Locher *et al.,* 1995; Atawodi *et al.,* 2003). In folk medicine, it is well known for the treatment of trypanosomiasis (Koeleman, 1972). Its leaves are component of many herbal remedies because they act as diaphoretic, expectorant, astringent and antipyretic. The leaves also have hypotensive and anti-histaminic properties. Leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice (Sidibe and Williams, 2002). As a phytomedicine, it is used in renal and bladder diseases, asthma, fatigue, diarrhea, inflammation, insect bite, ulcers etc, while in skin aliments root is recommended mostly as demulcent (Wood, 1969; Harborne, 1973). A variety of phytochemical compounds such as carbohydrate, protein, alkaloids, tannins, steroids, flavonoids, vitamins and mineral have also been reported from different parts of this plant (Hey *et al.*, 2007). The detailed proximate, analytical and chemical characteristics of the fruit (seed and pulp) of this plant were reported (Ayaz *et al.*, 2014).

Recently, the European Commission authorised the import of baobab fruit pulp as a novel food (Buchmann *et al.,* 2010) and it was approved in 2009 by the Food and Drug Administration as a food ingredient in the United States of America. Baobab products (e.g. bark, fruits, leaves and seeds) contribute to the livelihood of many populations of the world as it is a source of food, fibre and medicine (Wickens, 1982; Codjia *et al.,* 2001). There is no phytochemical and pharmacognostic report on the leaves of this nutritionally important plant. The basic aim of this study is to evaluate the phytochemical and establish standardization profile of leaves of *A. digitata* L.

2. MATERIAL AND METHODS

The fresh green leaves of *Adansonia digitata* (Bombacaceae) , were collected in the month of June from University garden and authenticated by Prof. Dr. Ghazala H. Rizwani (Meritorious), Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan. Specimen No. 103 was deposited in the museum of Department of Pharmacognosy, for further research.

2.1. *Preparation of Extract*

The fresh leaves of the plant were shade dried for eight days. The dried leaves were powdered and weighed. They were then passed through 80 mesh size and finally packed in an air tight container for further studies. The sample was weighed extracted and percolated in absolute ethanol followed by partitioning with

ethyl acetate (EtAc), butanol (BuOH), aqueous (H_2O) and n-hexane (H) (Sazada *et al.,* 2009). All semi-solid extracts were refrigerated and used for phytochemical analysis.

2.2. *Phytochemical Studies*

Preliminary qualitative analysis of all extracts was carried out by employing standard conventional protocols (Kokate *et al.,* 2006). The extracts (ethanol, ethyl acetate, butanol, aqueous and n-hexane) were tested for the presence of various types of phytoconstituents such as alkaloids, carbohydrates, flavonoids, glycosides, sterols, and tannins etc.

2.3. *Fluorescence Analysis*

Fluorescence characters of leaf material of *A. digitata* with different chemical reagents were determined under visible and ultraviolet light on both wavelengths (254 nm and 366 nm), respectively. Leaf sample (1 mg) was taken on a glass slide and treated with various reagents for the presence of their fluorescence characters under ultraviolet lamp (Chase and Pratt, 1949).

2.4. *Pharmacognostic Studies*

A thin section of leaf was prepared through midrib to perform microscopical studies. The section was cleared with chloral hydrate solution and then stained with phloroglucinol (1% in 90% ethanol) and concentrated hydrochloric acid, mounted in glycerin for the identification of lignified cells. Photomicrographs of the microscopical sections were taken with the help of MOTIC Digital Microscope (Evans and Trease, 2009; Kokate, 1994).

3. RESULTS AND DISCUSSION

Preliminary phytochemical analysis of leaves extract (ethanol, ethyl acetate, butanol, aqueous and n-hexane) showed presence of various phytochemicals of medicinal importance such as alkaloid, ascorbic acids, carbohydrates, flavonoids, glycosides, gums, phytosterols, protein & amino acid, tannins, tartaric and saponins, (Table 1). The change of color on treating with different chemical reagents are represented in Table 2.

Presence or absence of particular phytochemical which have therapeutic effect present in extract of plant is authenticated by qualitative phytochemical screening (Sunil *et al.,* 2012). The current phytochemical analysis showed that *A. digitata* leaves are rich source of carbohydrate, protein and vitamin C and may provide dietary supplements in certain conditions of ill health which could be utilized as necessary energy source (Ijomah *et al.,* 2000; Hall *et al.,* 1976). Vitamin C acts as an antioxidant and involves in hydroxylation reaction, collagen formation and wound healing (Champe and Harvey, 1994). Flavonoids present in hexane, ethyl acetate and ethanol extracts produce anti-inflammatory activity, profound antioxidant preventing from oxidative cell damage and also have strong anticancer activity. Ethanolic and n-hexanolic extract revealed the presence of alkaloids that could be used as CNS stimulant with pain relieving potential (Okwu and Josiah, 2006).

Transverse section of leaf lamina of *A. digitata* through mid-rib showed that the upper epidermis is composed of single layered rectangular cells with wavy wall; cuticularized covering and glandular trichomes (sessile – without stalk) and stomata in the stomatal chambers. Below upper epidermis two to four layers of collenchyma cells are present. The mesophyll region consists of two layers of compact and radially elongated cells of palisade and spongy parenchyma which are eight layered, loosely arranged with intercellular spaces in which deposition of calcium oxalate crystals were observed. Lower Epidermis is similar to upper epidermis, but with more trichomes and

Table 1: Preliminary Phytochemical Analysis of Various Extracts of *A. digitata* Leaves

Plant constituents	Tests	Extracts				
		Hexane	Butanol	Ethyl acetate	Ethanol	Aqueous
Flavonoids	Alkali reagent test	–	–	+	+	–
	Zn-HCl test	–	–	+	+	–
Phytosterols	Liberman's burchard test	–	+	–	+	+
	Libermans reaction	–	+	–	+	+
Glycoside	Keller killiani test	+	+	–	+	+
Alkaloid	Wagner test	+	–	–	–	–
Carbohydrate	Molish test	+	+	–	–	+
Saponins	Foam test	–	–	–	–	+
Gums	Benedict test	–	–	–	+	+
Proteins and amino acids	Millons test	–	+	–	+	+
	Xanthoproteinic test	–	+	–	+	+
	Biuret test	–	+	–	+	+
	Ninhydrin test					
Tannins	$FeCl_3$ test	–	–	–	+	+
	Acetic acid test	–	–	–	+	+
	Potassium di chromate test	–	–	–	+	+
	Iodine test	–	–	–	+	+
	Lead acetate test	–	–	–	+	+
Tartaric acid		+	+	–	+	+
Ascorbic acid (Vitamin C)		+	+	+	+	+
Fixed oil	Spot Test	–	–	–	–	–
Volatile oil		+	+	+	+	–
Anthocyanins		–	–	+	+	–

Respective plant constituents are represented as: present (+) or absent (–) in various extracts.

Table 2: Fluorescence Analysis of Powdered Drug of *A. digitata* Leaves

Leaves powder + Suitable solvent	Visible light	Ultraviolet (UV) (nm)	
		254	365
Distilled water	Green	Brown	Brown
MeOH	Light green	Light green	White
HCl (Conc)	Brown green	Brown	Brown
HCl (50%)	Brown green	Green	Green
HNO_3 (Conc)	Reddish yellow	Brown	Red
HNO_3 (50%)	Light yellow	Light yellow	White
H_2SO_4 (Conc)	Light green	Green	Blackish green
H_2SO_4 (50%)	Leaf green	Brown	Brown
NH_3 solution	Brown	Brown	Dark brown
NaOH in water	Blackish brown	Dark green	Blackish green
Pet. ether	Brown	Brown	Blackish brown
H_2O_2 (5%)	Green	Green	Yellowish green
Xylene	Yellow	Green	Brown
CCl_4	Apple green	Green	Purple
KOH (50%)	Dark green	Black green	Black
$K_2Cr_2O_7$	Dark brown	Black	Black
$AgNO_3$	Purple brown	Light brown	Black
Bromine water	Dark green	Brown	White
NaOH (1N) + MeOH	Light brown	Light green	Greenish red

Methanol (MeOH), Hydrochloric acid (HCl), Nitric acid (HNO_3), Sulphuric acid (H_2SO_4), Ammonia (NH_3), Sodium hydrooxide (NaOH), Petroleum ether (Pet. ether), Hydrogen peroxide (H_2O_2), Carbon tetrachloride (CCl_4), Potassium hydrooxide (KOH), Chromic acid ($K_2Cr_2O_7$), Silver nitrate ($AgNO_3$).

stomatal pores. The covering trichomes are 2-4 cells blunt, thick walled and pointed. Few cystoliths were also seen in cortical parenchyma. There was an arc shaped, collateral vascular bundle present at the center of leaf section (Figs. 2 and 3).

Studies on the nutritional value of fruit pulp and seed are abundant but no work has been done on the leaves of *A. digitata* and there is a need to evaluate its proximate composition, mineral and amino acid.

4. CONCLUSION

The aim of this research is useful in understanding of *A. digitata* L. via identification, pharmacognostic features, systemic classification along with preliminary phytochemical screening of different extracts of the leaves showing

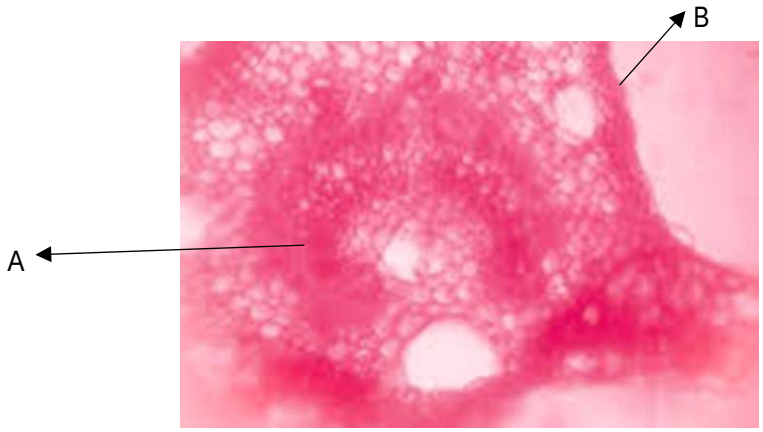

Fig. 2
Transverse section of *A. digitata* leaf

The dorsal view of *A. digitata* leaf showing: Vascular bundle; xylem and phloem (A) and Upper epidermis (B).

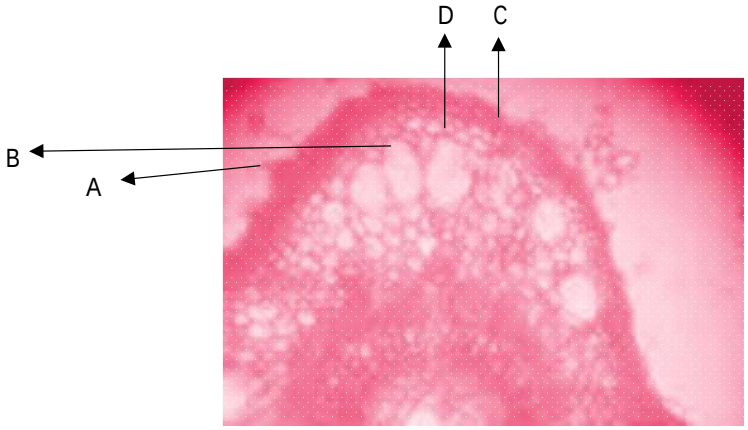

Fig. 3
Transverse section of *A. digitata* leaf

The ventral view of *A. digitata* leaf showing: Covering trichome (A), Vacuole (B), Lower epidermis (C) and Collenchyma (D).

presence of alkaloids, carbohydrates, flavonoids, glycosides, protein, tannins etc. These have been found useful in both curative and preventive medicine. These findings assures the proper and effective use of not only leaves but other parts of plant in different herbal preparation as a medicine, neutraceutical and industrial raw material as phytopharmaceuticals.

Conflict of Interest

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

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Comparative Studies on the Enzyme Status of Indian Major and Exotic Carps

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Abstract

Aquaculture is the farming of economically important aquatic animals and plants under controlled conditions. The analysis of enzyme profile of Indian major and exotic carps were carried out. The study revealed that there was fluctuation in physicochemical parameters. The enzyme profile in the present study the major carps showed significantly higher concentration than the exotic craps.

Keywords

Fish, Enzymes, Major carps, Physico-chemical parameters.

1. INTRODUCTION

Aquaculture is the farming of economically important aquatic animals and plants under controlled conditions of fresh water and brackish water culture. People of developing countries have viewed oceans as a means of providing feed and livelihood. It is as such will definitely reduce food shortage, it is major problem in developing countries. Aquaculture is a remarkable economic growth sector in many countries its growth is expected to continue most part of the world as the gap between supply and demand for fish products are widening .Most of the global aquaculture productions is from semi intensive and intensive management farms and follow a combination of part fertilization and supplementary feed inputs or corresponds aqua feed.

India is the second largest aquaculture producing country since 1995 (5.8% of global aquaculture productions). It is an excellent complement to human being to meet the protein rich food requirement of growing population. Modern aquaculture production technology is composed of contribution from many diverse technical specialities such as nutrition fish health, environmental physiology, genetics reproductive biology, water quality dynamics ,design of various specialized equipments and product quality assurance (Kannaiyan, 2002) proper management of fish pond is the key for higher yield (Vardia, 1999).

The physicochemical properties of water are of great ecological importance. The aquatic organism are influenced directly or indirectly by physical chemical and biological factors like temperature, pH, salinity etc., (Nasar *et al.*, 1974). The variability of physicochemical biological parameter between

outdoor fresh water lentic mescocomes has been estimated (Caquet *et al.,* 2001) and the effect of physicochemical parameter, growth and survival of the freshwater fishes in a culture pond has been known (Ramesh, 2002). Effect of pig dung on water quality productivity and growth of carps in polyculture system (Dhawan and Kaur, 2002). The limits of salinity and its effect on water quality productivity and growth performance of the Indian major carp *Labeo rohita* (Pillai *et al.,* 2003) and the relative acid phosphatase activity in the liver of nurrel species (Shrivastava *et al.,* 1989), analyzed the level of alkaline phosphatase activity during the early period of three bundh bred Indian major carps and exotic craps (Sarkar *et al.,* 1996). Hence an attempt has been made to compare the enzyme profile of Indian carps and some exotic carps.

2. MATERIALS AND METHOD

The physicochemical parameters such as pH, temperature, turbidity, alkalinity, total hardness, total dissolved solids, calcium, manganese, sodium and potassium were studied by using standard procedures (Eaton *et al* 1994). The samples for physicochemical analysis were collected consecutively for six months May-October (2013). For the enzyme analysis *Catla catla, Labeo rohita, Cirrhirus mrigala Cyprinus carpio and Hypo phthalmichthys molitrix* were collected from the experimental ponds, and acclimatized to the laboratory conditions, maintaining the same physico-chemical characters. The blood samples were collected from the caudal regions and four enzymes were used in the study alkaline phosphatase, acid phosphatase. Serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase were estimated (King, 1965 and Maiti, 1994). The data were subjected to statistical analysis (Palanichamy and Manoharan, 1994).

3. RESULTS

The physicochemical parameters of the experimental fish rearing pond were represented in Table 1. The temperature showed minor fluctuation during the experimental period all the other physicochemical parameters were optimum level throughout the study period. The acid phosphatase, alkaline phosphatase, serum glutamate pyruvate transamenase and serum oxaloacetate transamenase profile revealed that there was a significant difference between the major carps and exotic carps (Table 2).

4. DISCUSSION

The physico chemical and biological factors in the aquatic ecosystem either directly or indirectly affect the life activities of each and every organism in the culture system. According to Hossain and Konar (1990); Nawal Kishore and Sanjay Kumar (1992) reported that the water of the fish culture pond showed alkaline range (Fisher *et al.,* 1998) reported that the turbidity play an important role in the productivity of the pond there by controlling other physical factors. The penetration of light is affected by turbidity and therefore the temperature of water which also affected. In the present study transparency showed significant fluctuation but no seasonal variations. Total hardeness is another important hydro biological factor of the fresh water environment. It is mainly due to carbonate and bicarbonate components of water (Bhaskaran *et al*., 1991). According to *Gelman et al.,* (1992) the thermal properties of intestinal alkaline phosphatase were investigated with silver carp, arboreal fish and three species of tropical and subtropical tilapia, and relationship was observed between the thermal characteristics of the enzyme and the fish origin. Histological and enzyme histochemical changes were observed

Table 1: Physico-chemical Parameters of the Experimental Pond

Months	Temperature °C		pH	Turbidity (cm)	(ppm)		Total dissolved solid (ccl litre)	(mg/l)			
	Atmos-pheric	Water			Alkaliniy	Total hardness		Calcium	Magne-sium	Sodium	Potta-sium
May	36.2±0.1	31.1±0.1	7.±0.01	30±0.7	136.2±4.2	124.3±2.1	406.2±5.3	38.2±1.2	7.1±0.01	64.2±0.2	6±0.2
June	34.3±0.1	29.1±0.1	7.3±0.001	36±0.2	120.2±6.4	140±2.5	448.6±6.3	48±1.3	9±0.02	81.1±0.3	9.3±0.3
July	31.2±0.1	29±0.1	7.5±0.001	38±0.1	160±6.2	152±2.3	470.2±4.3	40±1.4	15±0.01	93±0.2	12±0.1
August	29.2±0.2	26.2±0.1	7.4±0.001	35.2±0.1	132±3.2	160±1.4	511.2±2.3	35±1.6	8±0.001	80.2±0.3	9.2±1.0
September	32.2±0.3	29.1±0.1	7.3±0.003	34±1	168±4.2	165±2.3	538.3±3.2	46±2.9	12±0.01	88.±0.6	10.2±1.3
October	27.1±0.1	25±1	7.2±0.002	37.2±0.3	147±2.5	168±1.6	553±4.2	44±2.0	14±0.01	95±0.4	11.2±1.4

Table 2: Enzyme Profile of Indian Major and Exotic Carps

S.No.	CARPS	ACP(IU/L)	ALP(IU/L)	SGPT(IU/L)	SGOT(IU/L)
1.	*Catla catla*	19.7±0.89	110±1.23	29±0.81	25±0.97
2.	*Labio rahita*	18.9±0.96	102±1.36	23±0.58	24±1.03
3.	*Cirrhinus mrigala*	19.8±0.92	97±1.07	42±0.97	27±1.24
4.	*Cyprinus carpio*	18.8±0.92	1091±1.27	28±0.79	17±0.81
5.	*Hypophthal michthys molitrix*	14.4±0.77	137±0.99	32±0.96	25±1.05

in the kidney of male *Cottus gobio* during the spawning period (Bucher and Hofer, 1993).

Vincent *et al.,* (1995) reported that the acid phosphatase is a non-specific enzyme which hydrolysis into ester. Acid phosphatase is separated from prostate cells, RBC, platelets and WBC. Acid phosphatase total value is increased in prostate cancer and highly elevated in bone metastatis of prostate cancer. According to Sinha (1979) alkaline and acid phosphatase were localized and distributed in the buccopharynx, intestinal bulb, intestine and rectum of *Cirrhinus mrigala* .

According to Vincent *et al.,* (1995) tissue homogenates of the Indian rivering major crap *Catla catla* revealed the extent of alkaline phosphatase acitivity to be higher than that of acid phosphatase activity.Alkaline phosphatase is a non-specific enzyme which hydrolysis aromatic components. Alkaline phosphatase is activated by magnesium and manganese. Zinc is a constant ion of alkaline phosphatase. The mineralization of bone is increased by increasing the activity of this enzyme. The alkaline phosphatase level is increased in blood serum during hepatic diseases**.**

The distribution of alkaline phosphatase along the carp intestine and also it association to the enterocyte merabrance was very interesting (Villanueva *et al.,* 1997). According to Mukhopadyay and Sinha (1983)analysed the alkaline phosphatase (ALpase), adenosine triphosphatase (ATPase) and indices of concentration of organic matter in pond water (Wilson *et al.,* 2002) reported that serum glutamate oxaloacetate transaminase (SGOT) which is also widely distributed in nature. It also exist isozyme form. It is present in highest amount in various tissues like heart, muscles, skeletal muscles, brain, liver, pancreas and kidney. Small amount of these enzymes normally liberated in blood stream. But when a small portion of heart muscle or liver muscle are injured by defect of coronary artery or hepatic artery a large amount of SGOT liberated in to the blood stream in *Cirrhinus mrigala*.. In *Cirrhinus mrigala* showed significant result in alkaline and acid phosphatase and lipase activity in gastrointestinal tract (Goel, 1975).

In Indian major craps *Labe rohita, Catla catla,* and *Cirrhinus mrigala* of alkaline phosphatase activity observed during the early stages of development (Sarkar *et al*., 1996)**.** Wilson *et al*., (2002) reported that using alkaline

phosphatase determination method for detection of Infections Salmon Anaemia Virus (ISAV) in tissue culture and tissue imprints.Thus the study revealed that there was significant difference between the common carps and exotic carps.

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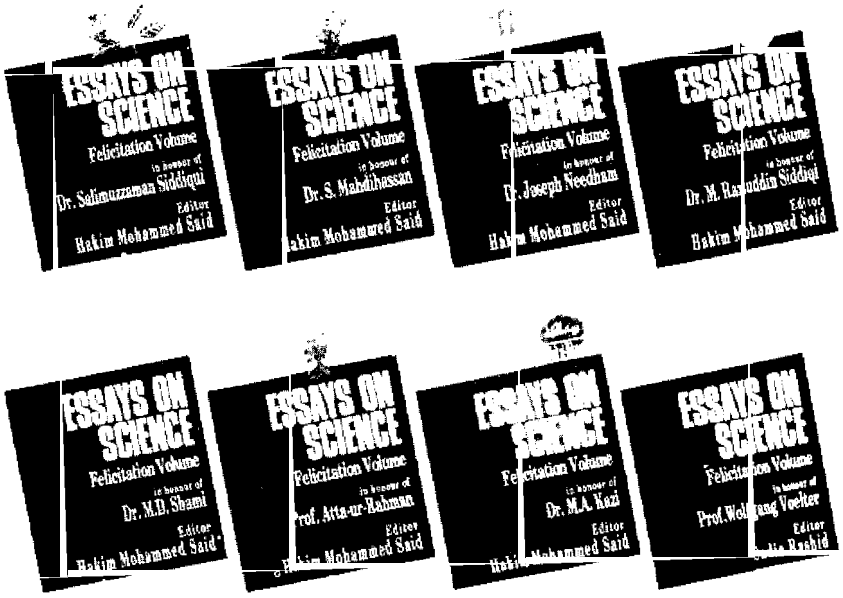

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