ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2014; 5(5): 103 - 109

RESEARCH ARTICLE

Investigation of Analgesic and Anti Inflammatory Potential of Ethanolic Extract of *Atylosia rugosa*

Prathibha Bharathi Mare[*1], Muralidhara Rao Dowlathabad[2]

[1]*Department of Biotechnology, Nirmala College of Pharmacy, Kadapa (DT),Andhra Pradesh, India*
[2]*Department of Biotechnology, Sri Krishnadevaraya University, Ananthapur (DT),Andhra Pradesh, India*

Received 09 Jul 2014; Revised 09 Oct 2014; Accepted 22 Oct 2014

ABSTRACT

Current study was designed to explore the topical analgesic and anti-inflammatory effects of the consistent extract isolated from *Atylosia rugosa*. It is a slender, twining herb with densely grey-dowry stems. The plant was reported to contain flavonoids, alkaloids, glycosides, terpenoids, tannins and phenolic compounds. Analgesic potential of ethanolic extract of *Atylosia rugosa* was investigated on swiss albino mice. For analgesic effect hot plate, tail flick and writhing method were used while for anti-inflammatory carrageenan, Histamine, and Dextran induced hind paw, edema were used and tested on wistar rats. The results of EEAR were compared with the efficiency of standard analgesics and anti-inflammatory drugs.

Key words: *Atylosia rugosa*, Analgesic, anti-inflammatory, flavonoid giycosides.

INTRODUCTION

Inflammation is an immunological defence mechanism elicited in response to mechanical injuries, burns, microbial infections, allergens and other noxious stimulus [1]. It develops in the classical forms of redness, swelling, heat and hyperalgesia. These symptoms result from the action of inflammatory agents such as bradykinin, serotonin, histamine, prostaglandins, leukotrienes and nitric oxide, which can originate locally or from cells that infiltrate at the site of inflammation [2]. Pain is a common symptom of various inflammatory diseases and an unpleasant sensory experience associated with actual or potential tissue damage [3,4]. Frequently, tissue damage leads to activation of nociceptors through the release of a variety of chemical mediator, such as excitatory amino acids, protons, peptides, lipids and cytokines, among others, which act on specific receptors and ion channels, contributing to the induction of pain and inflammation [5]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed therapeutics, primarily for the treatment of pain and inflammation. However, long-term clinical usage of NSAIDs is associated with significant side effects, such as gastrointestinal lesion, bleeding and peptic ulcers [7]. As an alternative, plant based medicines are getting an increased therapeutics market share due to their mild action and fewer adverse effects. *Atylosia rugosa* belonging to the family Fabaceae commonly known as pedda adavikandi, Adaviualva. It is a slender, twining herb with densely grey-dowry stems [8]. The plant was reported to contain flavonoids, alkaloids, glycosides, terpenoids, tannins and phenolic compounds. The whole plant or the roots are being crushed and prescribed for vitality to the mother after child birth, used in bronchitis and tooth paste. Nothing is reported on biological work of *Atylosia rugosa*. This study aimed at investigation of chemical constituents of the leaves of *Atylosia rugosa* grown in India, in addition to pharmacological evaluation.

MATERIALS AND METHO

Plant material:

The plant *Atylosia rugosa* was collected during from hill regions of Tirupathi. The plant was authenticated by Dr. K. Madhava Chetty, Taxonomist, S.V.University, Tirupathi, India. The collected plant was cleaned immediately and shade-dried for a week, powdered mechanically, sieved (10/44) and stored in air tight container.

***Corresponding Author:** Prathibha Bharathi Mare, **Email:** mprprathibha@gmail.com

Extraction:
5000grams of the powdered drug was accurately weighed and extracted using analytical grade solvents starting with highly non polar petroleum ether (60-80^{0}c) to successively increasing the polarity viz Petroleum ether, chloroform, acetone, ethyl acetate and ethanol (95%) following soxhlet method. All the extracts were concentrated by using rota vaccum evaporator (Buchi type, Mumbai, India) until a semisolid extract is obtained, dried at less than 50$^{0\ C}$, comminuted in a ball mill and preserved in air tight containers kept in desicators prior to its studies.

Preliminary phytochemical investigation:
A preliminary phytochemical investigation was carried out for all the extracts obtained from *Atylosia rugosa* [9] using analytical grade chemicals, solvents and reagents. The yields and phytochemical investigation results were given in (**Table 1 & 2**) respectively.

Pharmacological Screening:

Toxicity Studies:
The method described by Lorke [10] was followed to determine LD_{50} using ethanolic extract of *Atylosia rugosa* (EEAR) at different doses. Male swiss albino mice weighing 20 – 25 gms were used in the study. They were acclimatized for one week by individually housing in polypropylene cages, lined with husk renewed every 24hrs in well ventilated rooms at 22±2^{0}c under 12:12 light and dark cycle in hygienic condition. They were fed with standard laboratory pellet diet (Hindustan Lever) and water ad libitum before initiation of the experiment. The chemicals, solvents and reagents used in experiments were analytical grade.

The mice were divided into three groups (20 in each group) namely I, II and III. A suspension of extract (SPE) 2% v/v aq.Tween 80 was prepared freshly by using aliquot quantity of powdered EEAR prior to its use. The suspension was administered to group II and III animals respectively starting from the dose of 0.1gm/kg and increasing the dose every alternate days upto 3.2gm/kg, p.o..Group I animals were administered with 2% v/v aq Tween 80 (10ml/kg p.o). A similar study was also conducted by administering same doses intraperitionally. Body weight before and after administration as per schedule was noted. They were observed in the open field for 72 hrs and any changes in skin, fur, eyes, mucous membranes, behaviour pattern were observed. Signs of tremors, convulsions, salivation, diarrhoea, coma and number of deaths were recorded. The results were given in (**Table 3**).

Hot plate method:
The study was carried out according to the method of Eddy1[11]. Mice that showed nociceptive responses within 10 sec, when placed on an Eddy's hot plate (Techno, Lucknow, India) maintained at 55±0.5^{0}C were selected for study. The mice so selected were then grouped into five (6 in each group) namely I, II, III, IV and V. The group I was treated with 2% v/v, aq. Tween 80, 10ml/kg p.o which served as control and the II, III and IV groups were treated with the EEAR 100, 200 and 400mg/kg. p.o respectively and group V was treated with morphine 2mg/kg s.c. After 30 min of the above treatment each mouse was placed gently on the hot plate maintained at 55±0.5^{0}C and the reaction time was noted. The reaction time was taken as the time interval between the animals placed on the plate till the moment it began to lick its forepaws or jump. Four consecutive trials after a gap of 5 minutes were done and the mean value was calculated.

Tail flick method:
The method described by Kulkarni [12] was followed in this experiment. The animals were treated and grouped similarly as described in hot plate method except group V was treated with aspirin 20 mg/kg, p.o. After 30 minutes of the above treatment the basal reaction time for each mouse was noted by placing the tip (last 1-3cm) of the tail on the radiant heat source of the analgesiometer (Techno, Lucknow, India) and the time of withdrawal of tail from the heat source (Flicking response) shown within 5-6 sec were selected for study. A cut off period of 10 – 12 sec was observed to prevent the damage to the tail. Four consecutive trials after a gap of 5 minutes were done and the mean value was taken.

Acetic acid induced writhing test:
The method described by Koster *et al* [13] was followed in this study. The animals were treated and grouped similarly as described in Tail flick method. Thirty minutes after the above treatment each mouse was injected 10 ml/kg of 0.7% aqueous acetic acid intraperitionaeally. Each mouse was placed in a plastic transparent observation cage and number of abdominal constriction was cumulatively counted from 5 to 15 minutes. Results were expressed as percent inhibition of analgesia.

$$\%\text{Inhibition} = \frac{\text{Mean no. of writhes in control group} - \text{Mean no. of writhes in test group}}{\text{Mean no. of writhes in control group}} \times 100$$

Anti inflammatory activity:

Anti-inflammatory activity of ethanolic extract of *Atylosia rugosa* (EEAR) at doses 100, 200 and 400 mg/kg, p.o was studied by three different methods. The results were given in (**Table 4**).

Carrageenan – induced rat paw edema:

The study was conducted according to the method of Winter *et al* [14]. Male albino Wistar rats weighing 100 – 250 g were housed in wire netted cages in a controlled room temperature 22 ± 1^0C, relative humidity 60 – 70 % and with 12 h light and dark cycle. The animals were maintained with pellet diet and water ad libitum. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. All studies were carried out using six rats in each group. The chemicals, solvents and reagents used in the experiments were of analytical grade. Five groups of six animals each were used for the experiment. Group I, animals were administered with 10 ml/kg, p.o. of 2% v/v aq. Tween 80, which served as control. Ethanolic extract of *Atylosia rugosa* (EEAR) 100, 200 and 400 mg/kg, p.o. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was treated with Indomethacin 20 mg/kg,p.o. One hour after oral administration, edema was induced by subplantar injection (left hind paw) of 0.1 ml of 1% freshly prepared suspension of carrageenan (Sigma Chemical Co; USA) in normal saline to all the animals. The volume of the injected and the contra lateral paws were measured at 3 hour after induction of inflammation using Plethysmometer. The percent inhibition of inflammation was calculated by using formula.

Percentage of inhibition inflammation = (A – B/A) x 100

Where A and B denote mean increase in paw volume of control and drug treated animals respectively.

Histamine induced rat paw edema

In this model edema was induced by sub plantar injection (hind paw) of 0.05 ml of 1% w/v, freshly prepared solution of histamine to all animals, which were grouped and treated similarly as followed in carragenan induced rat paw edema method. The volume of the injected and the contra lateral paws were measured 3 h after induction of inflammation using Plethysmometer according to the method described by Winter *et al* [14].

Dextran induced rat paw edema

In this method edema was induced by sub plantar injection of 0.05 ml of freshly prepared 1% w/v solution of dextran into the right hind paw of the rats, which were grouped and treated similarly as followed in carragenan induced rat paw edema method [15].

RESULTS AND DISCUSSION

Results in (**Table 1**) suggest the extraction yields with various solvents. (**Table 2**) suggests the plant contains several constituents such as flavonoid, alkaloids, glycosides and phenolic compounds in appreciable quantities. Results of toxicity signs or deaths have been noted in the animals for the dose upto 3.2gms/kg. in case of intra- peritoneal administration of EEAR no clinical toxicity of sings or deaths has been noted in the animals for doses upto 1.6gm/kg,but at a dose 3.2gm/kg EEAR exhibited 20% mortality. Hence concluded that that the test drug is safe to administer in animals upto 3.2gm/kg orally and 1.6gm/kg intra peritoneally. Further no remarkable change in the body weight, skin, fur, eyes, mucous membrane and behavourial pattern were observed. No signs of tremors, convulsions, salivation, diarrhoea and coma were seen in all these cases. (**Table 4 & Fig 1**) represents the results of analgesic activity studies of three different methods. Several tests (acute and sub acute) which differ with respect to stimulus quality, intensity and duration, were employed in evaluating analgesic effect of EEAR to ascertain the analgesic properties of a substance using behavioural nociceptive tests .In the hot plate method the test drug showed16.137%, 35.99% and 76.07% of inhibition at dosage of 100, 200, 400 mg/kg respectively whereas morphine showed 78.264% under similar conditions. In the Tail flick method the latency of EEAR of the test drug showed 31.25%, 61.28% and 63.39% of inhibition at dosage of 100, 200, 400 mg/kg respectively where as morphine showed 80.8% under similar conditions. The drug under investigation (EEAR) showed significant dose-dependent inhibition of pain in the above models. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure, hot plate, tail flick models suggest that the effect on these pain models may act via centrally mediated pain. The abdominal writhing response induced by the acetic acid is sensitive

process to establish peripherally acting analgesics. The acetic acid induced writhing assay the test drug EEAR at the doses of 10 0,200,400mg/kg.p.o exhibited 59.92%, 61.75%, 73.53%, inhibition respectively. The aspirin at the doses of 100mg/kg. p.o exhibited 79.90% inhibition under similar conditions.

NSAID such as aspirin used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation mediating agent prostaglandin (PGE_2) from arachidonic acid [16-18].The pattern of anti-inflammatory and analgesic activities exhibited by these extracts were similar of that of aspirin which suggests that the plants activity may be mediated by cyclooxygenase I&II inhibition. The observation that the plant increased pain threshold of animals could be due to inhibition of sensitization of pain receptors by prostaglandins at the inflammation site [19]. After administration of acetic acid several mediators such as cytokines, eicosanoids and arachidonic acid are liberated from membrane after phospolipase A_2 activity leading to production of prostaglandins and leukotrienes [20]. The analgesic activity of the ethanolic extracts may be due to inhibition of phospolipase A_2 or even blocking cyclooxygenase (COX-1 and/or COX-2). The results suggest the EEAR also possess significant peripherially mediated analgesic effect. Hence it can be concluded that the EEAR possess analgesic properties which are mediated via peripheral and central inhibitory mechanisms.

The subplantar injection of carrageenan (1%w/v) develops edema of high intensity and persisted for 3hrs after injection the control groups. The oral administration of EEAR at the doses of 100, 200 and 400mg/kg.p.o showed significant and dose dependent % inhibition 48.97%, 48.45%, 34.59% respectively. The commercial anti-inflammatory drug, indomethacin showed % inhibition 55.84%, 50.50%, 37.91% respectively of the dose 20mg/kg.p.o. The development of carrageenan induced edema is biphasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, proteases, prostaglandins and lysosomes [21]. Prostaglandins play a major role in development of second phase of reaction that is measured at 3 hrs. These mediators take part in the inflammatory response and are able to stimulate nociceptors and thus induce pain [22].Carrageenan induced edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non steroidal anti-inflammatory agents that is inhibition of cyclooxygenase in prostaglandin synthesis (23). Based on these reports it may be concluded that the inhibitory effect of the ethanolic extract of 400mg/kg,in carrageenin induced inflammation in rats could be due to inhibition of the cyclooxygenase in prostaglandin synthesis. Hence EEAR was further investigated against paw edema induced by individual agents like histamine and dextran showed a maximum inhibition of 48.45% and 34.59% respectively at the dose of 400mg/kg .The results suggested that the EEAR at doses of 100, 200, 400mg/kg .p.o significantly reduced the edema produced by the several inducers are comparable with many standard drugs suggested in each model. It has been reported by researchers that flavonoids inhibit eicosonoids synthesis by inhibiting both cyclooxygenase and lipooxygenase activities as well as hamper the non enzymatic peroxidation of polyunsaturated fattyacids required for the activation of these oxygenases [24].

Table 1: Exraction yields of *Atylosia rugosa*

S. No	Extract(200gm) (EEAR)	Color in day light and consistency	Weight(gm)	% yield
1	Petroleum Ether	Solid Greenish	12.61	6.305
2	Chloroform	Solid Dark Brown	4.20	2.1
3	Acetone	Solid Dark Brownish Black	10.49	5.245
4	Ethyl Acetate	Soid Dark Brown	24.24	12.12
5	Ethanol	Semi-Solid Reddish Brown	28.16	14.08

Table 2: Preliminary phytochemical screening of *Atylosia rugosa*

Nature	Pet Ether	Chloroform	Acetone	EthylAcetate	Ethanol
Alkaloids	-	+	-	+	+
Amino acids	-	-	-	-	+
Flavonoids	-	+	+	+	+
Anthraquinon Glycosides	-	-	-	-	+
Triterpenoids	+	+	-	-	+
Reducing Sugar	+	+	+	+	+
Gums	-	-	-	+	+
Tannins and Phenolics	-	+	+	+	++
Saponins	+	+	+	+	+
Fixed oils	+	+	+	+	+

+ Present, - absent

Table 3: Toxicity studies of EEAR in mice

Route of Administration	Treatment	Dose mg/kg	No of animals	No of Survival	No of Death	LD_{50}
Peroral	Control	10 ml/kg	20	20	0	>3.2gms/kg,p.o
	SPE	100	20	20	0	
		200	20	20	0	
		400	20	20	0	
		800	20	20	0	
		1600	20	20	0	
		3200	20	20	0	
Intraperitonial	Control	10 ml/kg	20	20	0	>3.2gms/kg,p.o
	SPE	100	20	20	0	
		200	20	20	0	
		400	20	20	0	
		800	20	20	0	
		1600	20	20	0	
		3200	20	18	2	

Table 4: Analgesic activity studies of EEAR on male swiss albino mice

	Group I	**Group II**		**Group III**		**Group IV**		**Group V**	
Material administered	**2%Tween 80**	**EEAR**		**EEAR**		**EEAR**		**Morphine**	
Dose	**10 ml/kg**	**100 mg/kg**		**200 mg/kg**		**400 mg/kg**		**2mg/kg**	
Route of administration	**P.O**	**P. O**		**P.O**		**P. O**		**P.O**	**subcutaneous**
Method	**Reaction time in secs (mean±SEM)**	**Reaction time in secs (mean±SEM)**	**% inhibition**	**Reaction time in secs (mean±SEM)**	**% inhibition**	**Reaction time in secs (mean±SEM)**	**% inhibition**	**Reaction time in secs (mean±SEM)**	**% inhibition**
Hot plate	6.395±0.35	7.42±0.240*	16.137	8.697±0.189 *	35.99	11.80±0.182*	76.07	11.40.±0.42*	78.264
Tail flick	3.407±0.113	4.472±0.199 **	31.25	5.495±0.149**	61.28	5.567±0.161**	63.39	6.160±0.106**	80.80
Material administered								Aspirin 20mg/kg p.o	
Acetic acid induced									
Writhing	53.92±0.402	21.61±0.402*	59.92	20.62±0.332*	61.75	14.27±0.271*	73.53	10.91±0.138*	79.90

Each value represents the mean ±SEM n=6,no. of animals in each group. Values p was calculated comparing by Dunnets test $^{*}p<0.001$, $^{**}p<0.05$ compared to positive control

Table 5: Anti-inflammatory activity studies of EEAR on male albino Wistar rats

	Group I	**Group II**		**Group III**		**Group IV**		**Group V**	
Material administered	**2%Tween 80**	**EEAR**		**EEAR**		**EEAR**		**Indomethacin**	
Dose	**10 ml/kg**	**100 mg/kg**		**200 mg/kg**		**400 mg/kg**		**20mg/kg**	
Route of administration	**oral**	**oral**		**oral**		**oral**		**oral**	
Method	**paw volume after 3hrs (mean±SEM)**	**paw volume after 3hrs (mean±SEM)**	**% inhibition**	**paw volume after 3 hrs (mean±SEM)**	**% inhibition**	**paw volume after 3 hrs (mean±SEM)**	**% inhibition**	**paw volume after 3hrs (mean±SEM)**	**% inhibition**
Carrageenan	**53.9±0.288**	**36..3±0.327****	**32.65**	**32.00±0.357***	**40.63**	**27.5±0.925***	**48.97**	**23.80±2.02***	**55.84**
Histamine	**48.5±0.269**	**33..6±0.239***	**30.72**	**29.1±0.110***	**40.00**	**25.0±0.302 ***	**48.45**	**24.1±0.240***	**50.50**
Dextran	**42.2±0.225**	**32.3±0.239****	**23.45**	**30.2±0.350****	**28.43**	**27.6±0.237***	**34.59**	**26.2±0.316***	**37.91**

Each value represents the mean ±SEM n=6,no. of animals in each group. Values p was calculated comparing by Dunnets test $^{*}p<0.05$, $^{**}p<0.01$ compared to positive control.

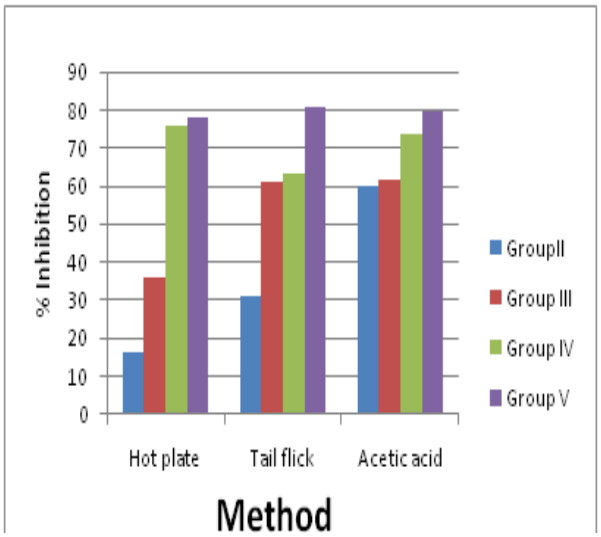

Fig 1: Analgesic activity studies of EEAR on male swiss albino mice

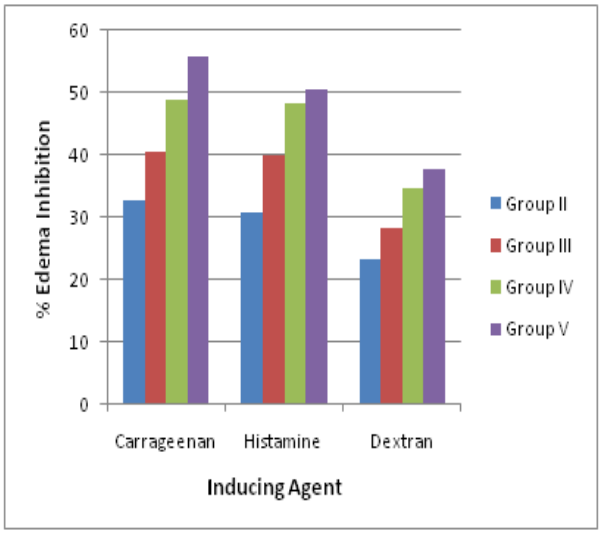

Fig 2: Antiinflammatory activity studies of EEAR on male albino Wistar rats

ACKNOWLEDGEMENT

We are thankful to the Management of Nirmala College of Pharmacy, Kadapa (A.P) for providing all the facilities to carried out of this work.

REFERENCES

1. Yoon. J., Baek. S.J.,. Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei Med. J. 2005, 46, 585-596.
2. Li, Y., Xian, Y.,Ip,S.,Su,J., Xie. Q.,Lai,X.,Lin.Z., 2011. Anti-inflammatory activity of patchouli alcohol isolated from pogostemonis Herba in animal models. Fitoterapia 82, 1295-1301.
3. IASP,. Pain terms; A list with definitions and notes on usage. Recommended by the IASP sub committee on taxonomy Pain 1979, 6, 249.
4. Silva. C.R., Oliveria , S.M., Rossato, M.F., Dalmolin. C.D., Guerra, G.P., Prudente, A.S., Cabrini. D.A., Otuki, M.F., Andre, E., Ferreira. J. The involvement of TRPA1 channel activation in the inflammatory response evoked by topical application of cinnamaldehyde to mice. Life Sci. 2011, 88, 1077-1087.
5. Sawynok. J. Topical and peripherally acting analgesics. Pharmacol. Rev. 55, 1-20.
6. Cunha, T.., Verri Jr., W.A., Silva, J.S., Poole. S., Cunha, F.Q., Ferreira, S.H., 2005. A cascade of cytokines mediates mechanical inflammatory hypernociception in mice. Proc. Nat acad. Sci. U.S.A.2003, 102, 1775-1760.
7. Corley. D.A., Kerlikowske. K., Verma. R., Buffler, P. Protective association of aspirin/NSAIDs and esophageal cancer; a systematic review and metaanalysis. Gastroenterology 2003, 124, 47-56.
8. Kirtikar, K. R., Basu, B. D. and An., I.C.S. 1993. Indian Medicinal Plants. Bishen Singh Mahendra Pal Sigh, India1993.
9. Kokate CK. Practical Pharmacognosy. 2nd edn; Vallabh Prakashan, Delhi, (1988) , 125.
10. Lorke D A. A new approach to practical acute toxicity testing. Arch. Toxical.1983, 54,275-287.
11. Eddy LB and Leimbach D Synthetic analgesics. II. Dithienyl-butenyl dithienbutylamine. J Pharmacol Exp Ther.1953, 107:385-393.
12. Kulkarni SK, Jain NK and Singh A. Role of cystenyl lukotrienes in nociceptive and inflammatory conditions in experimental animals. Eur J Pharmacol; 2001, 423:85-92.
13. R Anderson M and De Beer EJ. Acetic acid for analgesic screening. Federation Proceedings 1959,18,412.
14. Winter CA, Risley EA and Nuss GW. Carrageenin induced oedema in hind paw of rats as an assay for anti-inflammatory drugs. Proc Soc Exp Bio Med. 1962, 111:544-7.
15. Lin CC, Lin WC, Chang CH and Namba T. Anti-inflammator and hepatoprotective effects of Ventilago leiocarpa. Phtotherapy Research 9.1994, 11-15.

16. Parmar NS, Ghosh MMN. Current trends in flavonoid research. Indian J Pharm 1978; 12: 213-228.
17. Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AKN. Preliminary studies on the anti-inflammatory and analgesic activity of the methanol fraction of the root extract of *Tragia involucrate* Linn. J Ethnopharmacol2000; 72: 265-268.
18. Wu KK. Aspirin and other cyclooxygenase inhibitors: new therapeutic insights. *Seminar Vascular Med 2003;* 3: 107-112.
19. Barar FSK. Essential of Pharmacotherapeutics, 4th ed. S. Chand & company Ltd, New Delhi; 2006. p. 106-109&526.
20. Goodman LS, Gilman AG. The Pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill, New York; 2001. p.687-696.
21. Castro J, Saseme H, Sussman H, Bullette P. Diverse effect of SKF 52 and anti oxidants on CCL4 induced changes in liver microsomal P-450 content and ethylmorphine metabolism. Life Sciences1968; 7: 129-136.
22. Di Rosa M. Biological properties of Carrageenan. J Pham Pharmacol.1972; 24: 89-102.
23. Phadke JD, Anderson LA. Ethanopharmacology and Western medicine. J Ethnopharmacol1998; 25: 61.
24. Swain T. The evolution of flavonoids.In progress in clinical and Biochemical Research. Edited by Cody V, Middleton E, Harbone JB. Alan R. Liss, Newyork.1986; 213:1.