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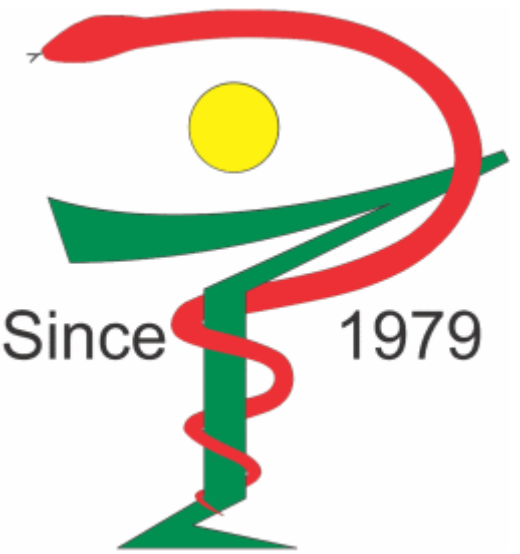

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CONTENTS

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EXTRACTION AND CHARACTERIZATION OF POLAR TERPENIC CONSTITUENTS FROM *Artemisia maritima*

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ABSTRACT

The chemica1 investigation based on finding the polar terpenic constituents in the *Artemesia maritima* has been carried out. Plant material was extracted with n-hexane and ethyl acetate. The ethyl acetate extract showed presence of polar terpenes, which were isolated by column chromatography. Further isolation and purification was carried out by thin layer chromatography and their tentative structures were explained on the basis of their physical properties.

Keywords: *Terpenes, Artemisia maritima*

INTRODUCTION

Terpenes are one of the most important classes of natural products and widely distributed in nature. Most of naturally occurring terpenes are either hydrocarbons having formula $(C_5H_8)n$ or their oxygenated derivatives. Isoprene (2-methyl-l, 3-butadiene) is the basic unit of all terpenoids. They are the important constituent of medicinally important essential oils.[1]

Artemisia maritima is a perenial, woody, branched, hairy herb. It is found in most parts of Khyber Pakhtoonkhwa, a province of Pakistan. Artemisia species are widely used as medicinal plants in folk medicine, and have been incorporated into the pharmacopoeias of several European and Asian countries. Ethnobotanical studies of plant have also been carried out by various workers.[2-7]

Santonin is an important constituent of Artemisia. It is chiefly reported in *Artemisia maritima* while other species show almost negligible content of it. It is an anthelmintic agent widely used previously.

A study on the extraction of santonin has been carried out by S. M. Khafagy *et a,l*[8] It also contains many bioactive sesquiterpenes and flavanoids. Extraction and analysis of sesquiterpenes and flavanoids from the plant has been described by Bilia *et al.*[9] Terpenoidal structure determination and analysis has been explained by various workers and biotransformation of terpenes has also been studied by Carla *et al.*[10-13]

Many pharmacologic activities have been reported for Artimisia plant. It is found to be antimicrobial, anticoccidial, antiplasmodial and anthelmintic.[14-16]

Its use in urinary tract infections and infections due to *Trichomonas vaginalis* has been studied. Fumigant toxicity of its essential oil against common pests in stored products has been studied by Negahban *et al*. It has also been used as a Forage Plant in northern areas of Pakistan.[17-20]

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MATERIALS AND METHOD

- *Artemisia maritima* was collected from Mansehra, Hazara Division, Khyber Pakhtoonkhwa (Pakistan).
- Melting points were recorded in glass capillary tube on Gallenkamp.
- The ultraviolet (UV) spectra were recorded in methanol on U-2000 Spectrophotometer.
- The infrared (IR) spectra were scanned on 270-30 Hitachi Infrared spectrophotometer.
- Thin layer chromatography was performed on glass plates (5 x 20cm) coated with slurry of silica gel G 60 (0.25 mm thick); the plates were activated in oven at 150°C for one hour. While the column chromatography was performed with silica gel 60 (70-230 mesh, E. Merk). The solvents used are given in Table I.

Table I: The solvents and their brands

Solvent	Brand
Methanol	E. Merk (DAB)
Chloroform	E. Merk (MW11938)
n-hexane	LAB-SCAN
Ethyl acetate	BDH Chemicals (Analar)

Spraying reagent used was ceric sulphate (0.1 g) suspended in 4 ml of water. One gram of trichloroacetic acid was added, the solution was boiled and sulphuric arid (conc.) was added drop wise until the turbidity disappeared.

Extraction and identification of terpenoids

The powdered plant (175g) was extracted with 700ml of n-hexane and filtered after three days of maceration. The 3.410 g residue obtained after filtration was extracted with 700 ml of ethyl acetate. The solvent was evaporated under reduced pressure. Removal of the solvent from dried filtrate gave 1500 mg of dark brown residue. Terpenoids were identified by thin layer chromatography (TLC) using methanol-chloroform (99:1) as mobile phase. The chromatographic plates were developed with ceric sulphate-sulphuric acid reagent which gave positive test for terpenoids.

Isolation of terpenoids

A column of kiesel Gel G type 60 was taken. Hundred gram kiesel Gel G 60 was prepared using n-hexane ethyl acetate (99:1) and dark brown residue extracted from the plant was chromatographed on this column using following ratios of eluting solvent as shown in Table II.

As a result of this separation twelve 12 fraction were obtained (Table II). Out of these 12 fractions, nine fractions (2, 3, 4, 5, 6, 7, 8, 9, 10) gave positive test for terpenoids while remaining gave no spots with ceric sulphate–sulsulphuric acid reagent, So further investigation was done on these fractions.

Table II: Isolation of terpenoids fraction using different concentrations of elution solvents by column chromatography

Fraction	n-hexane ethyl acetate ratio	Yield (mg)	Consistency of color	Result after spray with ceric sulphate reagent
1	99:1	61	White	No spot
2	95:5	212	Dark Greenish brown	Two spots
3	90:10	107	Light Greenish brown	One spot
4	80:20	76	Dirty yellow	One spot
5	70:30	118	Greenish yellow	One spot
6	60:40	78	Greenish yellow	One spot
7	50:50	240	Faint green	One spot
8	40:60	63	Faint green	One spot
9	30:70	77	Greenish yellow	One spot
10	20:80	81	Bright yellowish green	One spot
11	10:90	68	Bright yellow	No spot
12	0:100	54	Colorless	No spot

Purification of terpenoids

Terpenoids from each of the fraction 5, 6, 7, 8, 9 and 10 were purified by preparative TLC plates using the solvent system chloroform - methanol (Table III).

Table III: Fractions obtained by increasing concentrations of ethyl acetate

Fraction	Yield (mg)	Pure component (mg)
5	118	93
6	78	61
7	240	213
8	63	50
9	77	63
10	81	69

RESULTS AND DISCUSSION

The present study was undertaken to investigate the polar constituents from *Artemisia maritima*. The fractions obtained from column chromatography of the extract obtained from plant showed presence of terpenoids only in those fractions with increasing ethyl acetate concentration, when analyzed by TLC using ceric sulphate-sulphuric acid reagent. Other fractions were non terpenoid.

The physical constants like melting point, lambda max and infrared (IR) spectra of terpenoids were determined for each fraction. The findings of the above are given in Table-IV.

The UV results indicated that the faction 7 showed the maximum absorbance at 441.5 nm whereas Fraction 9 showed minimum absorbance at 295.5 nm. The IR data of fractions 5 to 10 also indicated that the present components were aromatic in nature along with the present of C-O, C-H, C=O, C=C bonds.

Table IV: Analysis of pooled fractions by UV/VS spectroscopy and IR spectroscopy

Fraction	M.P (C°)	UV max (nm) (Absorbance)	IR (cm-1)				
			C-O	C-H	C=O	C=C	Aromatic
5	169	426.5	1050	2900	1640	1640	1460
6	172	417.5	1048	2900	1820	1620	1460
7	167	441.5	1048	2900	1800	1620	1460
8	174	298	1048	2900	1720	1660	1440
9	176	295.5	1045	2925	1740	1640	1430
10	164	421	1050	2940	1640	1640	1480

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ANTIBACTERIAL ACTIVITY OF *Stachys palustris*

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ABSTRACT

In vitro antibacterial studies were carried out with the hexane, chloroform and ethanol extracts of leaves, stems and roots of *Stachys palustris*. All of them, a part from leaves, stems, roots hexane extracts and ethanol extract of leaves, exhibited antibacterial activity against both gram positive and gram negative bacteria. The chloroform extracts were more potent than the ethanolic extract.

KEY WORDS: *Stachys palustris,* Antibacterial activity

INTRODUCTION

Various herbs of the genus of the Stachys (*Labiatae/Lamiaceae*) are used in the traditional medicine as astringent, tonic, stomachache, vulnerary antiseptic, sedative, antidiarrhoeal, purgative, emetic, cystitis, and also used in asthma, neuralgia, swelling and infected wounds.[1] Many other species of the genus Stachys are also used as a general remedy for the treatment of spasm, cardiac debility (tachycardia), amenorrhoea and neurousis.[2]

Uterotionic, antitumor, cytotoxic and antiviral activities[3-6] have been reported in plant species of the genus *Stachys acteside.* Isolated compounds from *Stachys sieboldii* exhibited antinephritic and antianoxia (Hyaluronidase activity) actions.[7-8] Stachyrene obtained from *Stachys recta* showed antiinflammatory, antitoxic and hypoazothemic responses.[9] Xanthine oxidase and antioxidative effects have been reported by the isoacteside and tubloside isolated from the stems of *Stachys deserticola.*[10] Previous chemical investigation of Stachys species proved presence of alkaloids,[11] flavonoids,[12-15] iridoids tannins, diterpenoids, triterpenoids,[16-18] and other constituent i.e. citric acid, malic acid, oleic acid, bitter principles are also present along with the carbohydrates,[19-21] from different parts. Although the medicinal importance of *Stachys palustris* L. has not been claimed by any worker. Therefore, this study was carried out on extracts of the different parts of *Stachys palustris* for their antibacterial activity.

MATERIALS AND METHODS

Plant material

Stachys palustris L. (*Labiatae /Lamiaceae*) was collected by Gray and MeGill in September, 1997 from the Ground of Rosspriory, University of Strathclyde, Glasgow. Scotland, U.K, and identified by Alan. The voucher specimen (Col 5321) was deposited in the herbarium of Royal Botanical Garden, Kew, Richmond, Surry, TW93AB, U.K.

Preparation of plant extracts

Leaves, stems and roots were separated, dried under shade and successively extracted in soxhlet apparatus with hexane, chloroform and ethanol. Evaporation of solvents in vacuo (ROTAVAPOR-R BUCK SWITZERLAND) provided Leaves: hexane (yield 2.79%), chloroform (C, 2.79%) and ethanol extract (E, 6.99%). Stems: hexane (0.51%), chloroform (C, 0.49%) and ethanol extract (E, 7.69%) roots: hexane (0.68%), chloroform (C, 0.79%) and ethanol (E, 19.40%).

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Microorganisms

The above mentioned extracts were tested againsts a panel of bacteria including the Gram-positive bacteria *Bacillus pumilus* NCTC 10327, *Bacillus subtilis* NCTC 10073, *Staphylococcus aureus* NCTC 10788, *Straptococcus faecalis* NCTC 775, and Gram-negative bacteria, *Serratia marcescens* NCTC 1377, *Proteus vulgaris* NCTC 4175, *Escherichia coli* NCTC 9001 and *Pseudomonas aerugrinosa* NCTC 6750. The organisms were obtained from the microbiology lab., Department, of Pharmaceutical Sciences, Strathclyde University, Glasgow, Scotland, U.K., and maintained as a slant on nutrient agar in McCartney bottles and stored at 4°C .A loopful of the bacteria from the stock culture was aseptically transferred to sterile nutrient broth medium. The organisms were allowed to grow on rotary shaker for 18h harvested and used for the experiments.

Bioassay

The antibacterial study was carried out according to the reported procedure[22], by incorporating into molten nutrient agar plates inocula containing 10^5 to 10^6 bacteria per ml approximately, the plates were allowed to solidify and 6 wells of 8mm diameter were made in each plate. 5.50 and 100 mg/ml of *S. palustris* extracts, and standard antibacterial agents (ampicillin and streptomycin 1 mg/ml) were introduced into the wells. Gum acacia (4.5%) was the vehicle that served as a control. The plates were incubated at 37°C for 24h. The zones of inhibition were determined as means of six replicates.

RESULTS

In-vitro antibacterial activity of hexane, chloroform and ethanol extracts of leaves, stems and roots of *S. palustris* were determined by using 5.50 and 100 mg/ml concentration against four species of Gram positive bacteria (*Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Straptococcus faecalis*) and four Gram negative bacteria (*Serratia marcescens*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aerugrinosa*). The antibacterial spectra of different parts of the plant crude extracts are displayed in (Table I - III).

Table I: Antibacterial activity, as indicated by zone of inhibition of the leave extracts of *S. palustris*. Mean ±SD, N =6)

Treatment	Gram positive microorganisms				Gram negative microorganisms			
	B. pumilus	***B. subtilis***	***St. aureus***	***Str. Faecalis***	***S. marcescens***	***P. vulgaris***	***E. coli***	***P. aeruginosa***
Hexane extr.								
5 mg/ml	-	-	-	-	-	-	-	-
50 mg/ml	-	-	-	-	-	-	-	-
100mg/ml	-	-	-	-	-	-	-	-
Chloroform extr.								
5 mg/ml	16.44 ±0.24	13.34 ±0.17	- -	- -	- -	- -	- -	- -
50 mg/ml	17.42 ±0.53	19.58 ±0.26	- -	- -	- -	- -	- -	- -
100mg/ml	19.94 ±0.26	20.5 ±0.32	-	-	-	-	-	-
Ethanol extr.								
5 mg/ml	-	-	-	-	-	-	-	-
50 mg/ml	-	-	-	-	-	-	-	-
100mg/ml	-	-	-	-	-	-	-	-
Ampicillin	23.21 ±0.45	24.91 ±0.33	19.85 ±0.35	22.39 ±1.33	28.67 ±0.96	21.42 ±1.98	20.9 ±2.34	26.11 ±2.38
Streptomycin	22.4 ±0.5	25.17 ±0.36	19.42 ±0.33	1.48 ±1.25	26.42 ±1.88	22.64 ±1.87	23.4 ±1.84	26.69 ±2.62

Extracts were tested at 5, 50 and 100 mg/ml
Ampicillin and streptomycin were tested at 1 mg/ml
All values were not significantly different at $p > 0.05$
– inactive

Table II: Antibacterial activity, as indicated by zone of inhibition of the stem extracts of *S. palustris*. (Mean ±SD, N =6)

Treatment	Gram positive microorganisms				Gram negative microorganisms			
	B. pumilus	***B. subtilis***	***St. aureus***	***Str. Faecalis***	***S. marcescens***	***P. vulgaris***	***E. coli***	***P. aeruginosa***
	*							
Hexane extr.								
5 mg/ml	-	-	-	-	-	-	-	-
50 mg/ml	-	-	-	-	-	-	-	-
100mg/ml	-	-	-	-	-	-	-	-
Chloroform extr.								
5 mg/ml	22.13 ±0.88	20.44 ±0.83	11.04 ±0.31	10.75 ±0.13	- -	- -	- -	- -
50 mg/ml	23.18 ±1.09	22.55 ±0.71	10.50 ±0.23	11.03 ±0.05	- -	- -	- -	- -
100mg/ml	23.76 ±0.65	23.80 ±0.80	11.64 ±0.19	11.18 ±0.17	-	-	-	-
Ethanol extr.								
5 mg/ml	-	-	-	10.75 ±0.26	-	11.56 ±0.17	-	-
50 mg/ml	-	-	9.58 ±0.13	-	-	-	-	-
100mg/ml	-	-	-	-	-	-	-	-
Ampicillin	24.40 ±0.58	25.40 ±0.43	21.39 ±0.48	22.75 ±0.20	27.46 ±0.27	27.23 ±1.29	25.96 ±0.66	29.98 ±0.82
Streptomycin	25.79 ±0.80	24.33 ±0.20	19.68 ±0.31	19.18 ±0.62	26.57 ±0.40	25.46 ±0.68	25.04 ±0.44	28.20 ±0.43

Extracts were tested at 5, 50 and 100 mg/ml
Ampicillin and streptomycin were tested at 1 mg/ml
All values were not significantly different at $p > 0.05$
- inactive

All the hexane extracts as well as ethanol extract of leaves did not display any antibacterial activity against any of the tested bacteria in this study. All chloroform extracts exhibited the most prominent antibacterial effect against two gram-positive microorganism (*B. pumilus* and *B. subtilis*) when compared with the reference antibiotics such as ampicillin and streptomycin.

DISCUSSION

The ethanol extract of stems and roots displayed antibacterial activity which may be due to polar components like alkaloids, glycosides, saponins, polyols, resins and amino acids. The antibacterial activity exhibited by the chloroform extract of leaves, stems and roots may be due to slightly polar components which are present in these parts of the plant. Slightly polar components seem to be more potent in their antibacterial action than the polar components.

Table III: Antibacterial activity, as indicated by zone of inhibition of the root extracts of *S. palustris*. (Mean ±SD, N =6)

Treatment	Gram positive microorganisms				Gram negative microorganisms			
	B. pumilus	***B. subtilis***	***St. aureus***	***Str. Faecalis***	***S. marcescens***	***P. vulgaris***	***E. coli***	***P. aeruginosa***
Hexane extr.								
5 mg/ml	-	-	-	-	-	-	-	-
50 mg/ml	-	-	-	-	-	-	-	-
100mg/ml	-	-	-	-	-	-	-	-
Chloroform extr.								
5 mg/ml	-	-	-	-	-	-	-	-
			-	-		-	-	-
50 mg/ml	22.23 ±0.83	23.46 ±0.77	- -	- -	11.23 ±0.21	- -	- -	- -
100mg/ml	24.30 ±0.97	24.13 ±0.66	- -	- -	13.10 ±0.05	- -	- -	- -
Ethanol extr.								
5 mg/ml	-	-	-	-	-	-	-	-
	-	-			-	-	-	-
50 mg/ml	-	-	10.50 ±0.14	11.23 ±0.26	-	-	-	-
	-	-			-	-	-	-
100mg/ml	-	-	11.53 ±0.17	10.95 ±0.04	-	-	-	-
	-	-			-	-	-	-
Ampicillin	24.38 ±0.37	25.15 ±0.30	20.91 ±0.31	22.95 ±0.17	27.48 ±0.35	25.02 ±0.99	23.26 ±0.67	27.85 ±0.60
Streptomycin	24.77 ±0.83	24.04 ±0.28	19.14 ±0.23	20.45 ±0.36	27.04 ±0.55	23.86 ±0.47	23.68 ±0.31	26.50 ±0.38

Extracts were tested at 5, 50 and 100 mg/ml
Ampicillin and streptomycin were tested at 1 mg/ml
All values were not significantly different at $P > 0.05$
– inactive

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EFFECT OF ESSENTIAL OILS ON THE PERCUTANEOUS ABSORPTION OF DICLOFENAC DIETHYLAMINE

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ABSTRACT

The aim of present study was to evaluate the effect of various essential oils (eucalyptus, peppermint, turpentine and cod-liver oil) as enhancers on transdermal absorption of diclofenac diethylamine across full thickness, hairless rabbit skin using modified Franz diffusion cell. The receptor compartment was constantly stirred normal saline solution at 37°C. At set intervals up to 24hr, 5ml samples were removed from the receptor compartment and the amount of diclofenac diethylamine permeated through the skin were calculated by the UV absorbance at 276 nm. In the interpretation of results the lag time played an important role. Peppermint oil showed the smallest lag time indicating its rapid enhancing effect. The permeability coefficient calculated for diclofenac under the influence of enhancers showed that peppermint oil was a better enhancer as compared to others at the concentration under study. The enhancing effects were ranked as: peppermint oil > cod liver oil > turpentine oil > eucalyptus oil in this study. The 'Benchmark', flux rate of diclofenac under the influence of enhancer showed that almost all the enhancers increased the penetration of DDA through hairless rabbit skin. The rate constant showed fluctuations at various time intervals. With all enhancers decreased partition coefficients were observed but the diffusion coefficient values obtained were comparatively higher. The mode of action of these accelerants may be described by combined process of partition and diffusion, the diffusion process being dominant.

Keywords: Enhancers, Transdermal absorption, Franz diffusion cell, Partition coefficients

INTRODUCTION

During the past few years, skin has been shown to be a suitable delivery route for drugs formulated in transdermal therapeutic system.[1] Transdermal drug delivery involved the continuous administration of therapeutic molecules through the skin. It has the advantage of maintaining constant drug plasma levels and improving patient compliance.[2] The amount of drug bioavailable for targeting the sites of action is lower than via the oral route, but the absorbed dose appears to the adequate for therapeutic use, particularly because of the absence of side effects.[3]

The skin surface consisted of a highly shiny lipid film of various depths, depending on the location on the body. The stratum corneum, however, is first barrier and much interest has been shown in the percutaneous absorption of chemicals and drugs that elicit a therapeutic toxicological response following skin contact.

Volatile or essential oils are volatile in steam and differ entirely into both chemical and physical properties from fixed oils. With the exception of oils such as oil of bitter almonds, which are produced by hydrolysis of glycol-

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sides, these oils are obtained largely as such from the plant. Volatile oils are used for their therapeutic action, for flavouring (e.g. oil of lemon), in perfumery (e.g. oil of rose) or as starting material for the synthesis of other compounds (e.g. oil of turpentine). For therapeutic purposes they are administered as inhalation (e.g. eucalyptus oil), orally (e.g. peppermint oil), as gargles and mouthwashes (e.g. thymol) and transdermally (many essential oils including those of levander, rosemary and bergamot are employed in practice of aromatherapy.[4]

Diclofenac {(2-[2,6-dichlorophenyl) armino] phenyl-acetate} is a phenyl acetic acid non steroidal anti-inflammatory drug (NSAID) and is a potent inhibitor of prostaglandin synthesis. For the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and acute gouty arthritis therapeutic doses of diclofenac have been proven to be equi-efficacious when compared with other commonly used NSAIDs.[5-6] Diclofenac exhibited potent analgesic effects and is used clinically for the short term alleviation of post-operative pain, dysmenorrhoea and in various ocular conditions.[7] The aim of present study was to evaluate the effect of various essential oils (eucalyptus, peppermint, turpentine and cod-liver oil) as enhancers on transdermal absorption of diclofenac diethylamine.

MATERIALS AND METHODS

Materials and apparatus

Diclofenac diethylamine was supplied by Novartis (China origin), turpentine oil, eucalyptus oil, cod-liver oil and peppermint oil were purchased from MD Traders. Double distilled water, pH 6.8 was used throughout the experiment. Ethanol and sodium chloride were obtained from Merck.

Software-assisted U. V. Spectrophotometer (Agilent 2005) was used for determination of drug in sample (Agilent, Germany). Franz diffusion cell, fabricated by HEJ glass apparatus repairing workshop, Karachi (Pakistan) was used for the permeation experiments.

Animal Skin

In-vitro technique that was used to study transdermal absorption involves the use of animal excised skin; in many cases full thickness was used.[8] Dorsal full thickness skin of male rabbit (white, n=5, weighing 1-2 kg) was used as a permeation membrane. The fat was removed with the aid of scissor.

Control Solution

Ten milligram of diclofenac diethylamine was dissolved in 5 ml methanol in 100 ml volumetric flask and the volume was made up to the mark with normal saline. This was used as reference control solution without any enhancers.

Test Solution

Test solutions were prepared by dissolving 10 mg of diclofenac diethylamine in 5 ml methanol in 100 ml volumetric flasks and the solutions were made up to the mark with previously constituted solutions of enhancer (5% v/v) in normal saline.

Diffusion Cell

Diffusion cell[9] was fabricated locally after some modifications. The cell was in the form of two cylindrical glass half cells termed as upper half cell (donor compartment) and the lower half cell (receptor compartment) and inside diameter was 2 cm. The volume capacity of the donor and receptor compartments was 40ml and 35ml, respectively. The membrane was mounted in between the two half cells and the exposed penetration area was approximately 3.14cm^2. From the lower half of the receptor compartment at a distance of about 3.8 cm a side arm 4 cm in length is used for taking the sample and correcting the volume of receptor compartment with the help of normal saline solution by exposing the epidermal side toward the donor half cell. The two half cells, after clamping were mounted on a magnetic stirrer and small magnetic fleas were placed in the receptor compartment, and the receptor solution was stirred at 60 rpm.

Membrane Preparation

The membrane, full thickness skin was taken from the abdominal surface of the hairless rabbit. The skin at the lower abdomen was marked and hairs were cut and then rabbit skin was sacrificed and whole skin was removed and a rectangular section marked was excised from the animal with surgical scissors. Since the skin was not firmly attached to the viscera it was lifted easily from the animal after the incision was made. Prior to the skin removal, a uniform circle was made on the abdomen, marking the precise skin section to be positioned between the two half cells after the excised skin was trimmed into an oversized rough circle it was mounted between the half cells with the marked section centered. The skin was placed in a normal saline solution before mounting on to the diffusion cell.[1, 10]

Charging the cell

The receptor cell filled with normal saline was stirred by magnetic stirrer at 60 rpm for 30 minutes, at which time the compartments were evacuated with a syringe and refilled with fresh normal saline. Then the compartments were evacuated a second time, refilled, evacuated a third time and finally refilled with normal saline. The donor compartment of the cell was charged with a test solution.

Permeation

The donor compartment of the cell was charged with a test solution containing 1% of diclofenac diethylamine plus 5% v/v of each enhancer dissolved in 100ml of normal saline. The receptor cell contents were stirred and at predetermined time intervals, samples were taken and transferred to the small bottles having stoppers, using 10 ml syringe the time of charging the donor compartment was noted at the beginning of the diffusion runs and the receptor samples were reference to this time.

Sampling

From the side arm of the receptor compartment, 5 ml of the sample was drawn at each time interval with the help of 5 ml syringe and correcting the receptor half cell volume with pre-thermostated normal saline. The sample taken from the receptor cell, a portion of 3 ml was taken and was run on U.V. Spectrophotometer (Agilent2005; software version 2005) at λ 276 nm.

RESULTS AND DISCUSSION

The permeation profile of the receptor phase concentrations in microgram per 100ml is summarized in Tables I-II.

The lag time of the plots was calculated graphically by extrapolation from the pseudo steady state region of the graph of the total amount penetrated versus time to the X-axis.

The Flux (J) of a drug is directly proportional to its thermodynamic activity of the drug (Equation 1).

$$J = D\frac{d_c}{d_X} \ (\mu g.cm^{-2}.h^{-1}) \text{ Equation 1}$$

Where D is the diffusion coefficient and is a function of the size, shape and flexibility of the diffusing molecule as well as the membrane resistance; C is the concentration of the diffusing species; X is the spatial coordinate.

Although the solution for J with various boundary conditions and membrane heterogeneities can be very complex, the basic concepts regarding flux enhancement can be found in Equation (5). The concentration gradient is thermodynamic in origin, and the diffusion coefficient is related to the size and shape of permeant and the energy required to make a hole for diffusion.

Thus enhancement of flux across membranes reduces to consideration of:

- Thermodynamics (lattice energies and distribution coefficients).
- Molecular size and shape.
- Reducing the energy required to make a molecular hole in the membrane

The agents to alter the barrier energy to form hole is a somewhat empirical process but the task is being approached from a basic study of kinetics of expanding the structure of proteins and lipids.[11]. For example, the effects of agents on the compressibility of monolayer film are studied through Langmuir troughs. Various spectroscopic techniques are employed to investigate detailed molecular interactions.

Flux which measures the mass of material transported through the skin is more relevant parameter, therapeutically, than the permeability coefficient.[12]

The diffusion coefficients (D) of different concentrations of enhancers are calculated by dividing the square of the thickness of the rabbit incised skin by 6 × lag time (Equation 2).[13]

$$D = \frac{h^2}{6L} \ (cm^2.h^{-1}) \text{ Equation 2}$$

Where h is the thickness of the rabbit skin and L is the lag time.

Permeability coefficient (P) was calculated by dividing the diffusion coefficient by square of the effective absorption area of the skin in contact.[14]

$$P = \frac{D}{A^2} \ (cm.h^{-1}) \text{ Equation 3}$$

A is the effective absorption area of the skin in contact.

As a measure of the penetration enhancing activity of enhancers, the enhancement ratio (ER) was calculated as under.[15]

$$ER = \frac{P_a}{P_b} \text{ Equation 4}$$

Where P_a is P after application of penetration enhancer and P_b is P before application of penetration enhancer.

The values indicate that the penetration may be dependent on the lipoidal solubility of the drug moiety. However, the permeation may be complicated by charge effect and also may depend on the skin partition coefficient of the drug between the aqueous phase and lipid phase of the barrier.[13]

$$P_C = \frac{P}{D} \text{ Equation 5}$$

The diffusion coefficients presented in Table II reflects its effects on permeability coefficients of diclofenac diethylamine. The change in lag time, changes the diffusion coefficients of diclofenac diethylamine that increases with decrease in lag time.[10, 16]

Permeability rate constant of various concentrations of enhancers was calculated which are summarized in Table III. As is assumed that the whole penetration process is first order rate constant, the rate constant then can be calculated as under:[17]

$$\text{Rate Constant} = \frac{\text{Log}(y_2 - y_1)}{t_2 - t_1} \times 2.303 \quad \text{Equation 5}$$

Typical results have been shown in the Tables I to III and data were subjected to Microsoft Excel 2003 for analysis. Table I shows the lag time (h), diffusion co-efficient (D), initial flux value (J) and ER while the Table II shows permeability co-efficient (P) & Partition co-efficient (Pc) of diclofenac diethylamine before and after treatment of rabbit skin with enhancers.

Table I: Penetration of diclofenac diethylamine with or without enhancer

Enhancer	Lag time (hr)	Diffusion Coefficient (D) ($cm^2.h^{-1}$) $\times 10^{-5}$	Flux (J) ($\mu g.cm^{-2}.h^{-1}$) $\times 10^{-5}$	ER
Peppermint Oil	0.50	70.53	9.7	8
Turpentine Oil	6.75	5.22	7.2	5.9
Eucalyptus Oil	8	4.4	6	4.9
Cod-Liver Oil	8.75	4.03	5.4	4.4
Control	4	8.81	1.22	-

ER = The ratio of Flux with or without enhancer

Table II: Permeability coefficient & diffusion coefficient of diclofenac diethylamine with or without enhancer

Enhancer	Permeability Coefficient(P) ($cm.h^{-1}$)$\times 10^{-5}$	Partition Coefficient (P_C)
Peppermint Oil	4.88	0.0691
Turpentine Oil	0.36	0.0689
Eucalyptus Oil	0.30	0.0681
Cod-Liver Oil	0.27	0.0669
Control	0.61	0.0692

DISCUSION

It has long been known that the subcutaneous provides the skin's primary diffusion barrier.[18] Correlations of skin permeability coefficients, P_C, versus physical properties of a wide variety of permeants have shown that skin can be effectively modeled as a simple lipid barrier to compounds having at least moderate water and oil solubilities.[19-23] In combination with the structural detail and evidence from electron microscopy[24] and other physical characterization techniques[25-26], this observation has led many researchers to conclude that the primary transport pathway for most materials traversing the SC is intercellular.[23-25, 27] If this is true, it follows that the arrangement of the corneocytes within the lipid matrix is a key determinant of the skin's permeability, as it would influence the effective path length for diffusion. The cod liver oil demonstrated a long lag time (6.75hrs) and since the steady state flux could not be obtained within 12hrs of this study. Only the initial flux value was calculated

Table III: Effect of enhancers on permeability rate constant (R) of diclofenac diethylamine through hairless rabbit Skin

Enhancer	0-2 hours	2-4 hours	4-6 Hours	6-12 hours	12-24 hours
Peppermint Oil	1.8409	N.A	N.A	0.3966	0.2715
Turpentine Oil	0.915	N.A	0.0350	0.000073	0.1806
Eucalyptus Oil	0.629	0.4315	N.A	0.0881	0.1850
Cod-Liver Oil	1.0618	N.A	N.A	0.2184	0.2564
Control	0.970	- 0.574	N.A	- 0.0377	0.0756

The control value for "P" of diclofenac diethylamine in the untreated skin at 37 ± 0.5°C was 0.61×10^{-5} $cm.h^{-1}$ with a lag time of 4 hour.

The oils very significantly increased drug permeation across the skin. The most effective oil as enhancer for the drug permeation across the skin was peppermint oil (ER=8) ($P<0.01$) followed by turpentine oil (ER =5.9), eucalyptus oil (ER =4.9) and cod-liver oil (ER =4.4) showed less activity as enhancers than both the above oils. The steady-state permeation was observed for only ½ -1 hours increase of peppermint oil, turpentine oil and eucalyptus oil, while cod-liver oil showed negative steady-state permeation. As the diclofenac diethylamine concentration in the donor compartment of the cell was significantly higher than the receptor compartment; therefore, the decrease of permeability after the steady state may contribute to the wash up effect of enhancer in the diffusion cell.[28]

The treatment with cod-liver oil did not improve the partitioning whereas peppermint oil, turpentine oil and eucalyptus oil enhanced the partitioning. From the diffusion co-efficient values, it can be seen (Table II) that use of oils as enhancer have decreased the resistance to diffusion of drug.[29, 34]

Current drug permeation enhancers (oils) enhanced drug delivery through biological membranes (such as skin or mucosa) by causing some physicochemical changes with in the lipophilic membrane barrier and has been observed that the aqueous exterior of membranes could be just as effective barrier as the membrane itself.[30]

Cod liver oil is rich in unsaturated fatty acids and has been reported that the extracted fatty acids from the cod-liver oil showed significant enhancing effect and the fatty acid profile of the extract was almost identical to that of the oil . So it has been observed that penetration enhancing effect of cod-liver oil may be associated with unsaturated fatty acid portion of the extract, interestingly, cod-liver oil itself did not enhance transdermal drug delivery.[30]

Some essential oils and their terpenes constituents have been investigated as potential enhancers. Eucalyptus oil increased the total flux of diclofenac diethylamine (6.0×10^{-5} ($\mu g.cm^{-2}.h^{-1}$) permeating excised hairless rabbit skin. A series of terpenes and some essential oils have been investigated from their penetration enhancing effect toward drugs. In the present study, eucalyptus oil was found to be active as compared to other oils. So oils can be used as effective enhancers because their safety is well documented.[31-32] From the enhancing effect of the graded fractions of eucalyptus oil it can be observed that the fractions with higher boiling points exert more significant effect than the original oil. Eucalyptus oil is mainly composed of 1, 8-cineol, which has a boiling point higher than the constituents present. Therefore, fraction of eucalyptus oil obtained at 140°C under vacuum has caused about 83 fold increase in the drug flux, and this is in agreement with William et al. 2003,[33] who found that 1, 8-cineol caused a 95 fold increase of drug permeability.

CONCLUSION

The partitioning ratio suggested that the enhancers increased the partition of DDA into the skin. As the drug is less soluble in oil than water, the increased partition of drug into the skin can be contributed to the structure modification of the stratum corneum lipid bilayer. Therefore, it could be concluded that the enhanced permeation of drug may not be only increasing the partition of drug into the stratum corneum, but also by modifying intercellular lipids, disrupting their highly ordered structure and thus increasing the permeation of DDA through the membrane. Furthermore, the latter was more important than the former in permeation because the increased amounts of drug in the skin may also be the retention of the drug by the skin.

This study also verifies the idea that essential oils may offer a large and useful selection of relatively safe penetration enhancers to aid topical drug delivery.

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STUDY OF NON-COMPLIANCE AND ITS REASONS IN OUTDOOR PATIENTS WITH MENTAL ILLNESS OF A PUBLIC HOSPITAL

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ABSTRACT

Compliance with prescribed therapeutic regimen of outdoor patients was studied by interrogating patients about planning they make to take the medicine. A questionnaire was prepared to evaluate patient's understanding, behavior and motivation to take medication in accordance with prescribed regimen. The results showed that about 13% of patients did not come to refill the prescription, because of distant healthcare facility. Non-compliance was found to be 20% due to patient's own choice, 26% due to dissatisfaction on the treatment, 24% because patient could not understand the instructions of prescription and 17% because of multiple attendants, disturbance of working routine and cultural beliefs.

Keywords: Non-compliance, Prescription, Patient understanding

INTRODUCTION

Compliance to medication regimens has been monitored since the time of Hippocrates.[1] In the recent years, it has become a focus of increasing concern in the treatment of psychiatric disorders. Non-compliance is a common, prevalent and important issue in the treatment of psychiatric illnesses.[2] Compliance to treatment is the degree to which a patient carries out the clinical recommendations of the physician or pharmacist or in other words non-compliance is the failure of the patient to follow the prescribed treatment regimen. Non-compliance is a significant problem in all patient population ranging from pediatrics to the elderly patients.[3, 4] It applies nearly to all chronic disease states and settings. Nowadays, non-compliance is considered to be the major problem in the health services of both developed and developing countries.[5]

Non-compliance to drug therapy is very common in Pakistan. The reasons for non-compliance reported were the lack of awareness about the benefits of treatment, non availability of healthcare services, non affordability of medicine, side effects and unfriendly attitude of physicians.[6]. Poor infrastructure of society, lack of proper knowledge of mental illness to patients and multiple caregivers could also be considered as some of basic reasons for non-compliance.[7] The other reported reasons of non-compliance were the beliefs of patients that the medications were not working and the medicines have physical side effects.[8]

MATERIALS AND METHODS

Outdoor patients of Punjab Institute of Mental Health Lahore (PIMH) were selected as subjects for the study. The patients were included in the study on the basis of

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their willingness to discuss their drug related problems with pharmacist openly. This is a cross sectional study in which the behavior and motivation of patients towards medication therapy was assessed by interviewing the patients. Trained pharmacist with validated questionnaire interviewed the patients to note the compliant or non-compliant behavior of the patients. A total of 150 patients with their prescription were selected randomly over a period of fifteen days. According to this questionnaire, patient's personal information including name, age, weight, gender, marital status, education, attendant's relation, address and socio-cultural background was recorded. Then patients were asked about their compliance with medication according to format of the prepared questionnaire.

Most of patients (about 64%) were willing to give information. A total of 150 patients were asked about the compliance with prescription medicines. Some of them (36%) did not respond properly and were excluded from the study, while others were happy to receive extra attention from healthcare professionals. Most of the patients were illiterate (92%) and belonged to the rural areas (67%) and not have good understanding about their prescriptions.

A large number of patients were not complying with the prescribed medication therapy. The major reasons for non compliance observed in patients are summarized in Figure 1.

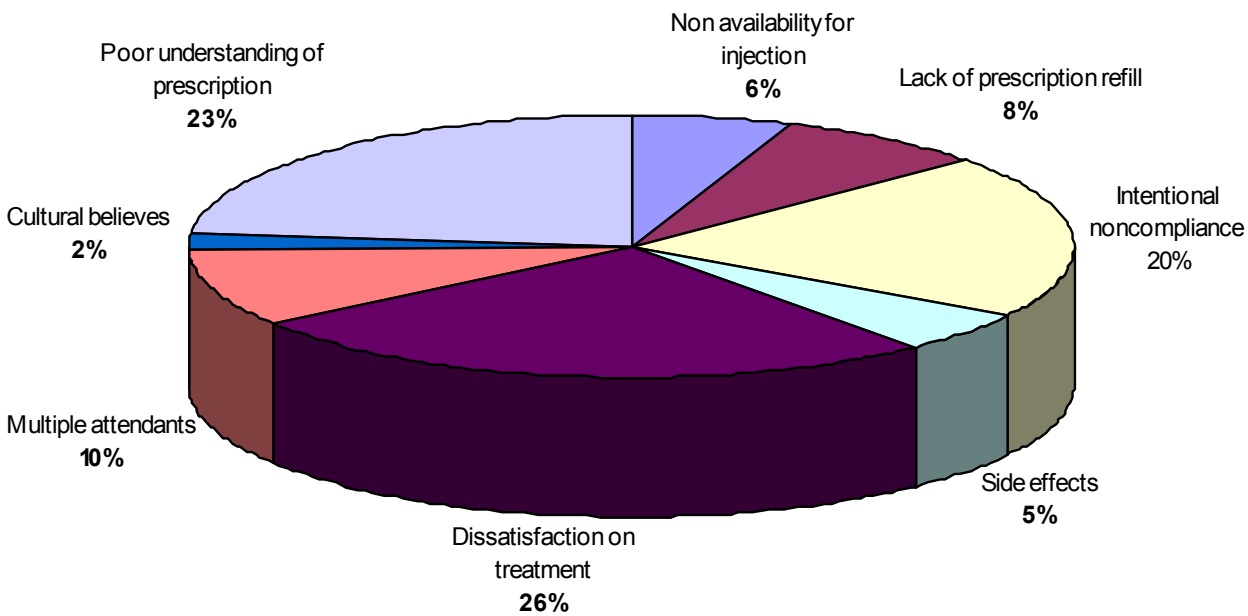

Figure 1: Several reasons of non-compliance along with their respective percentages

The result showed that the commonest reason of non compliance in psychiatric illness could be the hopelessness of patients that might lead to dissatisfaction on treatment (26%) and intentional non-compliance (20%). The reasons of hopelessness might be long term therapy and the supernatural believes. Patients did not want to continue the therapy for long time and were eager to get positive results of therapy as early as possible. As the psychiatric medications showed their results after a long time, hence, the patients become unsatisfied. The lack of prescription refill could be due to socio-cultural background and distant residences of patients from healthcare services (Average 120km) in 8% of patients. The other reasons might include patient's own choice to follow the schedule of doses because of disturbance of their routine work as the side effects of medication appeared (5%), multiple care givers (10%), fear of treatment (6%) and patient's inability to understand the prescription (23%) as shown in Figure 1. Many of these reasons of non-compliance were not

reported in any study previously. Our results conform the findings of Mahmood *et al*.[12]

The social and cultural stigma related to psychiatric illnesses and their treatment and doctor- patient relationship also play a significant role in patient compliance. The patients do not follow the medication regimen as per advice either due to aggressive behavior or forgetfulness to take medicine. Patients stop medication without consulting the doctor when they find themselves stable or skip the medicines due to sleep. Some patients also decrease the dose by their own perception as they feel side effects with prescribed doses. The illiteracy of patients was another reason of non compliance due to which they were unaware of their disease and mechanism of cure and time required for treatment with such medication.

Multiple care givers including mother, father, sister, brother or some times other relatives involved in the care of psychiatric patients might pose another problem in compliance. It is better to have only one care giver. In case of multiple care givers, patient and caregivers depend on each other and dose may be skipped. Medications used to treat mental illnesses are known to have an array of potentially unpleasant side effects ranging from restlessness and pacing to excessive sedation, tremor, dry mouth, constipation, impotence, weight gain, missed menstrual cycles and many others. This study showed that side effects of psychotropic drugs were also a reason for non-compliance.

In majority of the cases only attendants come to hospital to get medicine for their psychiatric patients. This is because many people were coming from distant areas and traveling with such patients in public transport was difficult and also there was chance that patient might fled away or lost. In this situation if the prescription had one or two injectable (s) non-compliance results. Many of such patients did not purchase injectables to be administered at home.

CONCLUSION

Non-compliance is common, prevalent and important issue in the treatment of psychiatric illnesses. Healthcare professionals especially pharmacists should take leading role to educate the patients and their caregivers about the course of disease and importance of complying with medication therapy along with others measures in the treatment of psychiatric illness.

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STUDY OF PHENYLBUTAZONE TOXICITY IN AVIAN SPECIES

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ABSTRACT

A vulture crisis is an important environmental problem, which happened because of utilization of unsafe anti-inflammatory and analgesic drugs. The clinical profile of phenylbutazone is not very much different from other NSAID's. Despite of sharing pharmacological usefulness, phenylbutazone also shares unwanted effects which may eventually lead to the serious therapeutic complications and ecological imbalance. Therefore; we have aimed this study to evaluate the effects of toxic doses of phenylbutazone in broiler chickens. Two hundred and twenty five (225) healthy broiler chickens were reared up to 28 days and were divided into 5 groups each comprising 25 birds. On day 29 four groups were dosed 50mg/kg body weight twice a day intra-muscularly for 4 days. Food and water were provided *ad libitum*. A physical examination, toxicity and mortality rate were recorded daily. Blood samples were drawn to determine the serum values of aspartame transaminase (AST), alanine transaminase (ALT), uric Acid, alkaline phosphatase (ALP), and creatinine. Postmortem was performed on day 41. In second experiment other 100 birds were divided into 5 groups, each comprising 20 birds. One of the groups was injected I/M phenylbuazone 100 mg/kg twice a day. Postmortem was performed after medication on day 5. Based on the necropsy findings and biochemical analysis, phenylbuazone was not found to be safe in the avian species. Thus, it is suggested that the veterinary use of phenylbuazone should be avoided.

Keywords: Phenylbutazone toxicity, Broiler birds, LFTs

INTRODUCTION

Phenylbutazone; (3, 5-Pyrazolidinedione, 4-butyl-1, 2-diphenyl-Butazolidin, $C_{19}H_{20}N_2O_2$) is a white to off-white, odorless, crystalline powder. Soluble in alcohol, water, acetone and ether. It has similar anti-inflammatory effects and different toxicity profile as compared to the other salicylates.[1] Like aminopyrine, phenylbutazone can cause retention of sodium and chloride ion, edema, nausea, vomiting epigastric discomfort.[2],skin rashes, peptic ulcer hemorrhage[3] perforation, hypersensitivty reaction, serum sickness, ulcerative stomatitis, hepatitis, nephritis, aplastic anemia, leukopenia, agranulocytosis and thrombocytopenia[4] A number of deaths have also been reported, especially from aplastic anemia and agranulocytosis. Keeping the above in view, we aimed this study to investigate the toxicity and evaluate the safety of phenylbutazone in avian species to avoid hazards in wild life.

MATERIALS AND METHODS

The experiment was conducted at experimental sheds of the Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore. One hundred and fifty (150) day old broiler chicks collected from the "Pakistan Hatchery, Lahore" were

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vaccinated according to the vaccination schedule given in Table I. The phenylbutazone (Orient Labs. Pvt. Limited) was injected twice a day.

Table I: Vaccination schedule

Age	Vaccine	Route
6th day	Newcastle disease	Eye
15th day	Gumboro (I.B.D)	Oral*
21st day	Newcastle disease	Oral*
25th day	Gumboro (I.B.D)	Oral *

*With drinking water

MATERIALS AND METHODS

Experimental Design

On day 28, 75 birds were randomly divded into 2 groups comprising; group A with 50 and group B with 25 birds. On day 29, phenylbutazone I/M 50mg/kg body weight was injected twice a day up to four days indivdually to each bird of group A, for four consecutive days. Group B was kept as control. The remaining 75 birds were divided into two groups C with 50 and D with 25 birds.

Each bird of group C was injected phenylbuazone I/M 100 mg/kg body weight twice a day for four days and group D was kept as controlled without medication. Food and water was provided *ad libitum*. A daily basis record of physical examination, sign and symptoms and toxicity was maintained regularly.

Sample Schedule and Parameters Determined

The sampling schedule and different parameters were investigated by Asif et al[5, 11]. Three ml blood sample from birds of each group (A, B, C and D) was collected before the start of medication on day 29. Then blood sample from the same birds were drawn from wing vein (vena cutanea ulnaris) on days 33, 37 and 41 after medication for determination of serum values of following parameters; aspartate transaminase, alanine transaminase,[6] uric acid, alkaline phosphatase (ALP)[7] concentration of creatinine in serum[8].

Clinical Findings and Statistical Analysis

The clinical findings mortality and postmortem were recorded during the study. The collected data were analyzed statistically with one way analysis of variance.[9] On days 41 and 47 the postmortem were done. Three parameters; postmortem, liver, kidney biopsies and staining of specimens were examined.

RESULTS

The biochemical parameters including uric acid, creatinine, alanine transaminase, aspartate transaminase and alkaline phosphatase were noted for test and controlled birds before and after phenylbutazone dose. The necropsy findings of experimental chicks were also recorded, as given below.

Biochemical Parameters of Phenylbutazone

Phenylbutazone I/M 50mg/kg and 100 mg/kg body weight were injected to each bird of group A and B respectively; twice a day for four days and following parameters were measured.

Uric Acid: As shown in Table II, the mean values of uric acid of phenylbutazone were 5.960040 mg/dl, 5.130200 mg/dl, 5.532480 mg/dl and 5.234160 mg/dl before medication, 1st, 5th and 9th days after medication, respectively. There was no significant difference in the mean values of uric acid of phenylbutazone Table III.

Table II: Biochemical parameters of phenylbutazone group. Mean±SEM, N=5

Time of sample collection	Uric Acid mg/dl	Creatinine mg/dl	ALT µg/L	AST µg/L	ALP µg/L
Before medication	5.960040 ± .331098	1.334880±0.1094	10.979760 ± 0.4342	184.13982 ± 6.05195	29.252640 ±2.70735
1st day after medication	5.130200 ± .218903	1.283580 ±0.1301	15.639820 ± 1.3141	299.63830 ± 4.95355	59.784000 ±2.07406
5th day after medication	5.532480 ± .292441	1.269640 ±0.1134	15.633520 ± 1.9852	242.31240 ± 7.90280	65.208000 ±2.05354
9th day after medication	5.234160 ± .363122	1.151620±0.1050	11.045560 ± 0.5830	187.74400 ±14.48778	59.564000 ±4.11303

Table III. Statistical analysis of phenylbutazone data

Parameters		Sum of Squares	Df	Mean Square	F	Sig.
Uric acid	Between groups	2.075	3	0.692	1.476	0.259
	Within groups	7.498	16	0.469		
	Total	9.573	19			
Creatinine	Between groups	9.001E-02	3	3.000E-02	.455	0.718
	Within groups	1.056	16	6.599E-02		
	Total	1.146	19			
ALT	Between groups	106.918	3	35.639	4.601	0.017
	Within groups	123.928	16	7.745		
	Total	230.846	19			
AST	Between groups	44401.552	3	14800.517	35.502	0.000
	Within groups	6670.280	16	416.892		
	Total	51071.832	19			
ALP	Between groups	4006.317	3	1335.439	32.606	0.000
	Within groups	655.312	16	40.957		
	Total	4661.629	19			

Biochemical Parameters in Control Group

No medication was given to group B and D.

Uric Acid: As shown in Table II, the mean values of uric acid of normal or control bird were 5.031800 mg/dl, 4.776720 mg/dl, 5.479140 mg/dl and 4.874880 mg/dl at 1st, 5th and 9th day, respectively. There was no significant difference in the mean values of uric acid of control birds.

Creatinine: The mean values of creatinine in control birds were 1.052080 mg/dl, 0.972660 mg/dl, 1.134440 mg/dl and 1.066040 mg/dl at 1st, 5th and 9th day, respectively. There was no significant difference in the mean values of creatinine in control birds.

Alanine Transaminase (ALT): The mean values of alanine transaminase of control birds were found to be 10.149740 µ/L, 10.205000 µ/L, 10.269660 µ/L and 10.351780 µ/L at 1st, 5th and 9th day, respectively. There was no significant difference in the mean values of ALT in controlled birds.

Aspartate Transaminase (AST): As shown in table IV, the mean values of aspartate transaminase of control birds were found to be 193.555 µ/L, 199.435 µ/L, 158.98660 µ/L and 166.81000 µ/L. There was no significant difference in the mean values of aspartate transaminase of control birds.

Alkaline Phosphatase (ALP): The mean values of alkaline phosphatase of piroxicam were 27.252000 µ/L, 34.470000 µ/L, 33.548000 µ/L and 27.824400 µ/L. There was no significant difference in the mean values of alkaline phosphatase of control birds.

Necropsy of Experimental Chicks in Different Groups

The birds were slaughtered at the end of experiment and different lesions in kidney, liver and muscles were recorded. Each bird of group C was injected with I/M phenylbutazone 100 mg/kg body weight, twice a day for four days. Each bird of group C was injected with I/M phenylbutazone 100 mg/kg body weight, twice a day for four days. Findings are given in Table IV.

Control Group: Group B and D acted as control (without medication).

Table IV: Necropsy of various drugs in broilers chicks, N=15

Drug	Dose	Postmortem lesions		
		Site of injection	Liver	Kidney
Phenylbutazone	50 mg/kg	10/15	7/15	0/15
	100 mg/kg	15/15	12/15	0/15
	No medication	0/15	0/15	0/15
Control	No medication	0/15	0/15	0/15

DISCUSSION

Phenylbutazone is a widely used non-steroidal anti-inflammatory drug with little anti-pyretic and analgesic effects. The adverse effects associated with Phenylbutazone include; peptic ulcer hypersensitivity reaction ulcerative stomatitis, hepatitis nephritis aplastic anemia and agranulocytosis. The current study showed that there was no toxic effect on kidneys in broiler chicks as indicated by the necropsy finding and biochemical analysis of serum uric acid and creatinine. GIT toxicity, edema of small intestine, erosions, and ulcers of large colon and development of renal crest necrosis were observed.[4] The results of the present study differed from the above observations which might be due to different species used for experiment. The findings of present study are partially in agreement with the observations of Kari and co-workers,[3] who investigated the toxic effects of phenylbutazone on the kidneys and liver in mice and reported different lesions as hemorrhage, centrilobular cytomegaly, fatty metamorphosis, cellular degeneration, coagulative necrosis and clear cell foci in liver. They also observed lesions in kidney which were not found in the present study which might be due to different animal species used for experiments.

Hepatotoxicity was observed in phenyl butazone treated group, while there no nephrotoxicity was found. Muscle necrosis was observed in almost all of the birds at the site of injection. There was no significant difference in serum uric acid and creatinine levels which indicated that phenylbutazone had no toxic effects on kidneys, but there was significant difference in the values of serum ALT, AST and ALP in phenylbutazone treated group as stated by Embert, 1986[10]

The levels of ALT, AST and ALP might be elevated due to cellular degeneration or destruction in liver muscles and acute hepato cellular necrosis or bilary obstruction. Similar observations were recorded in the present study. It could be concluded that phenyl butazone is hepato toxic in avan species even at the dosage rate of 50 mg/kg body weight.

The postmortem of the control group revealed no abnormalities particularly in liver, kidneys and muscles. Similarly there was no significant difference in the serum values of uric acid and creatinine ALT, AST and ALP in the samples collected at the different times during experiments in the group.

CONCLUSION

The phenylbutazone was studied for its toxicity in broiler chicks. No mortality was recorded in all groups. Based on the necropsy and biochemical studies, phenylbutazone was not found to be safe in avian species. In context with vulture's crises, phenylbutazone should be avoided in veterinary practice.

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A REVIEW OF *Euphorbia pilulifera L.*

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ABSTRACT

Plant extracts, that have common utilization in the traditional medicine as curative agents, are being used in modern medicine. Euphorbiaceae is a big plant family and contain many plants that are medicinal very important and have common utilization for the treatment of different disease conditions in the conventional medicine systems. *Euphorbia pilulifera* L is one of the plants of this family and have constituents that are valuable curative agents. In addition, the latex from its leaf and other parts of the plant is irritant to skin. It is used in many female disorders; it has property to increase the milk flow in woman and is also used in respiratory problems. In subcontinent it is used to treat worms in children. It is employed in a variety of treatments because of its different constituents. A review of this plant is being presented in this article.

Keywords: Euphorbia, Asthma, Irritancy

INTRODUCTION

Role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. For example, we have western medicine with origins in Mesopotamia and Egypt, the unani (Islamic) and Ayurvedic (Hindu) systems centered in western Asia and Indian subcontinent and those of the orient (China, Japan, Tibet, etc.)[1]. How and when such medicinal plants were first used is, in many cases, lost in pre-history. The plant kingdom thus presents an enormous reservoir of pharmacologically valuable materials still to be discovered[2]. Nearly 100,000 secondary plant products have so far been isolated and characterized.[3]

Medicinal plants used in the traditional medicines should therefore be screened for their safety and efficacy, in the light of modern scientific investigations.[4-7] In recent years, there has been growing trends to evaluate the chemical constituents of the medicinal plants, used in the traditional medicine on different biological and pharmacological parameters, which lead to a systematic therapeutic utilization.

During a general screening of medicinal plants from local flora, it was observed that most of the medicinal plants belong to famous family *Euphorbiaceae*. Several plants of this family are of high economic value.[8-9] Most of the species are poisonous, causing sicknesses or death if ingested. Dermatitis was also caused by many species, if juice of the plant contacts the skin. Even the rain water dripping from certain plants is enough to cause dermatitis.[8-9] *Euphorbia pilulifera* L. is used in different female disorders and also in the treatment of respiratory diseases like asthma and bronchitis. The chloroform extract of *Euphorbia pilulifera* L. is irritant to skin.[10]

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DESCRIPTION

Euphorbia pilulifera L. or *Euphorbia hirta* L.

Family*: Euphorbiaceae*

Euphorbia pilulifera

Vernacular Names and classification:

Urdu	*Dudhi*
Unani	*Dhudi Kalan, sheer jiyah*
Sanskrit	*Raktavinduchada*
English	*Australian asthma weed, snake, weed, cat's hair, milk weed*
Bengal	*Barakeru, Brokeruee*
Hind	*Dudhi*
Mah	*Nayeti, Dudhali, Goverdhan*
Telugu	*Bidarie, Nanbala*
Tami	*Amumpatchaiyariss*

Classification of *Euphorbia pilulifera*

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta– Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta–Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Euphorbiales
Family	*Euphorbiaceae* – Spurge family
Genus	Euphorbia
Species	*Euphorbia pilulifera* L

PLANT FAMILY *EUPHORBIACEAEcs*

Euphorbiaceae is a large family of angiosperms. It includes 300 genera and around 7500 species.[11] Most of the members of this family are herbs, sometime shrubs and trees. Some species of genera *Euphorbia* are xerophytic.[12] Family *Euphorbiaceae* is widely distributed throughout both hemispheres and ranges in morphological form, from largest desert succulent herbs to trees.[13]

GENERAL BOTANICAL DESCRIPTION

Euphorbia is one of the most diverse genera in the plant kingdom. Plants are often annual or perennial herbs, woody shrubs or trees with a caustic, poisonous milky sap (latex). The roots are fine or thick and fleshy or tuberous. Many species are more or less succulent, thorny or unarmed. The main stem and side branches of the succulent species are thick and fleshy, 15-91 cm (6-36 inches) tall. The deciduous leaves are opposite, alternate or in whorls. In succulent species the leaves are mostly small and short-lived. The stipules are mostly small, partly transformed into spines or glands. Like all members of family *Euphorbiaceae*, it has unisexual flowers.[14]

Distribution

Euphorbia pilulifera L. is common weed found throughout India on the waste grounds and in loamy soils.[15-16] It occur mostly in summer and rainy seasons but found occasionally in winter season along road side and waste grounds. It is also found in Pakistan and many tropical countries but it is exported mainly from India.[15-18]

Morphology

The plant is an annual, erect or ascending, 14-50cm high, covered with long yellowish hairs; stem usually terete, branched often four angled; leaves opposite, 2-3.5 X 1-1.5 cm. obliquely oblong or lanceolate or obovate-lanceolate, acute or sub-acute, serrate, dark green above, pale beneath. Base of the leaves usually unequal, acute or rounded, main veins 3-4, distinct; petioles short about 2-3mm. long; stipules pectinate, caduceus; flowers minute, numerous in globular, axillary, short peduncled, clusters; capsules hairy; seeds ovaoid; trigonous, light reddish brown.[15-16, 19-20] Capsules 1/20 inches across hairy; cocci compressed, keeled; seeds pale brown, ovoid, acutely angled, faintly transversely wrinkled. Flowers and fruit formed through out the year.[15] The flowers are very minute and crowded in dense axillary or terminal cymes; about 1cm in diameter. The fruit is minute, yellow, three celled capsule of about 1mm, each of the three carpels being distinctly keeled; containing a single seed.[17] The whole plant produces milky latex which is often irritating to the mucous membranes and skin.[21]

ETHNOPHARMACOLOGY

According to the doctrine of signatures, *Euphorbia pilulifera* L., has reputation for increasing milk flow in women, because of its milky latex and is used for other female complaints as well as in diseases of respiratory tract.[22] The plant has been used for female disorders but is now more important in treating respiratory ailments, especially cough, coryza, bronchitis and asthma. In India it is used to treat worm infections in children and for dysentery, gonorrhoea, jaundice, pimples, digestive problems and tumors. The fresh milky latex is applied to wounds and warts. Roots of the plant are used in sprains and inflammation, miscarriage, epilepsy, maggots in wounds and irregular growth of teeth.[23]

Chemical constituents

Euphorbia pilulifera L. contained 0.4 % of a lycosidal substance, tannin, fatty acids, phorbic acid, sterols, eciphosterol, Jambulol melissic acid and sugars, 0.1 % alkaloids.[24] Important constituents of the aerial parts were terpenoids, including triterpenes: α-amyrin, β-amyrin, friedlin, taxaxerol and esters of it: taraxerone, 11α-oxidotaraxerol, 12α-oxidotaraxerol, cycloartenol, 24-methylene-cycloartenol and euphorbol hexacosoate.[16,25] The aerial parts and roots also contain diterpenoid esters of the phorbol type and ingenol type, including 12-deoxyphorbol-13-dodecanoate-20-acetate, 12-deoxyphorbol-13-phenylacetate-20-acetate-ingenol triacetate, as well as highly toxic tinyatoxin, a resiniferonol derivative.[25] Other terpenoids isolated are sterols including β-sistosterol, campesterol, cholesterol and stigmasterol.[19, 25]

Tannins isolated include the dimeric hydrolysable dehydroellagitannins, euphorbin A, B, C, E and terchebin, the monomeric hydrolyable tannins geraniin, 2, 4, 6 tri-O-galloyl-β-D-glucose abd 1, 2, 3, 4, 6 penta-O-galloyl-β-D-glucose and the ester 5-O-caffeoylquinic acid (neochlorogenic acid) and 3,4-di-O-galloylquinic acid, and benzyl gallate.[26-27] Acids isolated include ellagic acid, gallic acid, tannic acid, maleic acid and tartaric acid.[26-27]

Flavonoids isolated include quercetin, quercitrin, quercitrol and derivatives containing rhamnose, quercetin rhamoside, a chlorophenolic acid, rutin, leucocyanidin, leucocyanidol, myricitrin, cyaniding 3, 5-diglucoside, pelargonium 3, 5-diglucoside and camphol. The flavonol glycoside xanthorhmnin was also isolated.[19, 28-29]

The latex contained inositol, taraxerol, friedelin, β-sitosterol, ellagic acid, kaempferol, quercitol and quercitrin.[19, 28-29] The mineral contents of dried leaves sample were: Ca 1.1 % and P 0.3%, Fe 0.03%, Mg 0.5%, Mn 0.01%, Zn 0.01% and Cu 0.002%. Fresh leaves from *Euphorbia pilulifera* of Nigerian origin were found to contain high levels of Mn (189ppm), Cu (30.5 ppm), Zn (152ppm) and NO_3 (4600ppm). Varying proportions of Fe, Mg, K, Ca, and Na were found.[30]

Pharmacological actions and medicinal uses

Polyphenolic extract of the whole plant inhibited the growth of *Entamoeba histolitica* with a minimal active concentration of less than 10µg /ml[31] and with 80µg /ml exhibited more than 70% inhibition of acetylcholine and / or KCl solution induced contractions on isolated guinea pig ileum.[32] It exhibited anti diarrheal activity against castor oil and prostaglandin E_2 induced diarrhea in mice.[33] The solvent extract of *Euphorbia pilulifera* showed selective cytotoxicity against several cancer cell lines. The plant was useful in effective treatment of cancer, particularly malignant melanomas and squamous cell carcinomas.[34]

Methanolic extract of the plant is non-cytotoxic and have antibacterial properties. The plant also had immunomodulatory activity. It affects lectin-induced lymphoblast transformation in vitro.[35]

Ethanolic extract of the plant exhibited antifungal activity when tested against the plant fungal pathogens such as Colletotrichum capsici, Fusarium palcidoroseum, Botryodiplodia theobromae, Alternaria alternate, penicillium citrinum; Phomopsis caricae-ppauae and Aspergillus niger.[36] An aqueous extract of Euphorbia pilulifera L. significantly inhibits aflotoxin production on rice, wheat, maize and ground nut.[37] E. pilulifera L. at a dose of 50mg/kg body weight reduced the sperm motility and density of cauda epididymal and testis sperm suspension significantly, leading eventually to 100% infertility.[38] This drug is also reported to have a relaxation effect on the bronchial tubes and a depressant action on respiration. It was shown that plant given to female guinea pigs before puberty, increased the development of mammary glands and induced secretion. Drug processed galactogenic activity.[39] Euphorbia extract has also been found to have depressant action on the cardiovascular system in general; musculature of heart is slightly depressed, a sedative effect on the mucous membrane of the respiratory tract and genitor-crinary tract; and produces a relaxation of the bronchioles by central action. The liquid extract of Euphorbia pilulifera L. was irritant to the mucous membrane of the stomach. In animals Euphorbia extracts produced broncho-dilatation.[40] Different fractions isolated through column chromatography, from the chloroform extract of the *Euphorbia pilulifera* L. were irritant to rabbit's skin.[11]

CONCLUSION

Euphorbia pilulifera Linne is a common herb. It grows among the grasses and in moist soil. It is very useful therapeutically. Valuable constituents of this herb can be screened and investigated for their potential as pharmacological candidates. Such herbs can be a source of revenue generation only once their chemical, structural and pharmacological studies have been established.

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THE PHARMACY ACT XI OF 1967: QUACKERY AND IRRATIONAL USE OF DRUGS

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Dear Editor!

The Pharmacy Act XI of 1967 is an Act to establish Pharmacy Councils to regulate the practices of pharmacy. And according to this act "Pharmacist" means a person who is registered under section 24 in Register A or Register B or Register C.[1] Among these three categories, the person registered in Register A is the only a pharmacy degree holding person, whereas, those registered in Register B and Register C are not having any formal or informal pharmacy education or even science education. Since the enforcement of this Act and Punjab Drug Rules 1988, the three types of "Pharmacists" were eligible to get license- 4 types- to practice pharmacy and provide pharmaceutical care to community.[2] Being competitive, the hiring of persons registered in Registers B and C is preferred by medical store/ retail pharmacy owners, which resulted in irrational dispensing of drugs and quackery. An effort was made in Punjab Drug Rules 2007 to provide better pharmaceutical care by drug sales license to only those registered in Register A and Register B.[3] In these rules, the role of person registered in Register B was restricted by prohibiting the selling drugs enlisted in Schedule G. [3] But the presence of provisions regarding the registration of pharmacists in the Pharmacy Act 1967 has encouraged influential people to cash the opportunity to make money by facilitating the medical store owners to get registration as a pharmacist in Register B and C, and at present Pharmacy Council of Pakistan has accredited four institutes to facilitate persons to be registered in Register B. Another astonishing practice is being exercised by Punjab Pharmacy Council for registration in Register B *viz* each pharmacist working in public sector in various capacities can grant certificate of eligibility to five persons to take examination of the Council, which has not only opened another door of corruption but quackery also. Nowadays, many quacks are in search of getting registration as a pharmacist in Register B to give cover to their illegal activity.

At present many public and private sector institutes are offering 5 years Pharm. D programme and adding more than 3000 pharmacists each year in a pool of unemployed/underpaid pharmacists. Additionally, a number of pharmacy institutes are in queue for accreditation of Pharmacy Council of Pakistan and few are in a process of development. From the prevailing scenario we can expect a tsunami of pharmacists in soon in Pakistan. Right now there is unemployment of pharmacists and those getting employment are receiving very low remuneration (Rs 5000.00 to 7000.00 per month) and situation will certainly be worsted in near future, if attention is not paid. Hence, it is a high time for government authorities, academia and practicing pharmacists to take inflexible notice to regulate/restrict pharmacy education and opening job opportunities for future pharmacists.

Keeping in view the above stated facts the suggestions made are given as follows:

1. The Federal Government of Pakistan need to amend the Pharmacy Act 1967 regarding the registration of Pharmacists. Only a person having pharmacy degree should be eligible for registration as a pharmacist.

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2. The Health Department, Government of Pakistan should acknowledge the role of pharmacist as a member of healthcare team for better pharmacotherapy outcome.
3. As highlighted by Hussain (2009) there is a dire need of a qualifying examination to get registration with the provincial pharmacy councils to enhance the standard of pharmacy education and pharmacy services, and to standardize the pharmacy examination system that is being observed in many pharmacy institutes.[4]
4. Mushroom growth of pharmacy institutes must be restricted and at the same time Pharmacy Council of Pakistan need to limit the number of admissions in its accredited institutes.
5. The Pharmacy Council of Pakistan and Higher Education Commission of Pakistan are required to put sincere efforts to bring curriculum of Pharm. D at par with the international standards because the present curriculum has many insufficiencies.[5]

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