Study of anti-inflammatory activity of *Antigonon leptopus* Hook. et Arn roots

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ABSTRACT

The anti-inflammatory activity of the metahnolic extract of *Antigonon leptopus* Hook.et Arn roots (MEAL) was investigated in mice and rats to find out the pharmacological basis for its ethnomedicinal use. The extract produced a significant inhibition of peritoneal and cutaneous vascular permeability induced by acetic acid, granuloma induced by cotton-pellet and migration of leucocytes and neutrophils induced by carrageenan in animals at the doses of 100, 200 and 400 mg/kg. Moreover, the extract markedly inhibited foot paw edema induced by formalin in rats at the doses of 100, 200 and 400 mg/kg. Acute toxicity studies were performed and produced no mortality in dose up to 2000 mg/kg, p.o. Preliminary phytochemical screening revealed the presence of steroids, flavonoids, tannins, alkaloids and glycosides. These results suggest that MEAL possesses promising anti-inflammatory activity against acute as well as sub acute inflammation, which appear to be due to prostaglandin inhibition and reduction of oxidative stress respectively.

Key Words: *Antigonon leptopus*, carrageenan, prostaglandin, cotton granuloma, oxidative stress, malondialdehyde.

Introduction

Antigonon leptopus Hook. et Arn (syn: *Corculum leptopus* family: Polygonaceae) or coral vine is grown in parks and gardens throughout India. It is most common in the upper Ganges plains and Himalayan regions. It is a fast growing climber with heart shaped green leaves, flowers through summer to autumn with coral pink to red flowers hanging in panicles up to 15 cm long and will climb up to 40 ft protect from frosts. Traditionally the leaves can be used to reduce swelling, a tea from the leaves can be made for diabetes and from the blossoms to treat high blood pressure. The vine is used to treat cough and throat constriction [1]. It has anticoagulant activity [2]. Information gathered from local herbal healers where the plant was collected revealed that the roots of the plant are useful to pain and inflammation. The purpose of the present study is to evaluate the possible anti-inflammatory effect by using formaldehyde-induced rat paw edema, acetic acid–induced vascular permeability, cotton pellet-induced granuloma, estimation of plasma MDA levels and carrageenan induced peritonitis models.

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Materials And Methods

Plant Material

The roots of *A.leptopus* Hook.et Arn were collected from Visakhapatnam, Andhra Pradesh. The plant material was authenticated by Prof V.S.Raju, Dept of Botany, Kakatiya University, Warangal. The voucher specimen (KU/UCPSc/15/2006) has been retained in the Dept of Pharmacognosy and Ethnopharmacology, University College of Pharmaceutical Sciences, Warangal.

Preparation of Extract

The roots were made into small pieces, shade dried and made into coarse powder subjected for maceration process with methanol at room temperature. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure at 50^0 – 55^0 C and stored in a vacuum desiccator. A fine suspension of the extract prepared in 2% gum acacia was used for the experiments.

Animals

Wistar rats (150-250 g) and albino mice (20-27 g) of either sex were used in this investigation. Animals were kept

under standard environmental conditions and had free access to feed and water *ad libitum.* All the animals were acclimatized to the laboratory environment for at least one week before the experimental session. All the animals were divide into different groups and each group consists of six animals. For experimentation, the animals were fasted overnight. Experiments on animals were performed by following guidelines of Institutional Animal Ethics Committee.

Chemicals and Drugs used

Formaldehyde (S.D. Fine chemicals Ltd. Mumbai), acetic acid (Ranbaxy laboratories Ltd., Punjab), diclofenac sodium (Dr. Reddy Labs, Hyderabad), indomethacin(Sun Pharma, Mumbai), ibuprufen(Natco Pharma, Hyderabad), evans blue (Sigma, St.Louis, Missouri, USA), gum acacia (Hi-media, Mumbai) and methanol (BDH, Mumbai). All other chemicals were of analytical grade and procured locally.

Phytochemical screening

The methanolic extract was screened for the presence of various phytoconstituents like steroids, alkaloids, tannins, flavonoids and glycosides by employing standard phytochemical tests [3].

Acute toxicity study

Acute oral toxicity was performed in mice by following Organization for Economic Co-operation and Development (OECD) guidelines AOT No 425[4].

Formalin - induced acute inflammatory model [5]

Formalin 0.1 ml (2% in distilled water) was injected into sub planter area of left hind paw. The extract at doses of 100, 200 and 400 mg/kg and standard diclofenec sodium 10 mg/kg were given 1 h prior to formalin injection. The paw volume was determined by plethysmographic method in order to measure degree of inflammation as shown in Table.1.

Acetic acid-induced Vascular Permeability test.

Whittle's method was used with some modifications [6]. In brief, male mice weighing 20-27 g were fasted for 10 h prior to the experiments and were given the test drugs and vehicle orally. Each animal was given an intravenous injection of a 1% solution of Evans blue as 0.1 ml/10 g at 30 min after the oral treatment. The vascular permeability inducer, 0.1 ml/10 g of 0.6% acetic acid in saline, was injected intraperitoneally at 30 min after Evans blue injection. After 20 min, the mice were killed by dislocation of the neck and 10 ml of normal saline was injected intraperitoneally, after which the washing solution was collected in tubes and then centrifuged at 2000 rpm for 10 min. The absorbance of the supernatant was read at 610 nm with a spectrophotometer. The control group was treated similarly except that they received an oral dose of vehicle alone. The vascular permeability was represented in terms of the absorbance (A_{610}) which leaked in to the cavity. Experiments were performed in triplicate.

Cotton pellet-induced granuloma [7]

The cotton pellet-induced granuloma in rats was studied according to the method D'Arcy *et al*. (1960). The animals were divided into five groups of six animals in each group. Cotton pellets weighing 10 ± 1 mg were autoclaved and implanted subcutaneously into both sides of the groin region of each rat. Group I served as control and received the vehicle (2% gum acaia). Group 2 received the standard drug, indomethacin (10 mg/kg body weight) for the same period. The extract MEAL at the concentration of 100, 200 and 400 mg/kg was administered orally to groups 3, 4 and 5, respectively for seven consecutive days from the day of cotton pellet implantation. On 8^{th} day the animals were anaesthetized and the pellets together with granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60^0 C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The anti-proliferative effect of MEAL was compared with control.

Plasma MDA (Malondialdehyde) estimation

After seven days drug treatment in cotton pellet granulation

Table.1

Effect of the methanolic extract of *Antigonon leptopus* root on formaldehyde-induced rat paw edema

Sl. No	Group	Dose (mg/kg)	Increase in paw volume (ml)					
			1h	2h	3h	4h	5h	24h
1.	Control	—	0.305±0.011	0.345±0.008	0.374±0.010	0.304±0.004	0.230±0.005	0.200±0.003
2.	Diclofenec sodium	10	0.208±0.003**	0.221±0.005**	0.203±0.006**	0.209±0.003**	0.142±0.002**	0.110±0.003**
3.	*A.leptopus*	100	0.260±0.008*	0.251±0.003**	0.253±0.021**	0.237±0.006**	0.206±0.004**	0.182±0.007*
4.	*A.leptopus*	200	0.257±0.009*	0.244±0.002**	0.243±0.002**	0.225±0.007**	0.179±0.004**	0.140±0.002**
5.	*A.leptopus*	400	0.221±0.016**	0.210±0.004**	0.206±0.002**	0.199±0.004**	0.162±0.004**	0.105±0.001**

Values are mean ± S.E.M. ($n = 6$).
** Experimental groups were compared with control ($p< 0.01$).

method, 3-5ml of blood was collected from inner canthus of eye from each animal using capillary tube, in a vial containing EDTA as an anticoagulant. Plasma was separated by centrifugation at 3000 rpm for 10 min. It was stored at -20^{0}C and used to estimate MDA levels. The reduced levels of MDA were taken as indicator of anti-lipoperoxidative activity, which can be taken as index of reduced oxidative stress.

Carrageenan induced peritonitis

Inflammation was induced by the modified method of Griswold et al., 1987 [8]. Male Swiss albino mice weighing 20-25 g were divided into five groups (n=6). Group I served as control, Group II served as standard and was dosed with indomethacin (10 mg/kg, p.o) and group III to V were dosed with MEAL at the doses of 100, 200, 400 mg/kg p.o. The standard drug and extract doses were administered orally one hour prior to the induction of peritonitis. After one hour, carrageenan (0.25 ml, 0.75% w/v in saline) was injected intraperitonially. Four hours later, the animals were sacrificed by cervical dislocation for further investigation. Two ml of Ca^{2+} and Mg^{2+}free phosphate buffered saline (PBS) was injected into the peritoneal cavity during the collection of peritoneal fluids. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined by microscopic counting [9.10]. The percentage of leukocyte inhibition was calculated using the following formula:

% of Leukocyte Inhibition (% L. I) = (1- T/C) X 100

Where 'T' represents the treated groups' leukocyte count and

'C' represents the treated control group leukocyte count.

Inhibition of Neutrophil migration was calculated by the following equation:

Inhibition of Neutrophil Migration = 100-{(N T/NC) X 100}

Where NT = Neutrophil counts of treated groups

NC = Neutrophil counts of control groups.

Statistical analysis

The experimental results were expressed as the mean ± SEM. Data were assessed by the method of analysis of ANOVA followed by Dunnet's t-test. P value of < 0.05 was considered as statistically significant.

Results

Phytochemical Screening

Preliminary phytochemical screening of methanol extract revealed the presence of steroids, flavonoids, tannins, alkaloids and glycosides.

Acute toxicity study

In acute toxicity study no mortality was observed during the 24 h period at the doses tested and the animals showed no stereotypical symptoms associated with toxicity, such as convulsions, ataxia, diarrhea or increased diuresis.

Formaldehyde induced paw edema

In formaldehyde induced paw edema method, the oral administration of MEAL in graded doses (100, 200 and 400mg/kg) produced significant reduction in paw volume in dose dependent manner in comparison to control. The maximum effect was seen in the oral dose of 400mg/kg which showed significant ($p<0.01$) reduction as 44.9% in paw volume in comparison to control. The anti-inflammatory activity in this dose of the test drug was comparable to standard, diclofenac sodium (10 mg/kg). The maximum anti-inflammatory effect was observed at 3 h in all the doses of test drug.

Vascular permeability test

The vascular permeability test is one of the acute inflammatory models. As shown in Table.2, the dye leakage induced by acetic acid was significantly inhibited by 32.6 and 39.5% in response to 400 mg/kg of MEAL and 10 mg/kg of indomethacin, respectively (compared with the control group). The anti-inflammatory activity of MEAL was less effective than the standard drug. However, the anti-

Table. 2

Effect of methanolic extract of *Antigonon leptopus* root on acetic acid-induced vascular permeability in mice

S.No	Group	Dose (mg/kg)	Amount of dye leakage (OD)	Inhibition (%)
1.	Control	—	1.38±0.035	—
2.	Indomethacin	10	0.835±0.018**	39.5
3.	*A.leptopus*	100	1.155±0.04**	16.3
4.	*A.leptopus*	200	1.01±0.07**	26.8
5.	*A.leptopus*	400	0.93±0.042**	32.6

Values are mean ± S.E.M. ($n = 6$).

** Experimental groups were compared with control *($p< 0.01$).*

inflammatory effect was statistically significant compared with the control group.

Cotton pellet granuloma

The effects of MEAL and indomethacin on the proliferative phase of inflammation are summarized in Table.3. It was seen that MEAL was responsible for anti-inflammatory effect, which would be calculated depending on the moist and dry weight of cotton pellets. According to these results, the antiproliferative effects of MEAL (400 mg/kg b.w.) and indomethacin (10 mg/kg b.w.) were calculated as 39.9 and 46.7 %($p<0.01$), respectively. After they were dried, the antiproliferative effects were calculated on the basis of dry weight pellets; the inhibition of inflammation by MEAL and indomethacin were established as 37.8 & 42.6 %($p<0.01$), respectively.

Estimation of MDA levels in plasma

In oxidative stress model, MEAL (400 mg/kg x 7 days, orally) produced significant ($p<0.01$) reduction in plasma MDA levels which was 37.75% in comparison to diseased control. However, standard drug reduced greater reduction of MDA levels (52.5%) as shown in fig.1.

Carrageenan induced peritonitis

The MEAL also inhibited peritoneal leukocyte migration at the rate of 38.6, 59.7 and 76.7% at the doses of 100, 200 and 400 mg/kg, respectively, whereas the inhibition produced by indomethacin (10 mg/kg) 60.7% was found to be in carrageenan-induced peritonitis model as shown in Table.4. The inhibition of neutrophils infiltration of MEAL was 33.1, 53.9 and 61.2% respectively, where as indomethacin shows 65.1%.

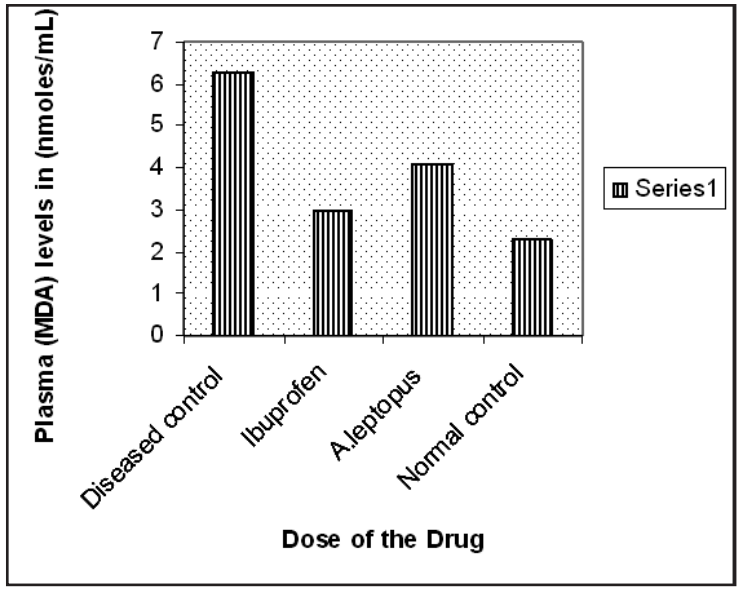

Fig.1. Oxidative stress by plasma estimation of Malondialdehyde (MDA)

Table. 3

Effect of the methanolic extract of *Antegonon leptopus* root on cotton pellet-induced granuloma in rats

S.No	Treatment	Dose (mg/kg)	Weight of cotton pellet (mg) ***(Wet)***	% Inhibition	Weight of cotton pellet (mg) ***(Dry)***	% Inhibition
1.	Control	—	142.0 ± 1.91	41.5 ± 2.11	—	—
2.	Indomethacin	10	75.67 ± 1.75**	46.71	23.83 ± 0.95**	42.58
3.	*A.leptopus*	100	101.3 ± 2.06**	28.66	31.17 ± 1.42**	25.07
4.	*A.leptopus*	200	94.67 ± 2.11**	33.3	27.3 ± 0.88**	34.22
5.	*A.leptopus*	400	85.33 ± 2.09**	39.9	25.83 ± 1.42**	37.8

Values are mean ± S.E.M. *(n=6).*

** Experimental groups were compared with control *($p<0.01$).*

Table. 4

Effect of methanolic extract of *Antigonon leptopus* root on leukocytes migration and neutrophils migration in peritoneal exudation in carrageenan-induced mice

S.No	Group	Dose (mg/kg)	Leukocytes (10^5 mL $^{-1}$)	Leukocyte Inhibition	Neutrophils (10^5 mL $^{-1}$)	% Inhibition of Neutrophil migration
1.	Control	––	4.07±0.08	––	2.45±0.1	––
2.	Indomethacin	10	1.6±0.14**	60.7	0.85±0.03**	65.3
3.	*A.leptopus*	100	2.5±0.05**	38.6	1.64±0.08**	33.1
4.	*A.leptopus*	200	1.64±0.12**	59.7	1.13±0.05**	53.9
5.	*A.leptopus*	400	0.95±0.07**	76.7	0.95±0.07**	61.2

Values are mean ± S.E.M. (*n=6*).
** Experimental groups were compared with control (*p<0.01*).

Discussion

The extract of *A.leptopus* Hook.et Arn root showed significant inhibition of formalin-induced rat paw edema. The formalin injection into rat paw produces localized inflammation and pain. This nociceptive effect is biphasic in nature, an early neurogenic component followed by a later tissue-mediated response [11]. The first phase is mediated through the release of histamine, serotonin and kinins where as the second phase is related to release of prostaglandin and slow reacting substances which are peak at 3 h [12]. Inhibition of formalin-induced pedal edema in rats is one of the most suitable tests to evaluate anti-proliferative activity and to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis [13, 14]. The MEAL produced dose dependent and significant inhibition of formaldehyde induced paw edema in both phases.

The vascular permeability was induced by acetic acid, which could cause an increase in peritoneal fluids of prostaglandin E_2 (PGE_2), prostaglandin $F_{2á}$ ($PGF_{2á}$), serotonin, and histamine [15]. This leads to a dilation of the arterioles and venules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravasated, and edema forms. Indomethacin and MEAL showed significant inhibition of acetic acid-induced vascular permeability in mice. This result suggested that MEAL probably has an anti-inflammatory property like indomethacin (nonselective COX inhibitor), acting through inhibition of the inflammatory mediators of the acute phase of inflammation.

Cotton pellet granuloma method is commonly used to evaluate the proliferative aspects of the tissue injury (inflammation). Subcutaneous implantation of pellets of compressed cotton provokes foreign body granuloma. Dose dependent percent inhibition of granuloma formation was observed with all doses. The wet weight of the cotton pellet correlates with the transuda and the dry weight of the cotton pellet correlates with the amount of the granulamatous tissue [16]. The present data support the hypothesis of the greater effect of the MEAL on the inflammation mediators in the immediate response of inflammation in rats.

The present study also showed significant reduction in MDA levels by MEAL. The oxidative stress is the condition where Reactive Oxygen Species (ROS) generation exceeds endogenous antioxidant defense [17] and it is well-known that in chronic and sub-acute inflammation ROS play an important role in modulating the extent of inflammatory response and consequent tissue and cell injury [18]. MDA is a metabolic product of lipidperoxidation, the level of which is increased in oxidative stress. Therefore, reduction of oxidative stress by anti-lipo peroxidative activity might possibly be the mechanism of anti-inflammatory action of MEAL in model of sub-acute inflammation.

Leukocyte aggregation at the site of inflammation is a fundamental event in the inflammatory process. Cell migration occurs as a result of much different process including adhesion and cell mobility [19]. The MEAL was found to inhibit leukocyte migration more potent than indomethacin. The extract (in peritonitis model) drastically reduced the migration of neutrophils.

These observations suggest that the methanol extract of *Antigonon leptopus* Hook.et Arn possess anti-inflammatory activity against acute as well as sub-acute inflammation which appears probably due to prostaglandin inhibition and reduction of oxidative stress respectively.

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A validated Chiral LC Method for the Enantiomeric Purity Determination of Fadrozole (S)-enantiomer on Amylose-based Stationary phase

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ABSTRACT

A new, simple and accurate normal phase high performance liquid chromatographic method was developed for quantitative determination of enantiomeric purity of 4-[(5*S*)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridin-5-yl]benzonitrile, (S)-enantiomer (Fadrozole) bulk drug samples. Chromatographic separation between Fadrozole and its opposite enantiomer (1*R*)-6,11-dioxo-1,2,3,4,6,11-hexahydropyridazino[1,2-*b*] phthalazine-1-carboxylic acid, (R)-enantiomer were achieved on a Chiralpak AD-H column using a mobile phase consisting of heptane and ethyl alcohol (70:30, v/v) at a flow rate of 0.8 mL/min. The resolution between the two enantiomers was found to be greater than 9. The developed method was found to be selective for Fadrozole, under exposed conditions of UV light and 60 ºC. The limit of detection (LOD) and limit of quantification (LOQ) of undesired (R)-enantiomer were found to be 200 and 700 ng/mL respectively, for 5 µL injection volume. The method precision for (R)-enantiomer at limit of quantification level was with in 8% R.S.D. Calibration curve for (R)-enantiomer was linear over the studied ranges (700-3000 ng) with correlation coefficient greater than 0.998. The percentage recoveries of (R)-enantiomer were ranged from 95.5 to 107.4% in the bulk drug samples of Fadrozole. The test solution and mobile phase were observed to be stable up to 24 h after the preparation. The validated method yielded good results of precision, linearity, accuracy, robustness and ruggedness. The optimized method was found to be selective and accurate for the quantitative determination of (R)-enantiomer in Fadrozole bulk drug samples.

Key Words: Fadrozole, Aromatase inhibitors. Chiral stationary phase, Enantiomeric separation, Validation and quantification

Introduction

Fadrozole, a single enantiomer 4-[(5*S*)-5, 6, 7, 8-tetrahydroimidazo [1, 5-*a*] pyridin-5-yl] benzonitrile, a potent, highly specific inhibitor of aromatase activity, has only been used as second-line therapy in treatment of post-menopausal women with advanced breast cancer. A prospectively randomised study was therefore undertaken to compare relative clinical efficacy of fadrozole as first-line treatment to that of tamoxifen. Fadrozole has good therapeutic effect as a second-line treatment in postmenopausal women with metastatic breast cancer [1, 2]. A normal phase chiral LC methods were reported in the literature for the enantioselective analysis of Fadrozole and its opposite enantiomer 4-[(5*R*)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridin-5-yl]benzonitrile ((R)-enantiomer) in the substituted [1-(imidazo-1-yl)-1-phenylmethyl)] benzothiazolinone and benzoxazolinone derivatives with one stereogenic center [3-6]. The resolution between the enantiomers was reported about greater than 1.5. Separation of enantiomers has become very important in analytical chemistry, especially in pharmaceutical and biological fields, because some stereo isomers of racemic drugs have quite different pharmacokinetic properties and different pharmacological or toxicological effects [7].

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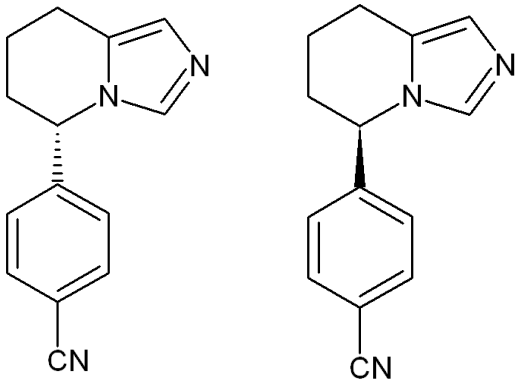
The developed chiral HPLC method was producing the superior chromatographic efficiency for enantiomeric separation of Fadrozole and its opposite enantiomer ((R)-enantiomer) using an amylose based chiral stationary phase (CSP), Chiralpak AD-H column. The developed chiral HPLC method was validated for quantitative determination of (R)-enantiomer content in Fadrozole bulk drug samples.

Experimental

Chemicals and Reagents

Samples of Fadrozole, (R)-enantiomer of Fadrozole and racemic samples were received from Process Research Department of Custom Pharmaceutical Services, a business unit of Dr. Reddy's Laboratories Ltd., Hyderabad, India and chemical structures are presented in Fig. 1.



Fadrozole **(R)-enantiomer of Fadrozole**

Fig.1: **Chemical structures of Fadrozole and (R)-enantiomer of Fadrozole**

The HPLC grade heptane was purchased from Qualigens fine chemicals, Mumbai, India, isopropyl alcohol was purchased from Ranbaxy fine chemicals, New Delhi, India, ethyl alcohol was purchased from Ranbaxy fine chemicals, New Delhi, India, and HPLC grade hexane was purchased from Qualigens fine chemicals, Mumbai, India.

Equipment

The Waters Alliance HPLC system equipped with 2695 separation module with inbuilt auto injector and 2996 photo diode array detector was utilized for method development and validation in laboratory A, The out put signal was monitored and processed using Empower software (Waters) on Pentium computer (Digital Equipment Co). The second LC system, Waters LCM1 plus HPLC system equipped with 515 HPLC pump, 717 Plus Auto sampler and 2487 dual absorbance detector was utilized in ruggedness study in laboratory B. The out put signal was monitored and processed using millennium 32-chromatography manager software on Pentium computer (Digital Equipment Co).

Sample preparation

The stock solutions of racemic, Fadrozole and (R)-enantiomer of Fadrozole samples were prepared separately by dissolving the appropriate amounts of the substances in ethyl alcohol. The target analyte concentration was fixed as 1000 µg/mL.

Chromatographic conditions

The chromatographic conditions were optimized using an amylose based chiral stationary phase Chiralpak AD-H 250 x 4.6 mm, 5µm (Daicel make) column that was safeguarded with a 1 cm long guard column. The mobile phase was heptane and ethyl alcohol (70:30, v/v). The flow rate was set at 0.8 mL/min. The column was maintained at 25 °C and the detection was carried out at a wavelength of 230 nm. The injection volume was 5 µL.

Method Validation

System suitability

System suitability test is an integral part of chromatographic methods and is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed [8, 9]. The system suitability test results of the chiral LC method on Chiralpak AD-H, Chiralcel OD-H and Chiralcel OJ-H columns are presented in Table 1.

Table 1

System suitability report

Column name	Compound (n=3)	*k*	R_s	*N*	*T*	*á*
Chiralcel OD-H	Fadrozole	6.2		2328	1.3	
	(R)-enantiomer	7.1	1.3	2345	1.8	1.1
Chiralcel OJ-H	Fadrozole	8.9		4614	2.1	
	(R)-enantiomer	9.9	1.2	1849	2.6	1.1
Chiralcel AD-H	Fadrozole	4.0		9401	1.0	
	(R)-enantiomer	6.7	9.8	8362	1.5	1.7

n= 3 determinations. *k*-capacity factor; R_s-USP resolution; *N*-number of theoretical plates (USP tangent method); *T*-USP tailing factor; *á*-enantioselectivity. Eluent conditions: (1) column: Chiralpak AD-H (250x4.6mm), 5µm; (2) mobile phase: heptane: ethyl alcohol (70:30, v/v); (3) flow rate: 0.8 mL/min; (4) column temperature: 25 ºC.

Specificity

Specificity is the ability of the method to measure the analyte response in presence of sample matrix [10]. Forced degradation was carried out for Fadrozole sample under UV light (254 nm) and heat (60 °C) for 10 days period. The exposed samples were tested for peak purity by using a photo diode array detector and confirmed the peak homogeneity. Fadrozole, racemic solution was tested for peak purity using photo diode array detector and confirmed the peak homogeneity for both the enantiomers.

Precision

Precision of the analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample [11]. The allowed limit of (R)-enantiomer in Fadrozole was fixed as 0.15%.

The precision of the method was checked by analyzing the replicate samples of Fadrozole (at the analyte concentration i.e. 1000 µg/mL) spiked with 0.15% (1500 ng/mL) of (R)-enantiomer and calculated the percentage relative standard deviation.

Limit of detection and Limit of quantification

The limit of detection (LOD) of an individual procedure is the lowest amount of analyte in the sample, which can be detected but not necessarily quantified as an exact value. The limit of quantification (LOQ) of an individual procedure is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy.

The limit of detection (LOD) and the limit of quantification (LOQ) for (R)-enantiomer were determined at a signal-to-noise ratio of 3 and 10 [12-14] by injecting a series of diluted solutions of (R)-enantiomer.

The precision of the developed chiral method for (R)-enantiomer at limit of quantification level was checked by analyzing six test solutions of (R)-enantiomer prepared at LOQ level and calculated the percentage relative standard deviation.

The accuracy of the method was checked for (R)-enantiomer at LOQ level by analyzing three replicate samples of Fadrozole spiked with (R)-enantiomer at LOQ level and calculated the percentage recovery.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of the analyte in the sample [13]. The linearity and range were established for (R)-enantiomer in Fadrozole from 700 (LOQ) to 3000 ng/mL (0.3% with respect to analyte concentration), (i.e. 700, 1000, 1500, 2000, 2500 and 3000 ng/mL).

The peak area and concentration of (R)-enantiomer were subjected to regression analysis to calculate calibration equation and correlation coefficient. Linearity was checked for 3 consecutive days in the same concentration range and the percentage relative standard deviation of the slope and Y-intercept of the calibration curve was calculated.

Quantification of (R)-enantiomer in bulk sample

Fadrozole bulk drug sample was provided by Process Research Department of Custom Pharmaceutical Services, Dr. Reddy's Laboratories, showed the absence of (R)-enantiomer. Standard addition and recovery experiments were conducted to determine accuracy of the present method for the quantification of (R)-enantiomer in bulk drug samples [15].

The study was carried out in triplicate at 0.12, 0.15 and 0.18% of the Fadrozole target analyte concentration. The recovery of (R)-enantiomer was calculated from the slope and Y-intercept of the calibration curve, drawn in the concentration range of 700 to 3000 ng/mL (Slope and Y-intercept values obtained in the linearity study).

Ruggedness

The ruggedness of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under variety of normal test conditions such as different laboratories, different analysts, different instruments, different days and different lots of reagents. The standard addition and recovery experiments carried out for (R)-enantiomer in Fadrozole bulk drug samples at the concentration levels tested in Laboratory A, were again carried out in laboratory B using a different instrument.

Robustness

The robustness of the method capability to remain unaffected by small but deliberate variations in the method parameters was studied in order to anticipate the problems, which may arise during the regular application of the developed method. To determine robustness of the method experimental conditions were purposely altered and chromatographic resolution between Fadrozole and (R)-enantiomer was evaluated.

The flow rate of the mobile phase was 0.8 mL/min. To study the effect of flow rate on the resolution of enantiomers, it was changed by 0.2 units from 0.8 to 0.6 and 1.0 mL/min. The effect of change in percent of ethyl alcohol on resolution was studied at 27 and 33% instead of 30%, while the other mobile phase components were held constant. The effect of column temperature on resolution was studied at 20 and 30 °C instead of 25 °C while the other components were held constant.

Solution stability and mobile phase stability

Stability of Fadrozole sample solution spiked with (R)-enantiomer at specification level (0.15%), was studied by keeping the solution in tightly capped volumetric flask at room temperature on a laboratory bench for 24 h. Content of (R)-enantiomer was checked for every 6 h interval up to the study period.

Mobile phase stability was carried out by evaluating the content of (R)-enantiomer in Fadrozole sample solutions spiked with (R)-enantiomer at specification level (0.15%), which was prepared freshly at every 6 h interval for 24 h. Same mobile phase was used during the study period.

Results and Discussion

Method development

The objective of this study was to separate the enantiomers of Fadrozole and accurate quantification of the undesired (R)-enantiomer in Fadrozole bulk drug samples. A 2000 µg/mL solution of racemic mixture prepared in ethyl alcohol was used in the method development. Three different chiral columns were employed during method development, namely Chiralcel OD-H, Chiralpak AD-H and Chiralcel OJ-H of Daicel. All the columns chosen were of 250 mm length, 4.6 mm internal diameter and 5 µm particle sizes. The chiral stationary phase (CSP) in Chiralpak AD-H, Chiralcel OD-H, and Chiralcel OJ-H columns are amylose tris (3,5-dimethylphenylcarbamate), cellulose tris (3,5-dimethylphenylcarbamate), and cellulose tris (4-methylbenzoate), respectively, coated on a silica gel. The mechanism of separation in direct separation methods is the interaction of chiral stationary phase (CSP) with analyte enantiomers to form short-lived, transient diastereomeric complexes [16]. The complexes are found as a result of hydrogen bonding, dipole-dipole interactions, pi bonding, electrostatic interactions, and inclusion complexation. From the initial studies, the Chiralpak AD-H column produced comparatively better results. Then, various experiments were conducted on Chiralpak AD-H stationary to select the best mobile phase composition and flow rate that would give optimum resolution and selectivity for the enantiomers.

Firstly, hexane: isopropyl alcohol (80:20, v/v) was used as a mobile phase and flow rate was used 1.0 mL/min. A baseline separation was observed on Chiralpak AD-H column (resolution was about 1.5 and USP tailing greater than 1.5) was observed on the Chiralpak AD-H column while using the above mobile phase. Secondly, heptane: isopropyl alcohol (80:20, v/v), flow rate was 1.0 mL/min, was tested on Chiralpak AD-H column, baseline separation was observed (resolution was about 1.6 and USP tailing greater than 1.5). Thirdly, heptane: ethyl alcohol (80:20, v/v), flow rate was 1.0 mL/min, was tested on Chiralpak AD-H column, baseline separation was observed (resolution was greater than 7 and USP tailing greater than 1.4). Finally, heptane: ethyl alcohol (70:30, v/v), flow rate was 0.8 mL/min, was tested and achieved the best chromatographic efficiency and resolution between the enantiomers (resolution was greater than 9 and USP tailing was 1.0). The results of method development mobile phase composition and flow rate selection details were described in Table 2.

In the optimized method, the typical retention times of Fadrozole and (R)-enantiomer were approximately about 12.4 and 19.2 min, respectively. The enantiomeric separation of Fadrozole on Chiralcel OD-H, Chiralcel OJ-H and Chiralpak AD-H columns are shown in Fig. 2.

Method validation

In the optimized chiral LC method, (R)-enantiomer was well separated from Fadrozole. In the case of stress study by UV light (254 nm) and heat (60 ºC), it was observed that rigorous stress of Fadrozole sample did not cause any significant degradation for 10 days study period. The proposed chromatographic conditions were found to be selective to the Fadrozole sample subjected to the applied stress conditions. Peak homogeneity was obtained for Fadrozole and (R)-enantiomer by overlay of the spectra captured at the apex, up slope and down slope using photo diode array detector, and no interference was noted for Fadrozole and (R)-enantiomer in stress samples. Hence, the developed method was found to be selective.

Table 2

Selection of mobile phase composition and flow rate

Mobile phase	Flow rate (mL/min)	Compound (n=3)	R_s	T
Hexane: isopropyl alcohol (80:20, v/v)	1.0	Fadrozole		1.6
		(R)-enantiomer	1.5	1.8
Heptane: isopropyl alcohol (80:20, v/v)	1.0	Fadrozole		1.6
		(R)-enantiomer	1.6	1.7
Heptane: ethyl alcohol (80:20, v/v)	1.0	Fadrozol		1.5
		(R)-enantiomer	8.1	2.1
Heptane: ethyl alcohol (70:30, v/v)	0.8	Fadrozole		1.0
		(R)-enantiomer	9.9	1.5

n= 3 determinations; R_s-USP resolution; *T*-USP tailing factor

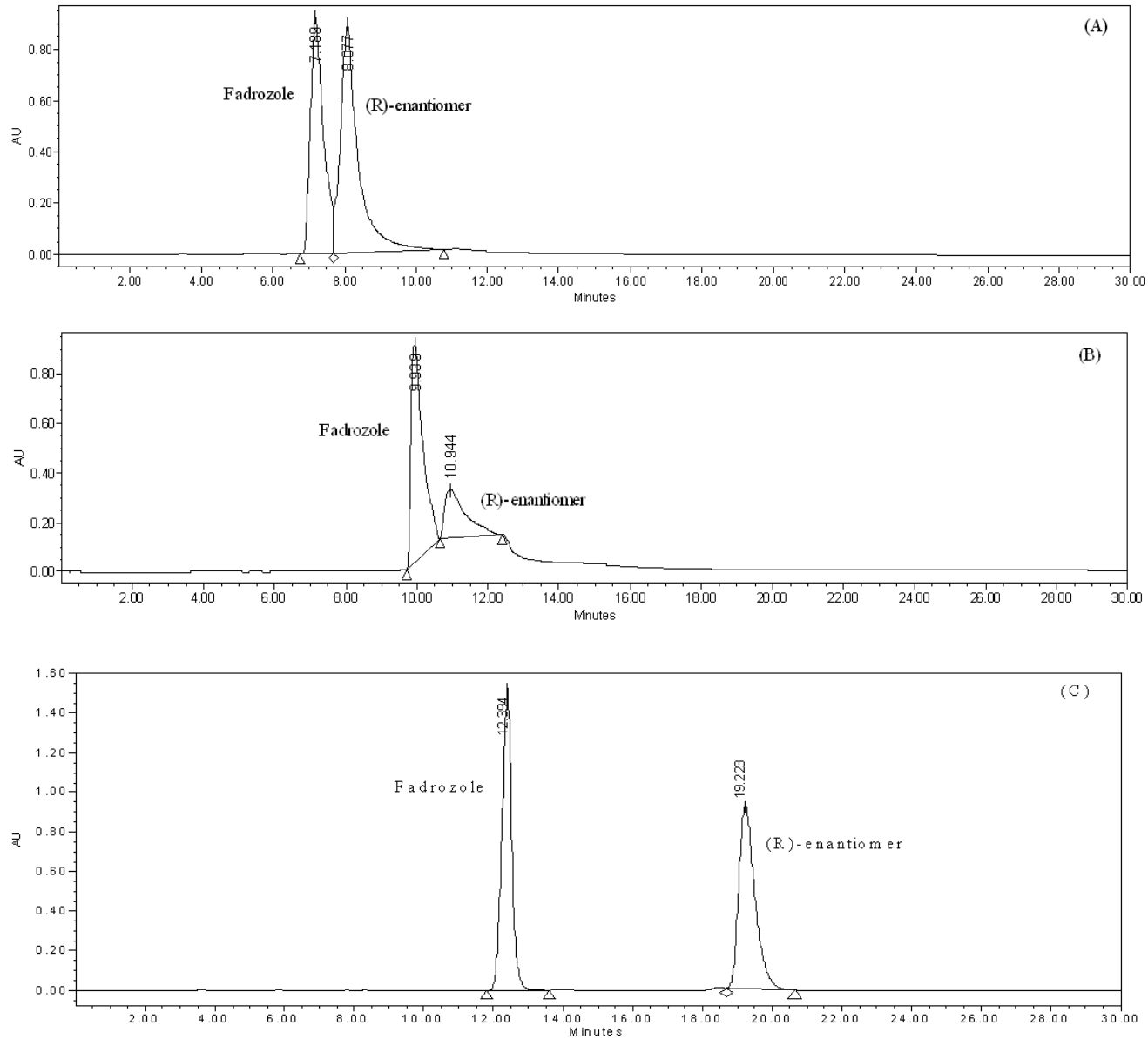


Fig. 2. **Enantiomeric separation of racemic Fadrozole on (A) Chiralcel OD-H, (B) Chiralcel OJ-H and (C) Chiralpak AD-H columns; mobile phase composed of heptane: ethyl alcohol (70:30, v/v); flow rate: 0.8 mL/min; UV:230 nm; column temperature: 25 °C.**

In the precision study, the percentage relative standard deviation of analysis repeatability for Fadrozole and (R)-enantiomer was found to be 0.6 and 4.9%, respectively, indicating the good precision of the method.

The limit of detection (LOD) and limit of quantification (LOQ) concentrations were found to be 200 and 700 ng/mL for (R)-enantiomer, when a signal-to-noise ratio of 3 and 10 were used as the criteria. The precision at limit of quantification for (R)-enantiomer was found to be less than 7.9% RSD (Table 3). The recovery of (R)-enantiomer at limit of quantification was 90.4% in the spiked Fadrozole samples.

Good linearity was observed for (R)-enantiomer over the concentration range of 700 – 3000 ng/mL (Correlation

Table 3

Precision results of (R)-enantiomer at LOQ level

Preparation	Peak area
1	14142
2	15900
3	17192
4	15626
5	14489
6	16942
	%R.S.D. 7.9

coefficient, R = 0.999). Linearity was checked for (R)-enantiomer over the same concentration range for three consecutive days. The percentage relative standard deviation of the slope and Y-intercept of the calibration curve were 2.7 and 4.9%, respectively. The results show that good correlation existed between the peak area and concentration of (R)-enantiomer. Based on the linearity study, (R)-enantiomer quantitative determination range has been considered from LOQ to 3000 ng/mL with respect to analyte concentration.

In the quantification of (R)-enantiomer in bulk samples of Fadrozole, standard addition and recovery experiments were conducted in triplicate at 0.12, 0.15 and 0.18% of analyte concentration. Recovery was calculated from slope and Y-intercept of the calibration curve obtained in linearity study and percentage recoveries were ranged from 95.5 to 107.4 (Table 4).

Table 4

Recovery results of (R)-enantiomer in bulk sample

Added (ng)	Recovered (ng)	% Recovery	% R.S.D.
1220	1165	95.5	8.3
1517	1574	103.8	5.2
1832	1967	107.4	6.7

n= 3 determinations

Standard addition and recovery experiments were also conducted for (R)-enantiomer in bulk drug samples using different system in Laboratory B at the same concentration levels tested in Laboratory A. The recovery results obtained in the Laboratory B were well in agreement with the results obtained in Laboratory A (Table 5). This confirms the ruggedness of the method.

Table 5

Ruggedness data of (R)-enantiomer in Laboratory B

Added (ng)	Recovered (ng)	% Recovery	% R.S.D.
1209	1152	95.3	7.5
1531	1612	105.3	6.1
1856	1789	96.4	8.8

n= 3 determinations

The chromatographic resolution of the Fadrozole and (R)-enantiomer peaks was used to evaluate the method robustness under modified conditions. Sufficient resolution for Fadrozole and (R)-enantiomer was obtained under all separation conditions tested (Table 6), demonstrating sufficient robustness.

Table 6

Robustness of the method

Parameter	USP resolution between Fadrozole and (R)-enantiomer
Flow rate (mL/min)	
0.6	11.1
0.8	9.8
1.0	9.0
Column temperature (°C)	
20	10.5
25	9.9
30	9.1
Ethyl alcohol percentage in mobile phase	
27	10.7
30	9.8
33	8.9

No significant change in the (R)-enantiomer content was observed in Fadrozole sample during solution stability and mobile phase stability experiments (Table 7). Hence, Fadrozole sample solution and mobile phase are stable for at least 24 h.

Table 7

Results of Fadrozole in solution stability and mobile phase stability

Time interval (h)	Solution stability (R)-enantiomer (% area)	Mobile phase stability (R)-enantiomer (% area)
0	0.16	0.16
6	0.15	0.17
12	0.16	0.14
18	0.15	0.15
24	0.14	0.14

Conclusion

A simple and accurate normal phase chiral LC method was developed for the quantitative determination of (R)-enantiomer content in Fadrozole bulk drug samples. Chiralpak AD-H, an amylose based chiral stationary phase was found to be selective for the enantiomers of Fadrozole. Method validation was carried out using Chiralpak AD-H column due to the best chromatographic results achieved on the column. The method was completely validated showing satisfactory data for all the method validation parameters

tested. The developed method is stability-indicating and can be used for the quantitative determination of chiral impurity ((R)-enantiomer) in Fadrozole bulk drug samples.

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Determination of nelfinavir mesylate in pharmaceutical dosage forms by reverse phase high performance liquid chromatography

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ABSTRACT

A new reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and used for the estimation of Nelfinavir mesylate (NEM) in bulk and pharmaceutical dosage forms using RPC-18 column using an isocratic HPLC system. The mobile phase consisted of acetonitrile and 0.05M potassium dihydrogen phosphate (pH 4.2) in the ratio of 50:50 at a flow rate of 1 mL/min. The run time was 15 min. Abacavir sulphate (ABS) (10mg/mL) was used as internal standard. The detection was carried out at 212 nm and the linearity was found to be in the range of 0.1-100 mg/mL. The retention times for drug (NEM) and internal standard (ABS) were 11.083 and 3.525 min respectively. Recovery studies have shown that about 100.07% of NEM could be recovered from the preanalyzed samples indicating high accuracy of proposed method. There was no intra-day and inter-day variation found in the method of analysis. The mean drug content in branded NEM tablet dosage forms was quantified and found to be between 99.76 and 100.21%. The method was found to be simple, precise, specific, sensitive, and reproducible.

Key Words: RP-HPLC, determination, Nelfinavir mesylate, pharmaceutical dosage forms.

Introduction

Nelfinavir mesylate (NEM) is an isoquinoline analog used against HIV-1 and HIV-2 in the treatment of AIDS. It is 2-[2-hydroxy-3-(3-hydroxy-2-methyl-benzoyl)amino-4-phenylsulfanyl-butyl]-N-tert-butyl-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-Carboxamide (CAS Reg. NO. 159989-64-7) classified under nucleoside reverse transcriptase inhibitors category of antiretroviral drugs[1]. Nelfinavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1[2-4]. Some analytical methods for the estimation of Nelfinavir mesylate were reported such as, HPLC [5-7] and LC-MS [8, 9]. The present study is aimed at developing a simple, reproducible, and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of NEM in bulk and pharmaceutical dosage forms using abacavir sulphate (ABS) as an internal standard (IS).

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Experimental

An isocratic HPLC system (Shimadzu®) consisting of LC-10 AT liquid pump, SPD-10A UV-visible detector, a ODS-18 RP column (4.6 mm I.D. X 25 cm length), 25 µL Hamilton® injecting syringe and MS Windows based Single channel software (Class VP®). Afcoset® electronic balance was used for weighing the materials. Pure samples of Nelfinavir mesylate and Abacavir sulphate were obtained from Matrix Laboratories, Hyderabad, India. Acetonitrile of HPLC grade and potassium dihydrogen phosphate of AR grade were purchased from E. Merck (India) Ltd., Mumbai. Water used was triple distilled prepared by all glass distillation apparatus.

Chromatographic conditions: The optimized chromatographic conditions were as follows:

Chromatograph	Schimadzu HPLC system
Mobile phase	Acetonitrile:0.05M potassium dihydrogen phosphate (pH 4.2) (50:50)

Column	ODS C-18 RP column (4.6 mm I.D. X 25 cm length)
Flow rate	1mL/min
Detection	UV set at 212 nm
Injection volume	20 mL
Temperature	Ambient
Retention time	
of Drug	11.083 min
of IS	3.525 min
Run time	15 min.

Procedure: Stock solutions of NEM and ABS were prepared by dissolving accurately weighed 25 mg of NEM and ABS in 25 mL of acetonitrile : 0.05M potassium dihydrogen phosphate (50:50) to obtain 1mg/mL solutions. From these solutions 2.5 mL was pipetted out into 25 mL volumetric flask and diluted with the same solvent system to obtain 100 µg/mL solutions. Working standard solutions of NEM each containing internal standard (ABS) solution in the concentration of 10µg/mL were prepared by taking required aliquots of NEM solutions and then diluted with the same solvent system. The standard solutions prepared above were injected five times into the column at a flow rate of 1mL/min. The ratios of AUC of drug to IS were calculated for each of the drug concentrations. The regression equation of drug concentration over the ratio of drug peak is to that of IS was obtained. The regression equation was used to estimate the amount of NEM in pharmaceutical tablet dosage forms.

The proposed HPLC method was tested for intra-day and inter-day variations. The recovery studies were carried out by adding known amounts of (10 µg and 30µg) of the NEM to the pre-analyzed samples and subjecting them to the proposed HPLC method.

Estimation of nelfinavir mesylate in its commercial tablet formulations: Contents of ten tablets containing NEM were pooled and powdered. The powder equivalent to 25 mg of NEM was extracted into acetonitrile and the volume was adjusted to 25 mL, mixed and filtered through a 0.45 µ filter. From the filtrate 0.1 mL was pipetted into a 10 mL graduated test tube and spiked with the required aliquot of IS solution and then the volume was adjusted to 10 mL with the mobile phase such that the concentration of IS in each sample was 10 mg/mL and was injected 5 times into HPLC column. The mean concentration of NEM corresponding to the ratio of AUC of NEM to that of IS was calculated from the standard graph. The same procedure was followed for remaining tablet brands.

Results and Discussion

The present study was carried out to develop a specific sensitive, precise and accurate HPLC method for the analysis of nelfinavir mesylate in pharmaceutical tablet dosage forms. A typical chromatogram is shown in **Fig. 1.** The column pressure varied from 205-230 kgf/cm2. The retention times for NEM and IS (ABS) were 11.083 and 3.525 min respectively. Each of the samples was injected five times and almost the same retention times were observed in all the cases.

The ratio of peak area of NEM to peak area of IS for different concentrations set up as above were calculated, and the average values for five such determinations are shown in Table-1. The peak areas of both drug and internal standard were reproducible as indicated by the low coefficient of variation (<2.96%). A good linear relationship (r = 0.9996) was observed between the concentration of drug and the respective ratio of peak areas. The calibration graph was found to be y = 0.0684x + 0.0456 (where y is the ratio of peak area of drug to that of internal standard and x is the concentration of drug in the range of 0.1 to 100 µg/mL). When NEM solutions containing 10µg/mL and 30µg/mL were analyzed by the proposed HPLC method for finding out intra-day and inter-day variation, a low coefficient of variation was observed (<2.41%) showing that the method is highly precise (Table-2). About 100.05% of NEM could be recovered from the preanalyzed samples indicating high accuracy of proposed method as shown in Table-3.

Table-1

Calibration of HPLC method for estimation of NELFINAVIR MESYLATE

Concentration of Nelfinavir mesylate (?g/mL)	Mean ratio of AUC of drug to IS (n=5)	CV (%)
0.1	0.1352	1.84
0.5	0.0484	1.38
1	0.0680	1.71
2	0.1693	1.83
5	0.4049	2.05
10	0.8102	2.14
20	1.3349	2.24
40	2.7891	2.96
80	5.4173	2.49
100	6.9791	1.68

C.V.= coefficient of variation, Regression equation (from 0.1 to 100 ?g/ml)

Table-2

Precision of the Proposed HPLC Method

Nelfinavir concen-tration (?g/mL)	Concentration of Nelfinavir mesylate mesylate (?g/ml) found on			
	Intra-day		Inter-day	
	Mean (n=5)	% CV	Mean (n=5)	% CV
10	10.18	1.84	10.26	1.49
30	30.11	1.73	30.24	2.41

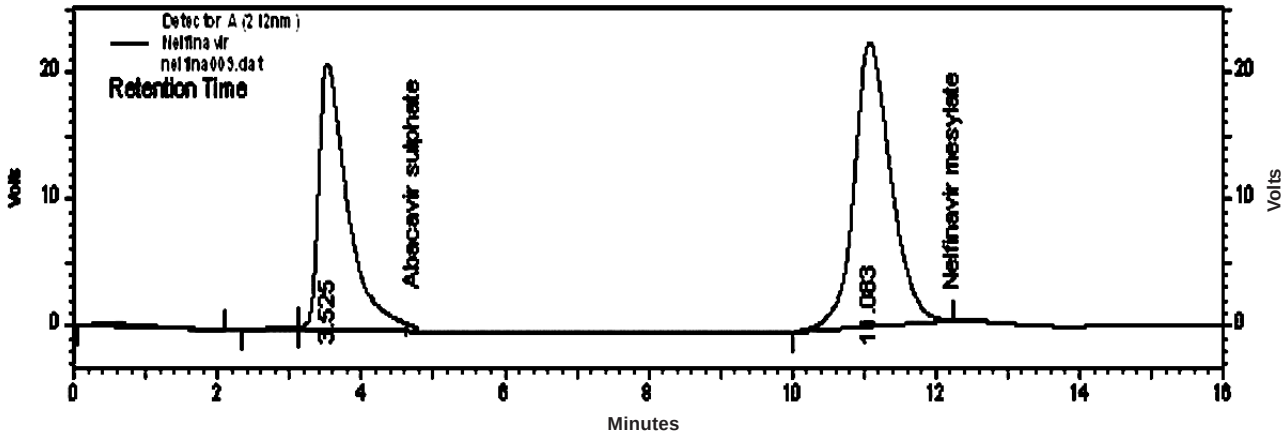

Fig.1: Typical Chromatogram of NELFINAVIR MESYLATE

Table-3

Recovery studies of NELFINAVIR MESYLATE

Amount of drug added (?g)	Mean (±s.d.) amount (?g) found (n=5)	Mean % recovery
10	10.022 (±0.025)	100.05
30	30.010 (±0.048)	99.95

Table-4

Assay of different Brands of NELFINAVIR MESYLATE tablets

Brand	Labeled amount of drug (mg)	Mean % of labeled amount (n=5)	%CV
A	250	99.79	1.38
B	250	100.15	1.82
C	250	100.21	1.96

The NEM content in branded tablet formulations was quantified using the proposed analytical method and details are shown in Table-4. The absence of additional peaks indicated no interference of the excipients used in the tablets. The tablets were found to contain 99.79 to 100.21% of the labeled amount. The low percent of CV (<1.96 %) indicates the reproducibility of the assay of NEM in the tablet dosage forms. The proposed method was found to be simple, precise, accurate, specific and economical. Hence this method can be employed to estimate NEM in bulk and tablet dosage forms effectively.

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Chemical characterization and *in vitro* antimicrobial activity of essential oil from the husk of *Bursera penicillata* (Sesse & Moc. ex DC.) Engl.

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ABSTRACT

The essential oil obtained from the husk of *Bursera penicillata* (common name: Linaloe or Indian lavender) has been studied by GC-FID and GC-MS. The essential oil chiefly consists of oxygenated monoterpenes (91.04%), monoterpene hydrocarbons (0.5%), sesqueterpene hydrocarbons (3.4%) and oxygenated sequeterpenes (4.9%). The essential oil constitutes twenty-eight chemical constituents which include twenty seven known (99.43%) and one unknown compounds. The principle component of the oil is linalyl acetate (65.9%) and accompanied by linalool (7.6%), nona – lactone (6.5%), neryl acetate (4.5%), a noticeable amount of 2-dodecanol (4.2%) while cis-linalool oxide, dihydro carvone, myrtenol were detected in much smaller amount. The oil exhibited a broad spectrum of antimicrobial activity against bacterial strains viz., *Bacillus megatherius, B. subtilis, Micrococcus luteus, M. roseus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pneumoniae* and fungal strains namely *Candida albicans* and *C. tropicalis*. The essential oil possess high ester value, antimicrobial activity with its pleasant odor took importance in soap and cosmetic industries.

Key Words: *Bursera penicillata*; Bursaraceae; GC-MS analysis; essential oil composition; linalyl acetate, linalool; antimicrobial activity.

Introduction

Bursera penicillata (Sesse & Moc. ex DC.) Engl. (syn. *B. delpechiana* Poss. ex Engl.), generally known as Indian lavender. It is introduced from Mexico and cultivated in South India. It is used as a substitute for true lavender oil obtained from *Lavandula angustifolia*, which is being imported. The oil is extensively used as a fixative for high-grade perfumes, cosmetic products and in the manufacture of transparent soaps. The oil is present in all parts of the plant, the highest yield can be obtained from husk of the berries [1]. The present investigation was emphasized on the antimicrobial activity of the essential oil of *Bursera penicillata* was hither to not report and the same was substantiated by the characterization of chemical compounds which might be responsible for the biological activity.

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Material and Methods

Plant material

The husk of *B. penicillata* was obtained from the Forest Plantation Storage Centre, Department of Forests, Hyderabad for the analysis.

Isolation of Essential oil

The berries were broken by disc huller and the husk was separated, ground and subjected to steam distillation for 24 h. Steam distillation was performed in a Clevenger – type apparatus for 5 h [2]. The oil was (6.81%) subjected to chemical and *in vitro* biological studies. The physico-chemical properties of the essential oil were determined by standard methods [3]

Chemical composition of essential oil

The chemical components of the oil were characterized by gas chromatography. The essential oil (5µL) was subjected to GC analysis (on a Perkin – Elmer 8500 gas chromatograph equipped with FID using BP – 1 (Diethyl

poly siloxane) column (30 x 0.32 mm i. d. and 0.25µ film thickness) and nitrogen as carrier gas at 10 psi inlet pressure, temperature programming was done from 60 – 220^0 C at 5^0 C/min. The split ratio was 1:80) and GC-MS was carried out using a Shimadzu Quadruple GC-MS 5050 QP, operated in EI mode. The sample was spiked on GC-MS on DB - 5 capillary column (30m x 0.32 inches diameter with 1.5-micron film thickness), programmed at 60^0 C for 3 minutes, 8-250^0 C for 10 min. Helium as carrier gas with 1.5 ml/min flow rate (the septum sweep at 1:30).

Identification of Components

The oil was spiked with a standard mixture of n-alkane series (C_8- C_{23}) and analyzed under the above mentioned conditions. The retention indices were calculated by application of a modified Kovat's procedure [4]. The individual chemical components of the oil (table 1) were identified based on their retention indices which was further confirmed by mass spectral data [5,6,7]

Antimicrobial assay

Bacterial and fungal strains were obtained from the Microbial Type Culture Collection Centre (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Microorganisms used in the present investigation were maintained on the appropriate media such as Nutrient agar, Nutrient broth, Mueller Hinton agar, Czepek Dox agar. Standard antibiotics like Ampicillin, Tetracycline and Vancomycin (30 µg/6mm disc), used for comparison were obtained from Hi-Media Laboratories, Mumbai, India. The media and the glassware were properly sterilized in an autoclave and all other manipulations were conducted under aseptic conditions.

Preparation of samples for antimicrobial assay

Before experimentation, the oil was diluted with equal volumes of TWEEN 80 at the concentrations ranges of 1:0 and 1:2. Antimicrobial susceptibility tests were determined by employing standard disc dilution technique [8]. Whatman no.1 filter paper discs of 6mm diameter placed in dry Petri-plates and sterilized in an autoclave at provided conditions. These sterile discs were dipped in the test oil samples and shaken thoroughly. These filter paper discs were allowed to dry and were care fully placed over the spread cultures and incubated at 35±2^0C, 24h for bacterial strains, while 28± 2^0 C, 48h for fungal strains. The paper discs dipped in TWEEN 80 alone were serves as negative controls [9]. The discs impregnated with antibiotics serve as positive controls and used in comparison with antimicrobial activity of the test oil. The zone of inhibitions surrounding the paper disc indicates antimicrobial activity, which was measured accurately to the nearest millimeter by means of metric ruler and illuminated colony counter. In all cases where the zone of inhibition was found more than 10 mm ascertained whether microbistatic or microbicidal. The microbicidal activity was confirmed by transferring a loop of culture from the inhibition zone transferred in to fresh sterilized nutrient broth and incubated under the standardized conditions. Simultaneously different standard antibiotics were tested for all microbes in similar conditions so as to compare the degree of inhibition exhibited by essential oil. The oils were subjected to the test of susceptibility and found free of microorganisms. Each plate carrying a disc with TWEEN 80 alone served as negative control. These experiments conducted for three times and the average of inhibition zones for each microorganism was recorded.

Table-1

Chemical composition of the essential oil from the husk of *Bursera penicillata*

Retention index	Compound	Per cent	Method of identification
991	myrcene	0.06	RI, GC-MS
1005	phellandrene	0.23	RI, GC-MS
1040	*cis*-Ocimene	0.17	RI, GC-MS
1074	*cis*- linalool oxide†	1.36	RI, GC-MS
1088	*trans* linalool oxide†	1.11	RI, GC-MS
1098	**linalool**	**7.66**	RI, GC-MS
1211	1- octyl – Acetate	0.11	RI, GC-MS
1134	pinocarveol	0.14	RI, GC-MS
1165	borneol	0.23	RI, GC-MS
1170	dihydrocarvone	1.08	RI, GC-MS
1194	myrtenol	1.18	RI, GC-MS
1272	1- decanol	0.19	RI, GC-MS
1257	**linalyl acetate**	**65.94**	RI, GC-MS
1255	geraniol	0.28	RI, GC-MS
1305	dihydrocarvyl acetate	0.14	RI, GC-MS
1315	**nona lactone**	**6.37**	RI, GC-MS
1350	terpenyl cetate	0.63	RI, GC-MS
1365	**neryl acetate**	**4.48**	RI, GC-MS
1351	cubebene	0.74	RI, GC-MS
1383	geranyl acetate	1.04	RI, GC-MS
1391	elemene	0.76	RI, GC-MS
1410	2 - do - decanal	4.26	RI, GC-MS
1479	murrolene	0.41	RI, GC-MS
1483	valanene	0.40	RI, GC-MS
1524	cadinene	0.06	RI, GC-MS
1568	dodecanoic acid	0.19	RI, GC-MS
1581	caryophellene oxide	0.63	RI, GC-MS
1835	Unknown compound	0.17	RI, GC-MS
MonoterpeneHydrocarbons		**0.46**	
Oxygenated Monoterpenes		**91.04**	
Sesqueterpene Hydrocarbons		**3.4**	
Oxygenated sesqueterpenes		**4.9**	

† furonoid form

RI, Retention index, GC-MS, Gas chromatography- Mass Spectrometry

Results and Discussion

The essential oil obtained (yield 6.8%v/w) from the husk of *B. penicillata* using steam distillation was analyzed for its chemical components and *in vitro* antimicrobial activities. The GC and GC-MS analysis of the essential oil revealed that twenty eight compounds of which twenty seven compounds were identified, represented 99.8%. The oil possessed monoterpene hydrocarbons (0.46%), sesquiterpene hydrocarbons (3.41%) and oxygenated sesquiterpenes (4.9%) while oxygenated mono terpenes constitute the major portion (91.9%). Linalool acetate is the major constituent of the essential oil (62.5%). However, the earlier report[1] indicate that it was only 47%, which may be due to the influence of ecoclimatic factors and improved cultivation practices. The mass spectral details appended for unidentified as well as linalool acetate, the compound which was found in high concentration (62.5%). In addition to the linalool (7.6%), the oil possessed neryl acetate (4.5%) and 2 do-decanol (4.26%) which were found as sub major compounds. The compounds cis-linalool oxide, t-linalool oxide, dihydrocarvone, myrtenol, geranyl acetate were found in traces while others reported in much smaller concentrations. The percentage of the compounds and the mode of identifications are listed in table-1. The mass spectral details of different compounds present in the oil are given in table 2.

The antimicrobial activity of the essential oil of *Bursera penicillata* is hither to not reported, hence gains the importance. The essential oil exhibited significant antimicrobial activity on test bacteria, viz., *Bacillus megatherius, B. subtilis, Micrococcus luteus, M. roseus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus pneumoniae* and fungal strains namely *Candida albicans* and *C. tropicalis* while *B. cereus* was found to be resistant to the tested oil. The inhibitory activity of the oil was similar to that of the standard antibiotics (Table 3). Interestingly, significant inhibition was recorded even at low concentrations (table 3) indicating the potential antimicrobial principle.

Linalool was reported to possess anti-inflammatory activity. The oxygenated mono terpenes constituting a

Table: 2

Mass spectra of certain interested compounds from the oil of *Bursera penicillata*

Name of the compound	Mass fragmentation peaks
Linalyl Acetate(65.94%)	93 (M+), 43, 55, 80, 107, 121, 136, 154, 224
Unknown compound (0.17)	43(M+), 67, 71, 82,109, 113, 137

Table: 3

Antimicrobial activity of essential oil of *Bursera penicillata* husk

S. No.	Microorganisms	Zone of Inhibition (mm^{-1})			
		Oil in Tween 80		MIC	Standard
		(1:1)	1:2	(µL/disc)	antibiotics
1	*Bacillus cereus* MTCC1429 (GP)	-	-	-	22 [a]
2	*Bacillus megatherius* (GP)	22	20	12	22 [a]
3	*Bacillus subtilis* MTCC121 (GP)	10	10	20	18 [b]
4	*Micrococcus luteus* MTCC1522 (GP)	16	14	15	36 [b]
5	*Micrococcus roseus*MTCC 2522	14	12	16	24 [b]
6	*Staphylococcus aureus* MTCC737 (GP)	14	12	16	14 [b]
7	*Streptococcus pneumoniae* (GP)	20	18	14	16 [b]
8	*Pseudomonas aeruginosa* MTCC1688 (GN)	12	12	17	20 [b]
9	*Escherichia coli* MTCC1687 (GN)	14	12	16	22 [b]
10	*Candida albicans* MTCC183 (fungal species)	12	12	17	24 [c]
11	*Candida troicalis*MTCC 187 (fungal species)	12	10	20	20 [c]

*a, Ampicillin; b, Tetracycline; c, Vankomycin; MIC, Minimum Inhibitory Concentration of oil; GP, Gram positive; GN, Gram negative;

major portion of the oil, which were reported to be involved in antibacterial and antifungal activity [10, 11] also involved in the present investigation. The antimicrobial activity of the oil might be due to a heterogeneous mixture of different terpenes as shown in the table-1. Thus, this oil can be considered as a protective agent against waterborne pathogenic microorganisms when used topically.

Conclusion

The husk oil of *B. penicillata* is potential commercial value as it substitutes the costly lavender oil [2] in cosmetic industry, which can be introduced as an alternate tree crop. The high ester value and non-sticky nature of the oil may find importance in the manufacture of natural soaps and cosmetics.

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Analgesic activity *Momordica cymbalaria* Hook. F. Fruit extract

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ABSTRACT

The analgesic activity of the methanol extract of *Momordica cymbalaria* Hook.F. fruit extract (MEMC) was investigated in chemical models of nociception in mice. MEMC at doses of 200, 400 and 600mg/kg i.p. produced an inhibition of 23.3, 47.7, and 65.8%, respectively, of the abdominal writhes induced by acetic acid in mice. In the formalin test, the administration of 200,400 and 600mg/kg i.p. had no effects in the first phase (0 to 5 min) but produced a dose dependent analgesic effect on the second phase (15 to 40 min) with inhibitions of the licking time of 25.4, 46.9 and 58.9%, respectively. These observations suggest that MEMC possesses some analgesic activity.

Key Words: *Momordica cymbalaria,* acetic acid, aspirin, MEMC, analgesic activity.

Introduction

Momordica cymabalaria Hook. F. belongs to the Cucurbitaceae family. The plant is a perennial herbaceous climber either allowed to trail on the ground or to climb on supports with the aid of tendrils. It is found in the south Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharastra and Tamil Nadu as a weed. The plant is allowed to grow along bunds (boundary of fields), fences and even in the fields for the sake of fruits. However no regular cultivation is practiced. The plant has a tuberous root, which helps to maintain perennial habits, pubescent or sub glabrous. i.e., the plants dry and disappear at the end of the season. The tubers remain in the soil and emerge in the next season. Flowering occurs during October; fruits are harvested from November to January. The yield of each plant is 1.25 to 1.5kg. The tender fruits closely resemble those of a small variety of bitter gourd Athalakkai is used as a vegetable by the rural people of South Tamil Nadu and North Karnataka, India [1]. The phytochemicals reported in this plant are tannins, alkaloids, phenols, proteins, amino acids [2]**,** Vitamin C, carbohydrate and ß-Carotene [1]. The fruits of this plant reported anti diabetic and antihyperlipedimic activities [2], hepatoprotective and antioxidant [3], antimicrobial [4]**.** The tubers were reported as antiovulatory activity [5].

Furthermore, literature survey of *M..cymbalaria* revealed that no researcher has not yet reported analgesic activity of the fruit. Therefore, it is worth conducting an investigation on the analgesic activity methanol extract of *M.cymbalaria* fruits (MEMC).

Materials and Methods

Plant material

The fruits of *Momordica cymbalaria* Hook F. was collected in November 2006 from the Bellary, Karnataka, India. The fruit material was taxonomically identified by the Regional Research Institute, Karnataka, India, and the Voucher specimen RRI/BNG/DSRU/F53/2006-07. The fruits were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

Preparation of extract

The powder obtained was subjected to successive soxhlet extraction with the solvents with increasing order of polarity i.e. Pet. Ether (60-80°), Chloroform (59.5-61.5°), Methanol (64.5-65.5°) and water. Yield 3.29, 6.19, 11.70, and 15.71% respectively.

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Phytochemical screening

A preliminary phytochemical screening of methanol extract was carried out as described by Khandelwal K.L [6].

Animals used

Mature albino mice (20-25g) were used for the present study. The animals were kept in constant temperature (22±2°C), humidity (55%) and light-dark conditions (12/12 light/dark) and provided with standard pellet diet (Hindustan Lever) and water *ad libitum.* The experiments were performed under the guidance of Ethical committee of Rural College of Pharmacy (Registration No: 129/99/CPCSEA).

Analgesic Activity

Inhibition of acetic acid-induced writhing in mice

Male albino mice were used in groups of six animals per dose of drugs. The animals were pretreated with methanolic extract of *M.Cymbalaria* (MEMC, 200, 400 and 600 mg/kg i.p.) or aspirin (100 mg/kg, i.p.) for 30 min or 20 min, respectively, prior to intra peritoneal injection of 1% acetic acid (0.1 ml/10 g). Five minutes after the *i.p* injection of acetic acid, the number of writhes was counted for ten minutes. Control mice received normal saline. The responses of extract treated groups were compared with those of animals receiving aspirin 100mg/kg (as standard drug), as well as with the controls [7].

Formalin test

Male Albino mice were used in groups of six animals per dose of drugs Male. Each animal was tested once only. The MEMC (200,400 and 600 mg/kg .) and aspirin (100 mg/kg) were suspended in Tween 80 plus 0.9% (w/v) saline solution and administered *i.p,* in a volume of 0.2 ml. Control group received only drugless vehicle (0.2 ml).. One hour before testing, the animal was placed in a standard cage that served as an observation chamber. 20ìl of 1.0% formalin injected to the dorsal surface of the right hind-paw. The mice were observed for 40 min after the injection of formalin, and the amount of time spent licking the injected hind-paw was recorded. The first 5 min post formalin injection is known as the early phase and the period between 15 min and 40 min as the late phase. The drugs were administered 30 min before injection of formalin. The total time spent on licking or biting the injured paw (pain behavior) was measured with a stopped watch. The activity was recorded in 5 min interval [7].

Statistical analysis

Results are presented as mean± SEM. Statistical analysis of data performed using ANOVA followed by Turkey's test.

Results

Preliminary phytochemical studies revealed that the presence of tannins, alkoloids, proteins, aminoacids, flavanoids, triterpenoids, sterols and Vitamins.

Inhibition of acetic acid-induced writhing in mice

Table-1 shows the pain behavior of writhing response, which is presented as cumulative abdominal stretching response. The treatment of animals with MEMC extract (400 and 600mg/kg/ b.w i.p.) produced a significant ($P < 0.01$) and dose dependent inhibition of the control writhes. The inhibition by MEMC (600 mg/kg) was similar to that produced by aspirin (100 mg/kg).

Formalin test

MEMC (400 mg/kg, 600 mg/kg) produced significant ($P < 0.001$) inhibition in the late phase of formalin induced pain; respectively (Table-2). The positive control aspirin (100 mg/kg) also produced significant ($P < 0.001$) inhibition in the late phase.

Discussion and Conclusion

The methanol extract of fruit of *M.cymbalaria* (MEMC) given i.p. at doses of 200, 400 and 600 mg/kg significantly inhibited the acetic acid-induced writhing response in a dose-dependent manner. Acetic acid, which is used as an inducer for writhing syndromes and, causes analgesia by releasing of endogenous substances, which then excites the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to

Table 1

Analgesic effect of methanol extract of *Momordica cymbalaria* (MEMC) in acetic acid-induced writhing test

Design of treatment	Number of writhings [a]	Inhibition (%)
Control	38.6±2.1	–
MEMC (200mg/kg)	29.6 ± 2.8	23.3
MEMC (400mg/kg)	20.2 ±2.4*	47.7
MEMC (600mg/kg)	13.3 ± 1.9**	65.8
Aspirin (100mg/kg)	11.4 ±1.2**	70.5

[a] Values are mean±S.E.M.

* $P<0.05$, ** $P<0.01$ significant compared with control values.

Table 2

Analgesic effect of methanol extract of *Momordica cymbalaria* (MEMC) in formalin test

Design of treatment	Licking(s) [a]		Inhibition (%)
	0-5min	15-40min	
Control	59.2± 4.1	119.7±11.2	–
MEMC (200mg/kg)	57.8±3.4	89.2±9.6*	25.4
MEMC (400mg/kg)	56.9±3.1	63.4±5.9**	46.9
MEMC (600mg/kg)	57.3± 3.6	49.2±6.3***	58.9
Aspirin (100mg/kg)	56.8± 4.2	44.7± 3.8***	62.6

[a] Values are mean±S.E.M.

* $P<0.05$, ** $P<0.01$ *** $P<0.001$ significant compared with control values.

prostaglandins [8]. Also, it is well known that, the acetic writhing test is normally used to study the peripheral analgesic effects of drugs. Although this test is a nonspecific (e.g., anti-cholinergic, antihistaminic and other agents also show activity in the test), it is widely used for analgesic screening and involves local cholinergic and histaminic receptors, and the mediators acetylcholine and histamine [9]. This result indicates that the analgesic effect of MEMC might be mediated by its peripheral effect.

The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. Formalin test produced a distinct biphasic response and different analgesics may act differently in the early and late phases of this test. Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic effect [10]. Centrally acting drugs such as opioids inhibit both phases equally [9], but peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [11, 12]. The effect of MEMC on the late phase of formalin test suggests that its activity may be resulted from its peripheral action when compared with aspirin activity in this respect. Based on the results of this study, we suggest that the analgesic effect of *M.cymbalria* may be attributed to inhibition of prostaglandin release and other mediators involved in this test. A preliminary phytochemical study reveals that presence of tannins, alkaloids, flavanoids, triterpenoids and sterols. Hence, the present analgesic activity of *M.cymbalria* may attribute to the presence of tannins, flavonoids, triterpenes and sterols.

In conclusion, this study has demonstrated using pharmacological models of pain that *M.cymbalaria* possesses analgesic effect and this provides a rationale for its use in folk medicine. However, more work is needed to evaluate the mode of action and identify the compounds responsible for its analgesic effect.

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Hypoglycemic activity of aqueous extract of leaves of *Talinum cuneifolium* Linn. in rats

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ABSTRACT

The hypoglycemic effect of aqueous extract of leaves *Talinum cuneifolium* (AETC) was evaluated in normal, glucose fed and alloxan- induced diabetic rats. Oral administration of extract (200 and 400mg/kg body wt) for 7 days resulted in a significant reduction in blood glucose level. The effect was compared with 0.5gm/kg (i.p) glibenclamide.

KEY WORDS: *Talinum cuneifolium*, AETC, alloxan, hypoglycemic.

Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by ineffectiveness of insulin produced such a deficiency result in increased concentration of glucose in the blood, which in turn damage of many of the body's systems in particular the blood vessels and nerves. As the number of the people with diabetes multiply world wide, the disease taken an ever increasing production of national and international health care budgets. It is projected to become of the world's main disablers and killers with in the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could be rise to two-to-three-folds than the present rates. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plants medicines are used throughout the world for a range of diabetic presentation [1]. The synthetic hypoglycemic agents used in clinical practices have serious side effects like hematological effects, coma, disturbances of liver and kidney. In addition they are suitable for use during pregnancy [2]. Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects [3].

Talinum cuneifolium Linn. (Portulaceae) commonly known as Ceylone Bachalli. In Indian System of Medicine, the various parts of plants includes leaves and roots are used as treatment of diabetic, mouth ulcer, and aphrodisiac, cough, gastritis, pulmonary tuberculosis, diarrhea and stomachic [4,5].

In the light of the above information the present investigation was undertaken to have evaluate the glucose lowering effects of aqueous extract of leaves of *Talinum cuneifolium* in alloxan hyperglycemic rats to establish pharmacological evidence in support of the folklore claim.

Materials and Methods

Plant material

Fresh leaves were collected from S.V.U campus, Tirumala gardens of Chittor District of Andhra Pradesh of India and authentified by Dr.K.Madava Chetty, Asst. Professor, Department of Botany, S.V.University, Tirupathi. Andhra Pradesh, India. Voucher specimen [No.TCA1/PRRMCP 06- 10] was deposited at Department of Pharmacognosy for further reference.

Extraction

The leaves, shade dried powder in a grinder mixture to obtain a coarse powder and then passed through 40-mesh sieve. The powdered leaves (430g) were defatted with hexane and later extracted with water (cold maceration). The extract evaporated to dryness, gave a residue 20.5%w/w.

Phytochemical screening

A preliminary phytochemical screening of Aq. Extract of TC was carried out as described by Khandelwal K.L (Khandelwal, 2003) [6].

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Animals

Wistar albino rats (200-250g) of both sexes were procured from Sri Venkateshwara Enterprises, Bangalore. Before and during the experiment rats were fed with standard diet (Gold Mohr, Lipton India Ltd). After randomization in to various groups and before initiation of experiment, the rats were acclimatized for a period of 7days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived off food and water for 16 hours *ad libitum*. Ethical clearance for animal study was obtained from the institutional animal ethics committee. (IAEC/PRRMCP/2006/07)

Toxicity study

An acute toxicity study relating to the determination of LD_{50} value was performed using different doses of the extract according to the method described by Ghoshet.al [7] from the toxicity study; it was observed that the extract is non-toxic upto dose of 5.0 g/kg body weight. It is safe and was used in different doses for further studies.

Experimental Design

Effects of AETC on blood glucose levels in normoglycemic rats

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the mentioned dosage schedule. Animals were divided into three groups of six rats in each group. Group-1, 2 and 3 receives 1% Sodium carboxy methyl cellouse (2ml/kg), 200 and 400 mg/kg orally of Aq. extract of TC respectively. Blood glucose levels were determined at 0 (before drug challenge) 60, 120min, after drug administration.

Effect of AETC on blood glucose level on glucose fed hyperglycemic rats (Oral Glucose Tolerance Test)

In this study the entire groups of animals were fasted over night and administered with respective drugs as per the mentioned dosage schedule. Animals were divided into four groups of six rats in each group. Group-1, 2, 3 and 4 receives glucose 2g/kg only, glibenclamide 0.5mg/kg, i.p, 200 and 400mg/kg and glucose 2g/kg orally half an hour before administration of standard and test extract respectively. Blood glucose levels were determined at 0 (before glucose challenge) 30, 60, 90, 120thmins after glucose administration.

Effect of AETC on blood glucose level in alloxan induced diabetic rats

Different groups of rats were used to study the effects of Aq. extract of TC. The rats were divided into five groups each consisting of six rats. Group-1: Normal control animals received 1% Sodium carboxy methyl cellouse 2ml/kg body wt. per orally. Group-2: Alloxan (150mg/kg body wt.) induced diabetic animals received 1% Sodium carboxy methyl cellouse 2ml/kg body wt. per orally. Group-3: Alloxan (150mg/kg body wt.) induced diabetic animals received glibenclamide 0.5mg/kg, body wt. per orally. Group-4: Alloxan (150mg/kg body wt.) induced diabetic animals received AETC 200mg/kg, body wt. per orally. Group-5: Alloxan (150mg/kg body wt.) induced diabetic animals received AETC 400mg/kg, body wt. per orally. Significant hyperglycemia was achieved within 48 hours after Alloxan (150 mg/kg b.w. i.p.) injection. Alloxan induced diabetic rats with more than 200 mg/dl of blood glucose were considered to be diabetic and used for the study.

In acute study all the surviving diabetic animals and normal animals were fasted over night. Blood samples were collected from the fasted animals prior to the treatment with above schedule and after administration at each day up to 7days. For glucose determination, blood was obtained snipping tail with sharp razor [8]. Then the blood glucose levels were determined by using Haemo-Glukotest (20-800R) glucose strips supplied by M/s Boehringer Mannheim India Ltd. These methods, which permit the measurement of blood glucose levels with minimum injury to rat, was previously validated by comparison with glucose oxidase method [9-11].

Statistical Analysis

All values were expressed as mean ± SEM .The data were statistically analyzed by ANOVA followed by Dunnets't' test [12].

Results and Discussion

Phytochemical screening

The preliminary phytochemical studies of Aq. extract of TC revealed that presence of alkoloids, tannins, flavanoids, proteins and carbohydrates.

Toxicity study

From the toxicity study it was observed that AETC is non-toxic and caused no death up to 5 g/kg orally. The results presented in Table-1.

Effect of AETC on blood glucose in normoglycemic rats

At dose 200mg/kg and 400mg/kg of Aq. extract of TC on fasting blood sugars level were assessed in normal rats at various time interval is shown in table-2. The mean blood glucose level decrease from 76.00 mg/dl to 76.40 mg/dl at dose of 200mg/kg body weight of Aq. extract of TC and 77.00 mg/dl to 75.80 mg/dl at dose of 400mg/kg bodyweight in rats treated with Aq. extract of TC.

Effect of AETC on blood glucose level in glucose fed hyperglycemic rats

At dose 200mg/kg and 400mg/kg of Aq. extract of TC blood sugar level were assessed in glucose fed rat at various intervals as shown in table-3. The blood glucose levels decreased from 77.65 mg/dl to 77.83 mg/dl at 200mg/kg bodyweight and 81.00 mg/dl to 79.53 mg/dl at 400mg/kg body weight.

Table-1

Toxicity Study of AETC

Treatment	Dose(mg/kg body wt)	No. of animals	No. of survival	No. of death	Percentage of morality	LD_{50} valve
Control	1% NaCMC	10	10	0	0	-
EETC	100	10	10	0	0	-
	200	10	10	0	0	-
	400	10	10	0	0	-
	800	10	10	0	0	-
	1600	10	10	0	0	-
	3200	10	10	0	0	-
	5000	10	10	0	0	> 5.0g/kg body wt.

Table -2:

Effect of AETC on Blood glucose in normoglycemic rats

GROUPS	Blood glucose levels (mg/dl)		
	Initial	60min	120 min
Group I (n=6)	79.33 ± 1.145	80.00 ± 1.204	78.66 ± 1.364
Group II (n=6)	76.00 ± 0.866	75.90 ± 1.77	76.40 ± 1.82
Group III (n=6)	77.00 ± 0.966	66.83 ± 0.175	75.80 ±1.501

The values are expressed as mean ± SEM. n = number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's –'t' test. The 60th and 120th min values are compared with initial value.

Table-3

Effect of AETC on Blood glucose in glucose fed hyperglycemic normal rats

Groups	Blood glucose levels (mg/dl)				
	Initial	30 min	60min	90 min	120 min
I	82.16±01.30	116.83±0.70	119.50±0.74	104.80±1.75	85.66±1.47
II	77.66±01.20	118.83±1.01*	106.33±1.22*	82.00±1.06*	75.66±1.38*
III	77.65± 1.20	116.53±0.80*	103.00±1.06*	88.53±1.45*	77.83±0.21*
IV	81.00±01.25	120.16±1.38*	111.26±1.08*	94.66±1.30*	79.53±1.25*

The values are expressed as mean ± SEM. n = 6 number of animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's –'t' test. The blood glucose values of group II, III and IV are compared with control animal's values. P< 0.05 were taken as* Significant.

Effect of AETC on blood glucose level in alloxan induced diabetic rats

The antihyperglycemic effect of the extracts on the blood sugar level on diabetic rats is shown in Table-4. The blood glucose level of diabetic animal significantly ($p<0.05$) reduced from 210.15 mg/dl to 105.18 mg/dl at 200mg/kg body wt. of Aq. extract of TC and 209.01 mg/dl to 99.73 mg/dl at 400mg/kg body wt. of Aq. Extract of TC. These results are comparable with 0.5mg/kg of glibenclamide.

Discussion

In the recent times many traditionally used medicinally important plants were tested for their anti-diabetic potential by various investigators in experimental animals. These properties were attributed to different formulations, extracts and active principles. Working on the same line, we have undertaken a study on *Talinum Cuneifolium* for its anti-diabetic property.

The AETC at a dose of 200mg/kg body wt. per orally did not significantly suppress blood glucose levels in over night fasted normoglycemic animals. The same effect was observed at a higher dose level of 400mg/kg body wt. Per orally of the Aq. extract of TC in over night fasted normoglycemic animals after 1st ,2nd and 3rd hour of oral administration, when compared with control group of animals.

The AETC showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process with concomitant decrease in glycogenolysis and glyconeogenesis [13]. However, the effect was less significant when compared to standard drug glibenclamide.

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that alloxan causes diabetes by rapid depletion of â cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states [14]. In this study significant hyperglycemia was achieved within 48 hours after Alloxan (150mg/kg b.w. i.p) injection. Alloxan induced diabetic rats with more than 200mg/dl of blood glucose were considered to be diabetic and used for the study.

The studies on antidiabetic activity in alloxanised rats, significant reduction of blood glucose was observed from the 2nd day of the study. The comparable effect of the extract with glibenclamide may suggest similar mode of action since alloxan permanently destroys the pancreatic β cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extent possesses extra pancreatic effects. From the Phytochemical analysis it was found that the major chemical constituents of the extract were flavonoids, and tannins. Over 150 plant extract and some of this active principle including flavonoids are known to be used for the treatments of diabetes [15-18] on the basis of the above evidences it is possible that the presence of flavonoids and tannins are responsible for the observed antidiabetic activity [19, 20].

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Table -4

Effect of AETC on Blood Glucose level in Alloxan induced Diabetic Rats

Groups	Blood glucose levels (mg/dl)						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
I	81.00 ± 0.59	81.33 ±0.44	80.91 ± 0.43	80.66 ± 0.54	81.00 ± 0.36	81.00 ± 0.53	81.33 ± 0.49
II	204.83 ± 1.25	212.66 ± 1.45	219.83 ± 1.35	228.16 ± 1.40	237.66 ± 1.80	246.66 ± 2.124	255.83 ± 2.54
III	207.00 ± 1.63	184.00 ± 1.77*	163.83 ± 1.66*	143.16 ± 2.18*	121.83 ± 2.85*	101.33 ± 3.01*	85.33 ± 1.35*
IV	210.15 ± 0.95	199.15 ± 1.20*	180.43 ± 0.95*	160.01 ± 0.95*	137.83 ± 1.75*	117.83 ± 1.30*	105.18 ± 1.60*
V	209.01 ± 1.31	187.50 ± 1.64*	171.86 ± 2.75*	156.42 ± 2.52*	135.00 ± 1.86*	110.83 ± 2.30*	99.73 ± 1.53*

The values are expressed as mean + SEM. n = 6 number of animals in each group.
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. P< 0.05 were taken as* Significant.

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In Vitro Antimicrobial and Antitubercular Activity of Some new Hydrazides, Hydrazones and Sulfonamides of Quinoxalines

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ABSTRACT

Some new 2 - substituted hydrazino / benzylidino / methyl hydrazones and 7 -sulfonamides of 1H, 4H – 3- oxo - quinoxalines were synthesized from 1H, 4H quinoxalin- 2, 3 - diones and characterized by IR, H^1 NMR, LC – MS and CHN analytical data. All the eleven synthons of the work were evaluated for their *in vitro* antimicrobial activity against the bacteria *S. aureus*, *E. coli*, *P. vulgaris*, *P. aeroginosa*, the fungi *C. albican and A. niger and* the mycobacterium *Mycobacterium tuberculosis* H_{37} *Rv* by agar plate disc diffusion method and Microplate Alamar Blue Assay (MABA) method, respectively. It was concluded that 2 – hydrazino, 7 – sufonamido and 2, 3 – dichloro substitution on quinoxaline showed potent antitubercular, antibacterial and anti fungal spectrum, respectively.

Introduction

In the recent years, 2, 3 – disubstituted quinoxalines have been extensively studied and reported to posses significant antimicrobial potential against bacteria, fungi and mycobacterium [1-2]. Design of quinoxaline antibiotics have undertaken by several workers, but they posses limited application due to their toxic effect. It is believed that the antimicrobial potency of the quinoxaline due to the facilitate approach of the structure to prevent DNA directed RNA synthesis by virtue binding to CpG site on DNA [3]. This is in accordance with the conclusion derived in literature survey, it was worthwhile to introduce lipophillic moiety into the 1H, 4H- quinoxalin – 2, 3 – dione system to make the structure as DNA targeted potent antimicrobial agent. On other view, the sulfonamides, hydrazones, hydrazides of several heterocycles have created considerable attention as carrier and lipophillic core in the area of synthetic medicinal chemistry [4]. Based on the above observations and in connection with our earlier studies [5-6], the present work was undertaken to synthesize some new 2 - substituted hydrazino / benzylidino / methyl hydrazones and 7 - sulfonamides of 1H, 4H – 3- oxo - quinoxalines from 1H, 4H quinoxalin- 2, 3 – diones. Herein, we also report the results of antimicrobial and antitubercular activities as structural activity relationship of 1H, 4H – quinoxalin- 2, 3 – dione system.

In the present work, quinoxalin – 2, 3 – dione (1) was synthesized by Phillips procedure [7] which on treatment with phosphorus oxy chloride ($POCl_3$) produced 2, 3 – dichloroquinoxaline (2). The IR spectra of compound 2 confirmed the conversion of keto group(C=O) into chloro group (C-Cl) by the presence of absorption band at 990 Cm^{-1} (C-Cl) and by the absence of band at 1681Cm^{-1} (C=O). The compound 2 was treated with 50% hydrazine hydrate to afford 3-chloro- 2- hydrazino- quinoxaline (3) and it was supported by the presence of absorption band at 3343 Cm^{-1} (NH).

All the above synthons (Compound 1, 2 and 3) were converted into 7- sulfonamide derivatives (4 – 6) by commercial procedure and the sulfonamide formation was confirmed by the presence of absorption band at 1179 Cm^{-1}. In the same series the compound 1 was treated with 50% hydrazine hydrate and converted into 2 – hydrazino quinoxalin – 3 – one (7) and it was well supported by the presence of NH- stretching band at 3332 $Cm^{-1.}$ Further the hydrazino derivatives are converted into hydrazones (8 a-b) and hydrazide (9 a-d) derivative by treating with corresponding aldehydes and acids. The presence of absorption band at 1685 Cm^{-1} (CO-NH) and sharp band 1637 Cm^{-1} confirm hydrazides and hydrazones, respectively. **(Scheme – 1).** The purity of the compounds was checked on silica gel coated TLC plates and by the concurrent melting point upon recrystallization. The structures of the compounds were established by CHN analysis, Mass (LC-MS), H^1 NMR and FT- IR spectral studies.

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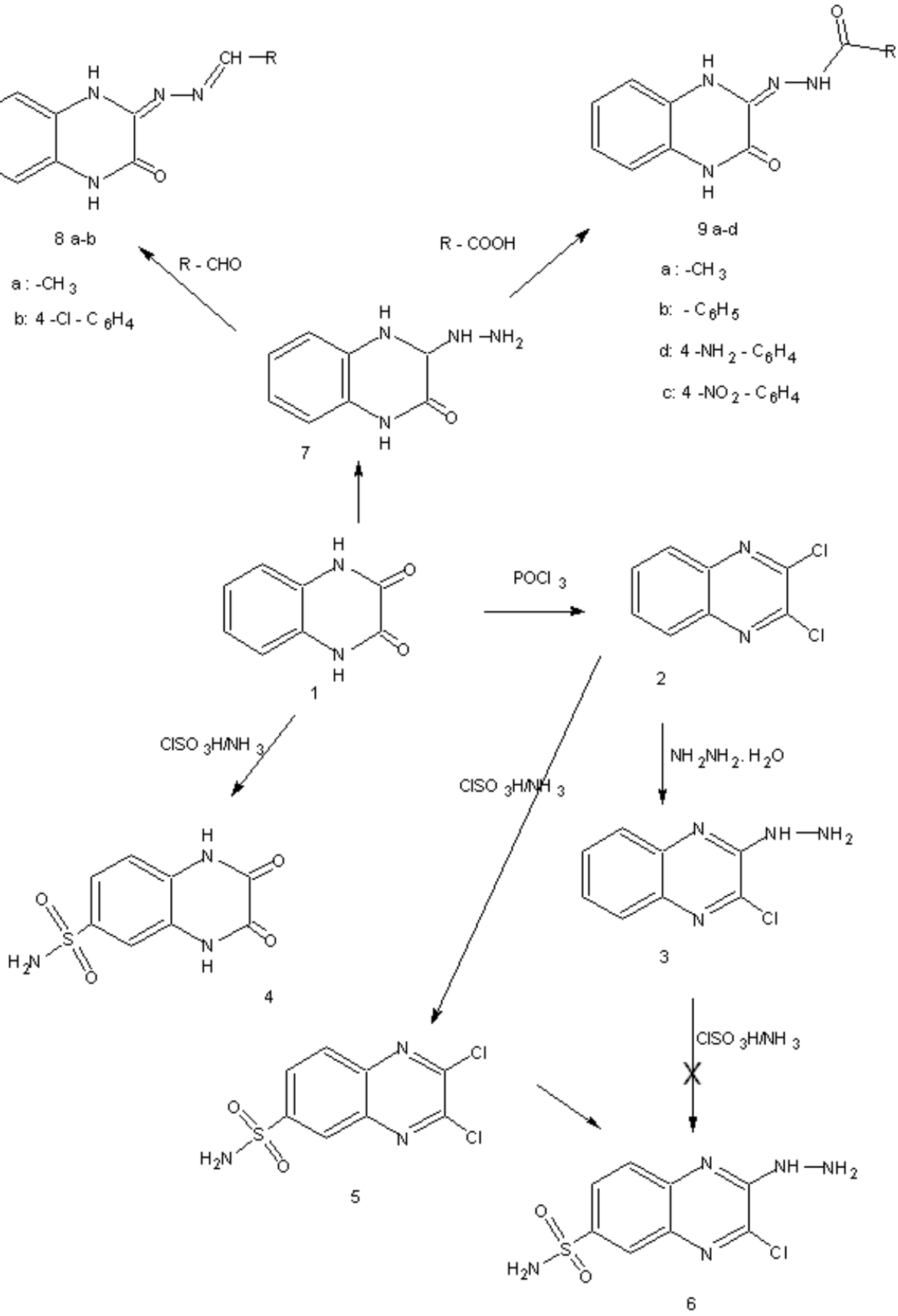
8 a-b
a : -CH 3
b: 4 -Cl - C 6H4
R - CHO
R - COOH
9 a-d
a : -CH 3
b: - C6H5
d: 4 -NH 2 - C6H4
c: 4 -NO 2 - C6H4
7
1
POCl 3
2
ClSO 3H/NH 3
NH 2NH 2. H 2O
ClSO 3H/NH 3
4
3
ClSO 3H/NH 3
5
6

Scheme - 1

Materials and Methods

TLC checking was done on silica gel G coated plates using benzene: chloroform (7:3) as irrigant. Melting points are determined by open capillary method and are uncorrected. IR (KBr in cm^{-1}) spectra were recorded on FT-IR Spectrophotometer at the resolution of $4cm^{-1}$. H^1 – NMR (δ ppm) spectra was obtained using TMS as internal standard. LC–MS spectra were scanned on a Shimadzu – LCMS –2010A spectrometer. Elemental analysis was performed on a Flash EA 1112 – CHN – Thermo Finnigan analyzer.

Synthesis of quinoxalin 2, 3 – dione (1) and its conversion into 2, 3 – dichloroquinoxaline (2) were established based on the reported method [7].

Synthesis of 3- chloro - 2-hydrazino-quinoxaline (3):

To the DMF solution of 2 (0.01 mol), 0.015 moles of 50% hydrazine hydrate was added and refluxed for 6 hours at 150°-The reaction mixer was cooled and kept over night for crystallization. The crystals were filtered and recrystallized from ethanol. Yield: 54%, m.p >300°C. IR (KBr Cm^{-1}): 3238(NH str), 3045, 3009 (C-H str, Aromatic) 1637 (C=N str), 1560 (C=C str), 1501 (C-H deform), 990 (C-Cl), 772 (C-H Ar out of plane deform).

Synthesis of sulfonamide derivatives (4 –6); General procedure:

0.01moles of quinoxaline derivatives was treated with chlorosulfonic acid under ice-cold conditions in fuming cupboard with constant stirring. The stirring was continued until the reaction reaches room temperature. The resultant mixture was poured into water to give sulphonyl chloride derivatives. The formed product was refluxed with 50% NH_3 solution for 1 hour. Then the reaction mixture was cooled and poured into water to get sulfonamide derivatives. The crude product was recrystalized from 90% ethanol.

Compound 4: 1H, 4H –7 – sulfonamido - quinoxalin – 2, 3- dione. Yield: 60%, m.p 270°C. [Found: C, 40.065; H, 3. 051; N, 18.218. $C_8H_7N_3O_4S$ requires C, 39.83; H, 2.92; N, 17.42]. IR (KBr Cm^{-1}): 3045, 3009 (C-H str, Aromatic), 2780 (C-H Str, Aliphatic), 1684 (C=O), 1612 (C=N str), 1560 (C=C str), 1499 (C-H deform), 1390 (C-N str). H^1 NMR (δ ppm): 7.6-7.9(m, 3H, Ar), 7.0 -7.2 (weak, 2H, $-SO_2NH_2$), 11.8 (s, 2H, NH quinoxaline). LC- MS: m/z 242 (M, 100%).

Synthesis of 2-hydrazino-quinoxalin- 3- one (7):

To the DMF solution of compound 1 (0.01 mol), 0.015 moles of hydrazine hydrate was added and refluxed for 6 hrs at 150°-c. The reaction mixer was cooled and kept over night for crystallization. The crystals were filtered and recrystallized from ethanol. Yield: 77%, m.p >300°C. IR (KBr Cm^{-1}): 3332(NH str), 3048, 3007 (C-H str, Aromatic), 1681(broad, CO –NH cyclic) 1630 (C=N str), 1500 (C=C str), 752, 759 (C-H Ar out of plane deform).

Synthesis of 2-oxo- Quinoxalin-3-yl – hydrazones (8 a-b); General procedure:

To the DMF solution of 0.01mol compound 7, 0.01 moles of 0.01 moles of respective aldehydes were added and the reaction mixer was stirred for 2 hr in room temperature followed by stirring for 2hrs at 70° C. The resultant reaction mixture was filtered, concentrated and allowed for crystallization. The formed crystals were filtered and recrystallized from 90%ethanol.

Compound 8a: 2-oxo- 1H, 4H Quinoxalin-3-yl- methyl hydrazones. Yield: 65%, m.p 300°C. [Found: C, 59.22; H, 5.03; N, 29.86. $C_{10}H_{10}N_4O$ requires C, 59.40; H, 4.98; N, 29.71]. IR (KBr Cm^{-1}): 3238(NH), 3045, 3009 (C-H str, Aromatic), 2780 (C-H Str, Aliphatic), 1684 (C=O), 1637 (C=N str), 1524, 1505 (C=C str), 1499 (C-H deform), 1390 (C-N str). LC- MS: m/z 202 (M, 100%).

Synthesis of 3-(N^2-substituted) hydrazine - Quinoxalin-2-one (9 a-d); General procedure:

To the DMF solution of 0.01mol compound 7, 0.01 moles of 0.01 moles of respective acids were added and the reaction mixer was stirred for 1 hr then refluxed for 3hr at 120° C. The resultant reaction mixture was concentrated and allowed for crystallization. The formed crystals were filtered and recrystallized from ethanol.

Compound 9a: 3-(N^2-acyl) hydrazino, 1H, 4H Quinoxalin-2-one. Yield: 62%, m.p > 300°C. [Found: C, 55.067; H, 4.685; N, 24.489. $C_{10}H_{10}N_4O_2$ requires C, 55.04; H, 4.62; N, 25.68]. IR (KBr Cm^{-1}): 3048, 3009, 2882 (C-H Str), 1681 (C=O), 1615 (C=N str), 1560, 1543, 1500 (C=C str), 1473 (C-H deform), 1391 (C-N str). H^1 NMR (δ ppm): 3.03 (s, 3H, $-CH_3$) 6.9-7.09(m, 4H, Ar), 7.62 (CONH acyl), 11.77 (s, 2H, NH quinoxaline). LC- MS: m/z 217 (M, 15%)

***In Vitro* Antimicrobial Activity**

All the compounds were evaluated for their *in vitro* antimicrobial activity *against the* gram-positive bacteria *Staphyllococus aureus, the* gram-negative *Psudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli,* the fungi *Aspergillus niger, Candida albicans* and the *Mycobacterium tuberculosis* $H_{37}Rv$ species. Antibacterial and antifungal screening was carried out by agar plate disc diffusion method [8] at 100µg/disc concentration and its results were reported as zone of inhibition in millimeter **(Table – 1)**. The antitubercular screening was performed by Microplate Alamar Blue Assay (MABA) method [9] at 6.25 µg/ml concentration using DMSO as solvent. Nalidixic acid (100 µg/disc) and Clotrimazole (50 µg/disc) were used as standard respectively for antibacterial and antifungal screening.

Results and Discussion

Antimicrobial screening data revealed that the compounds exhibited significant and comparable antibacterial that of standard but it was more selective

Table-1:

Antimicrobial data of the compounds synthesized

Comp. code	Antibacterial activity at 100 µg/disc (Inhibition zone in mm)				Antifungal activity at 100 µg/disc (in mm)		Antitubercular activity at 6.25µg/ml (in % inhibition)
	S.aureus	*P.vulgaris*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*	*M. tuberculosis* H_{37} *Rv*
1	20	21	19	17	17	19	51
2	18	15	14	13	24	18	41
3	13	20	25	18	—	20	78
4	21	34	33	26	16	21	64
5	21	33	38	30	23	20	57
6	19	32	29	27	—	—	50
7	24	21	18	18	21	18	83
8a	15	18	29	20	21	14	94
8b	16	19	28	21	19	17	51
9a	23	30	30	26	19	18	90
9b	22	33	32	29	18	18	64
9c	23	34	32	30	20	19	59
9d	20	32	33	27	17	16	61
Std	24	33	32	33	24	20	100
Blank	—	—	—	—	—	—	—

towards gram – ve species. Antifungal activity was found to be moderate. Antitubercular screening showed that the hydrazino derivative **3** exhibited antitubercular activity with inhibition of 78% but sulfonamide derivatives showed in between 41 – 64 %. Methyl hydrazones and acylhydrazide side chain exhibited superior inhibition against *Mycobacterium tuberculosis* H_{37} *Rv* with 94% and 93 %, respectively.

Interpretation of results as Structural activity relationship revealed that the conversion of cyclic keto group into chlorine diminishes the antibacterial and antitubercular spectrum with enhanced antifungal activity. Introduction of hydrazino side chain at 2^{nd} position as methyl hydrazones or/and acyl hydrazides showed more significant (94% inhibition) antitubercular activity and antibacterial activity. The benzylidino and benzoyl hydrazino side chain showed less antitubercular activity but exhibited greater spectrum against gram – ve bacteria.

Conversion into 7 - sulfonamide derivatives exhibited greater inhibition against gram –ve bacteria and fungi but not with mycobacterium. However, it was concluded that 2 – hydrazino, 7 – sufonamido and 2, 3 – dichloro substitution on quinoxaline potentiates antitubercular, antibacterial and anti fungal spectrum, respectively.

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Antihyperlipedemic effect of *Derris Trifoliata Lour* in triton induced hyperlipedemia in rats

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ABSTRACT

The plant *Derris trifoliata Lour* is a large woody climber, which is distributed worldwide. *Derris* species is traditionally being used as a pesticide and reported for its anti-inflammatory, antioxidant, anticancer and antimicrobial activity. In the present study, the methanolic extract of leaves and stems of *Derris trifoliata* Lour were evaluated for its hypocholesterolaemic and hypoglyceridaemic effect using Triton WR-1339 induced hyperlipidemic rats as experimental model. The group of animals treated with methanolic extract of *Derris trifoliata* Lour exhibited a significant decrease ($p<0.01$) in levels of triglycerides and cholesterol after 7 hrs as compared to the control group. The level of HDL also significantly increased ($p<0.01$) in *Derris trifoliata* Lour treated groups after 7 hrs as compared to the standard drug fenofibrate. Hence it can be concluded that Derris *trifoliata* Lour, has significant antihyperlipidemic effect owing to its ability to reduce the levels of total cholesterol, triglyceride with an increases in the level of HDL.

Key Words: - Hyperlipidemia, *Derris trifoliata* Lour, Triton WR 1339.

INTRODUCTION

Increased plasma lipid levels, mainly total cholesterol (TC), triglycerides (TG), along with decrease in high density lipoproteins (HDL) are known to cause hyperlipidemia which is core in the initiation and progression of arthrosclerosis impasse. The prime consideration in therapy for hyperlipidemia is to enervate the elevated plasma levels of TC and TG along with increase in HDL lipids levels. The ideal approach to prevent or to treat arthrosclerosis and CVS complications is to target the lipid profile of hyperlipidemic patients using lipid lowering drugs or by improving the diet [1]

Derris trifoliata Lour is one of the genus of leguniminosae family. It is a species of woody climbers and trees distributed worldwide. *Derris* species is traditionally being used as a pesticide and reported for its anti-inflammatory, antioxidant and anticancer, antimicrobial activity. It is also documented for the presence of flavonoids, steroids, isoflavonoids, chalcones, tannins, sterols and glycosides [2-4]. However literature survey reveals no report of the antihyperlipidemic activity of *Derris trifoliata* Lour. Hence the present study was designed to investigate the effect of *Derris trifoliata* Lour on Triton WR 1339 induced hyperlipidemia in rats.

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Materials and Methods [5-10]

Collection and extraction:

The fresh leaves and stem of *Derris trifoliata* lour was collected from mature plants growing near Vengurla sea face region; district Sindhudurga, Maharastra, India. Its botanical identification was confirmed by Botanical survey of India, Pune.

(Voucher specimen number BSI/WC/TECH/2005/1146). The collected plant material (leaves and stem) was shed dried, crushed and extracted with methanol. Total extract was evaporated in vaccum to yield 13.3 %(W/W) dark green semi solid.

Drugs

Fenofibrate (Zydus Cadila), Triton WR 1339 (Sigma Aldrich USA), Carboxy methyl cellulose (CMC) and other chemicals used were of analytical grade. Fenofibrate was administered orally in saline solution, the methanolic extract was administered as an aqueous suspension in 1% cmc, and Triton WR 1339 was injected i.p. in saline solution.

Animals

Wistar rats of either sex weighing 150–180 g used for experiments were obtained from National Toxicological Center, Pune. Animal were housed in controlled room with 12 hr light and dark cycle at room temperature and feed with standard chow diet and water.

Experimental animal protocol

Experimental rats, starved for 18 hr, were provided water ad libitum. The rats were divided in 6 groups containing 5 animals each. Treatment protocol for each group was given as follows,

Group I- Normal Control (NC):- 1% CMC, (1ml/kg, p.o.)

Group II- Hyperlipidemic (HG):- Triton (200mg/kg, i.p.)

Group III-Fenofibrate (FG):- Triton (200mg/kg, i.p.) + Fenofibrate (65 mg/kg, p.o.)

Group IV- (DTE 175):- Triton (200mg/kg, i.p.) + DTE (175 mg/kg, p.o.)

Group V- (DTE 350):- Triton (200mg/kg, i.p.) + DTE (350 mg/kg, p.o.)

Group VI- (DTE 700):- Triton (200mg/kg, i.p.) + DTE (700 mg/kg, p.o.)

DTE- *Derris trifoliata* Lour extract group.

Hyperlipidemia was induced by Triton WR 1339 (200mg/kg i.p.) in group II, III, IV, V, VI. Group III received Fenofibrate (65mg/kg, p.o) while groups IV, V, VI received the *Derris trifoliata* Lour methanolic extract 175, 350 and 700 mg/kg p.o. respectively immediately after injection of Triton. In the following period of the study (48 hr) animals had access only to water.

Biochemical estimation

Blood samples were collected after 7, 24 and 48 hr of Triton injection by retrorbital puncture. Blood was immediately centrifuged (2500 rpm for 10 min.) and serum was analyzed for total cholesterol, triglyceride and HDL level using biochemical kits.

Statistical analysis:-

Data obtained was analysed by unpaired "t " test and ANOVA followed by Dunett test. Values were expressed as mean ± SEM and P Values < 0.05 was considered significant.

RESULTS

Induction of hyperlipidemia with Triton WR 1339

The level of serum total cholesterol, triglyceride, HDL in groups NC, HG, DTE 175, DTE 350 and DTE 700 after 7, 24, 48 hr from treatment are reported in tables 1, 2 and 3 respectively.

In HG group, significant increase ($p<0.0001$) in the level of total cholesterol and triglyceride was observed at 7, 24 and 48 hr after induction with Triton as compared to NC Group.

The increase in level of serum total cholesterol and triglyceride after 7 hr was observed to be 76.53% and 29.09 % respectively. After 24 hr, the further elevation in the levels of total cholesterol and triglyceride were found to be 192.3 % and 234.03 % respectively. After 48 hrs, the level of total cholesterol and triglycerides were found to be 66.95 % and 16.16 % respectively. No significant change was observed in levels of HDL after 7, 24, and 48 hrs.

Effect of *Derris Trifoliata Lour* on lipid profile in hyperlipidemic rat Effect on triglyceride levels in serum (Table 1)

The groups treated with methanolic extract of *Derris trifoliata* Lour showed significant decrease ($p<0.01$) in the level of triglycerides at all the doses after 24 hr .However a significant decrease ($p<0.01$) was observed only in groups DTE 350 and DTE 700 after 7 hrs itself and which persisted upto 48 hr of treatment.

Table-1

Effect on serum level of triglycerides

Group	After 7 hr	After 24 hr	After 48 hr
NC	64.21	59.98	63.45
SEM	± 1.796	± 1.844	± 1.242
HG	106.12 # #	265.888 # #	99.94 # #
SEM	± 3.068	± 2.029	± 1.478
Fenofibrate	81.74 **	84.678 **	83.726 **
SEM	± 2.262	± 1.406	± 1.978
DTE 175	99.726	218.05 **	96.576
SEM	± 1.266	± 3.833	± 1.971
DTE 350	93.792 **	145.548 **	88.122 **
SEM	± 2.143	± 4.134	± 2.364
DTE 700	86.05 **	100.05 **	85.4 **
SEM	± 2.104	± 2.275	± 3.492

DTE 175-*Derris trifoliata lour (*175 mg/kg, p.o.),
HG-hyperlipidemic Group
DTE 350-*Derris trifoliata lour* (350 mg/kg, p.o.),
NC-Normal Control,
DTE 700-*Derris trifoliata lour* (700 mg/kg, p.o.),
SEM-std. error of mean

Effect on cholesterol level in serum (Table 2)

The groups treated with methanolic extract of *Derris trifoliata* Lour showed significant decrease ($p<0.01$) in the level of total cholesterol at all the doses after 7 and 24 hr, where as a significant decrease ($p<0.05$, $p<0.01$) in level of total cholesterol was observed in groups DTE 350 and DTE 700 after 48 hr respectively.

Effect on HDL level in serum (Table 3)

The levels of HDL significantly increased ($p<0.01$) in *Derris trifoliata* Lour treated groups DTE 350 and DTE 700 after 7 and 24 hr .However a significant increase ($p<0.01$) was observed only in Group DTE 700 after 48 hr of treatment.

Table-2

Effect on serum level of total cholesterol

Group	After 7 hr	After 24 hr	After 48 hr
NC	66.38	69.146	66.138
SEM	± 2.098	±1.542	±1.721
HG	132.434 ##	226.642 ##	103.764 ##
SEM	±3.757	±2.718	±1.688
Fenofibrate	74.034 **	77.724 **	100.714
SEM	±2.184	±2.440	±2.222
DTE 175	108.46 **	204.804 **	99.674
SEM	±2.456	±4.248	±1.955
DTE 350	90.756 **	150.53 **	95.668 *
SEM	±2.467	±3.516	±1.248
DTE 700	78.818 **	90.352 **	90.68 **
SEM	±2.224	±2.765	±1.849

DTE 175-*Derris trifoliata lour (*175 mg/kg, p.o.),
HG-hyperlipidemic Group
DTE 350-*Derris trifoliata lour* (350 mg/kg, p.o.),
NC-Normal Control,
DTE 700-*Derris trifoliata lour* (700 mg/kg, p.o.),
SEM-std. error of mean

Table 3

Effect on serum level of HDL

Group	After 7 hr	After 24 hr	After 48 hr
NC	23.29	23.22	24.218
SEM	± 1.514	±0.4544	±1.403
HG	18.96 ##	20.2444 ##	21.638 ##
SEM	± 1.283	±0.6332	±1.230
Fenofibrate	22.678	23.756	22.386
SEM	±1.028	±1.225	±1.380
DTE 175	21.67	21.512	21.056
SEM	±0.4584	±1.139	±0.8776
DTE 350	30.11**	37.028 **	23.066
SEM	±1.486	±1.592	±1.457
DTE 700	37.52 **	44.318 **	31.558 **
SEM	±1.815	±2.105	±1.864

DTE 175-*Derris trifoliata lour (*175 mg/kg, p.o.)
,HG-hyperlipidemic Group
DTE 350-*Derris trifoliata lour* (350 mg/kg, p.o.),
NC-Normal Control,
DTE 700-*Derris trifoliata lour* (700 mg/kg, p.o.),
SEM-std. error of mean

Table-4

Percent reduction in level of total Cholesterol and Triglyceride in Triton induced hyperlipidemic rats

Groups	After 7 hr		After 24 hr		After 48 hr	
	CH (%)	TG (%)	CH (%)	TG (%)	CH (%)	TG (%)
FG	76.53	29.09	192.3	234.03	60.95	16.16
DTE175	50.53	12.15	136.2	183.86	7.70	3.31
DTE350	63.89	17.73	160.2	196.72	11.56	11.76
DTE700	72.91	25.03	186.7	228.74	16.36	14.48

TC: Triglyceride, CH: Cholesterol, FG: Fenofibrate group (65 mg/kg, p.o.),
DTE 175: *Derris trifoliata lour (*175 mg/kg, p.o.),
DTE 350: *Derris trifoliata lour* (350 mg/kg, p.o.),
DTE 700: *Derris trifoliata lour* (700 mg/kg, p.o.) .

Effect of fenofibrate on lipid profile

Fenofibrate (65 mg/kg, p.o.) treated group showed significant decrease ($p<0.01$) in the level of total cholesterol after 7 and 24 hr and in the levels of triglyceride after 7, 24 and 48 hr. However no significant changes were observed in the level of HDL after 7, 24 and 48 hr of treatment.

The percent reduction in the levels of serum total cholesterol and triglyceride after 7, 24 and 48 hr in Fenofibrate and Derris trifoliate Lour treated groups are shown in table 4.

DISCUSSION

Systemic administration of Triton WR 1339 (ionic surfactant) in fasted rat induced hyperlipidemia. The maximum plasma triglyceride and total cholesterol were reached at 20 hr followed by decline to normal values .The plant constituents like steroids, flavonoids and saponins are reported to possess lipid lowering activity. The plant steroids reduce the absorption of cholesterol and thus increase fecal excretion of cholesterol. Flavonoids augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays an important role in the incorporation of cholesterol into HDL (this may increase the level of HDL). Several studies have showed that increase

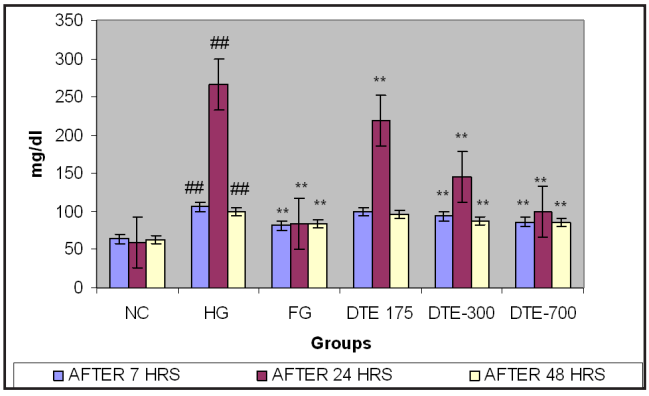

Figure-1: Effect on Serum level of Triglycerides

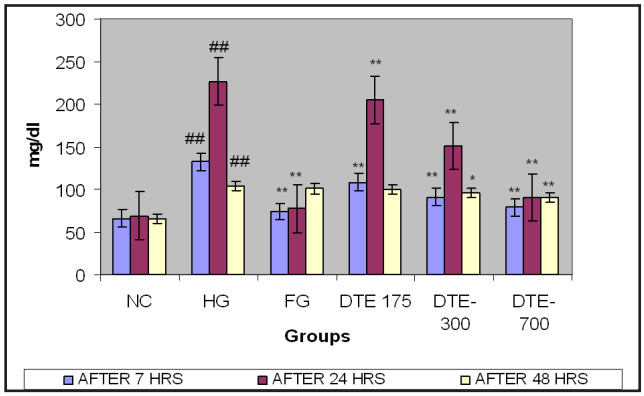

Figure-2: Effect on Serum level of cholesterol

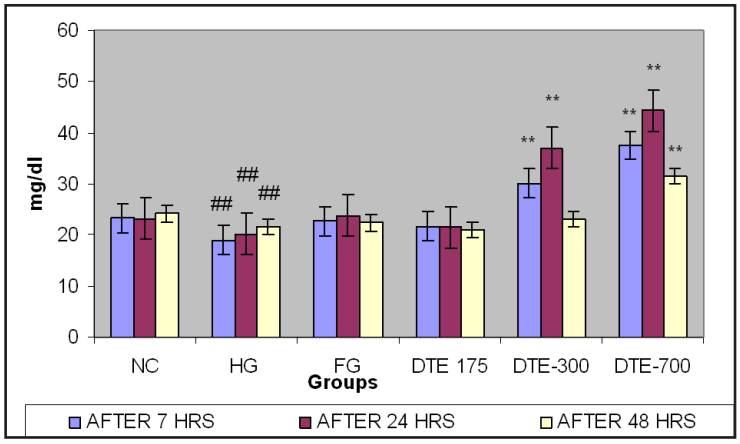

Figure-3: Effect on Serum level of HDL

in HDL is associated with decrease in cardiovascular diseases. Saponins also act as antihyperlipidemic agents by binding with cholesterol in intestinal lumen, so that cholesterol is less readily absorbed, besides increasing lipoprotein lipase activity which helps in removal of VLDL from the circulation.

In the present study, a decrease in serum triglyceride and cholesterol levels in groups of rats treated with methanolic extract of *Derris trifoliate*. *Derris trifoliata* Lour may act by inhibiting cholesterol synthesis with increased excretion of cholesterol. The extract also increased the levels of HDL.

Hence it can be concluded that *Derris Trifoliata* Lour has significant antihyperlipidemic effect owing to its ability to reduce level of total cholesterol, triglyceride with an increase in HDL levels. Further research with regard to fractionation of extract, isolation, purification and characterization of active constituents responsible for antihyperlipidemic activity and elucidation of the possible biochemical mechanism is underway.

Acknowledgment

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