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Research Article

ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITIES OF *RUBUS ELLIPTICUS* SMITH. LEAF METHANOL EXTRACT

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ABSTRACT

Objective: The investigation was undertaken to explore the anti-inflammatory, analgesic and antipyretic activities of *Rubus ellipticus*.

Materials and Methods: The methanolic leaf extract (up to 2000 mg/kg b. wt.) of *R. ellipticus* was used to observe acute toxicity. Anti-inflammatory activity was evaluated using carrageenan induced paw edema in rats and croton oil induced ear edema in mice. Analgesic activity was evaluated using acetic acid induced writhing and Eddy's hot plate mediated pain models. Antipyretic activity was investigated by yeast induced pyrexia in rats.

Results: The results demonstrated no mortality in the toxicity testing. The extract (200 and 400 mg/kg) showed significant anti-inflammatory, analgesic and antipyretic activities. Both the doses significantly reduced the paw and ear edema compared to indomethacin (20, 10mg/kg) in the anti-inflammatory study, the doses also reduced the writhing responses and increased the latency period significantly in analgesic activity compared to aspirin (100 mg/kg) and morphine (10 mg/kg). The rectal temperatures of the rats were found to be decreased significantly compared to paracetamol (100 mg/ kg).

Conclusion: The present study will put forward a scope to develop an effective drug from *Rubus ellipticus* against inflammatory disorders, as this plant has been used in folk medicine to treat various other ailments.

Keywords: *Rubus ellipticus,* Anti-inflammatory, Analgesic, Antipyretic.

INTRODUCTION

Use of medicinal plants as a source of relief and cure from various illness is as old as humankind. Even today, medicinal plants provide a cheap source of drugs for majority of world's population. Plants have provided and will continue to provide not only directly usable drugs, but also a great variety of chemical compounds that can be used as a starting points for the synthesis of new drug with improved pharmacological properties [1].

It is estimated that 20% of all cancers death are associated with chronic infection and inflammation. Although inflammation acts as host defence mechanism against infection or injury and is primarily a self limiting process, inadequate resolution of inflammatory responses lead to various chronic disorders associated with cancers [2]. Cancer-related inflammation leads to the hypothesis that anti-inflammatory agents may have potential as cancer preventative agents. In terms of nonsteroidal anti-inflammatory agents, there are several observational studies suggesting that aspirin reduces risk of certain cancers [3].

The genus *Rubus* is very diverse, includes over 750 species in 12 subgenera, and is found on all continents except Antarctica [4]. Due to the ethnomedicinal richness; *Rubus* species has been used in folk medicine [5]. *R. ellipticus* root paste is used as poultice for the treatment of bone fracture, applied on forehead during severe headache; fruit is edible [6]. Ripe fruits are laxative and are used in the case of constipation, paste of young fruits are taken in case of gastritis, diarrhea and dysentery [7]. The root juice drunk against urinary tract infection and its fruits are edible and were listed in the top ten wild edible medicinal plants in Tanahun district of Western Nepal [8]. *R. ellipticus* is used for curing different ailments by the Lepcha tribe of Dzongu valley in North Sikkim, India. The young shoot is chewed raw to relieve sudden stomach pain. Root decoction given to the children to get rid of stomach warm. The inner root bark of the plant is valued as a medicinal herb in traditional Tibetan medicine, including its use as a renal tonic and antidiuretic [9].

Even though this plant has immense ethnomedicinal value; a survey of literature revealed that the anti-inflammatory, analgesic and antipyretic properties of this plant using animal models have not yet been evaluated. Keeping this in view, the present investigation has been undertaken to study the *in vivo* anti-inflammatory, analgesic and antipyretic activities of *R. ellipticus* leaf methanol extract.

MATERIALS AND METHODS

Plant material collection and identification

The fresh plant parts of *R. ellipticus* were collected from Shola forest of Marayoor, Kerala, India, during the month of September 2010. The collected plant material was identified and authenticated by (Voucher specimen No. BSI/SRC/5/23/2010-11/Tech.1659) Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

Processing and extraction

Collected plants were cleaned properly, separately shade dried and powdered. The powdered leaf was extracted in Soxhlet apparatus using methanol. The extract was concentrated to dryness under reduced pressure in a rotary evaporator to yield dried methanol extract.

Animals and acute toxicity study

Healthy Wistar albino rats (150-180g) and mice (25-30g) of either sex and of approximately the same age were used for the study. They were fed with standard chow diet and water *ad libitum* and were housed in polypropylene cages in a well maintained and clean environment. The experimental protocol was subjected to scrutiny of institutional animal ethical committee for experimental clearance (KMCRET/Ph.D/03/2011).

The acute toxicity was performed as per Organization for Economic Co-operation and Development guidelines [10]. Wistar albino rats and Swiss albino mice were used to assess the toxicity level. The methanol extract at dose of 100, 500, 1000 and 2000 mg/kg was administered to 3 rats and 3 mice in a single dose orally. The rats were fasted over-night and mice were fasted 3 h prior to the dosage. Animals are observed individually after drug administration at least once during the first 30 minutes, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days.

Anti-inflammatory activity
Carrageenin induced paw edema

The acute inflammation in rats was induced by carrageenan according to the modified method described by Galani and Patel [11]. Four groups of six animals each were used for the study. Paw thickness was increased by sub-plantar injection of 0.1 mL 1%

carrageenan in normal saline into the right hind paw. 200 and 400 mg/kg methanolic leaf extract was administered orally 60 min before carrageenan induction. Indomethacin 20mg/kg p.o. was used as standard drug. The control group received vehicle only. The mean increase in paw size was measured with vernier caliper at 0, 1, 2, 3, 4, 5, 6 and 7th h after carrageenan injection in each group. 0th h reading was considered as the initial paw size of the animals.

Croton oil induced ear edema

To estimate the inhibitory activity of methanol extract against acute inflammation, croton oil-induced mice ear edema was performed according to the method of Wang et al. [12] modified by Lin et al., [13]. Briefly, 10 µL acetone solution containing the 5% croton oil was applied topically to the right ear of mice. The left ear received an equal volume of acetone. Methanolic extract were administrated orally at a dose of 200 and 400 mg/kg. About 60 min before the croton oil treatment. The left ear received vehicle alone. As a reference, the non-steroidal anti-inflammatory drug (NSAID), Indomethacin (10mg/kg), was used. Six hours later, the mice were sacrificed and both ears were removed uniformly by a sharp scissors and individually weighed on a sensitive balance. The edematous response was measured as the weight difference between the two plugs.

Analgesic activity

Acetic acid induced writhing test

Male Swiss albino mice were divided into four groups with six animals each. Group 1 served as control group 2 received standard drug Aspirin (100 mg/kg); group 3 and 4 received leaf methanol extract at doses of 200 mg/kg and 400 mg/ kg. The acetic acid 0.6% v/v (10 ml/kg, i.p.) was injected intraperitoneally 1 h after administration of the drugs. After administration of acetic acid, number of writhes (abdominal muscle contractions) was counted over a period of 15 min and immediately after acetic acid injection (0 time) [14,15]. Analgesic activity was expressed as the percentage protection against writhing produced by the tested extract compared with writhing at 0 time. Aspirin and methanol extract were suspended in carboxy methyl cellulose (0.1%) before oral administration.

Eddy's hot plate mediated pain reaction

The hot-plate test was performed to measure response latencies according to the method described by Eddy and Leimback [16]. Male Swiss albino mice were divided into four groups of six animals each. Group 1 served as control; group 2 served as standard which received morphine (10 mg/kg); group 3 and 4 served as plant extract at a dose of 200 mg/kg and 400 mg/kg respectively. The animals were placed on the hot plate, maintained at (55±1) ºC. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the hot plate. The time taken for the mice to react in this fashion was obtained using a stopwatch and noted as basal reaction time (0 min). A latency period of 15 seconds (cut-off) was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury [17]. The reaction time was reinvestigated at 30, 60 and 120 min after the treatment and changes in the reaction time were noted.

Antipyretic activity

Yeast induced pyrexia in rats

Hyperpyrexia was induced in rats by subcutaneous injection of 20 mL/ kg b. w. of a 15% aqueous suspension of Brewer's yeast in the back below the nape of the neck [18]. Animals were divided into four groups of six each. The animals were then fasted approximately for 24 h, having free access to water. Control temperatures were taken 18 h after the yeast injection to determine the pyretic response to yeast. The rats which showed a rise in temperature of at least 0.6ºC were taken for the study. The extract suspension (200 and 400 mg/kg) and the standard drug (paracetamol, 100 mg/ kg) were given orally after 18 h of yeast injection. Initial rectal temperatures of the rats were recorded (0 h). The temperatures were recorded at 1-6 h after the drug treatment [17].

Statistical analysis

All the results were expressed as mean± SEM. Statistical significance was determined by using the one way ANOVA followed by Dunnett's multiple comparison tests. $p < 0.05$ was considered statistically significant.

RESULTS

Acute toxicity

In the acute toxicity studies; four groups of rats and mice were administered with methanolic leaves extract in graded doses of 100, 500, 1000 and 2000 mg/kg p.o., respectively. The animals were kept under observation for the change in behavior or death up to 14 days following the drug administration. The extract administration neither caused any significant change in the behaviors nor the death of animals in all the test groups. This indicates that the methanolic leaves extract of *R. ellipticus* was safe up to a single dose of 2000 mg/kg body weight. Hence we selected 200 and 400 mg/kg doses to evaluate anti-inflammatory, analgesic and antipyretic activities.

Anti-inflammatory activity

Carrageenan induced paw edema

Carrageenan-induced inflammation in the rat paw represents a classical model for studying the acute inflammation; that was used for evaluation of anti-inflammatory activity of methanolic leaf extract of *R. ellipticus* (Table 1). Pre-treatment with The standard drug; Indomethacin (20 mg/kg) and methanolic extract at doses 200 and 400 mg/kg significantly (p<0.05) prevented the increase in thickness of paw edema in a dose dependent manner compared to the control rats. It has been found that, methanol extract showed good activity after 7 h, the standard drug produced a significant inhibitory effect (80.89%) followed by 400 and 200 mg/kg plant extract (66.47% & 45.43%) respectively.

Table 1: Shows Effect of *R. ellipticus* leaf methanol extract in carrageenan induced paw edema

Groups	Paw thickness (mm) before carrageenan induction (Initial)	Paw thickness (mm) after carrageenan induction							Mean increase in paw thickness (mm) after 7th hr	%Inhibition
		1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr	7th hr		
Control	4.67	6.46	6.69	7.01	6.70	6.41	6.38	6.34	1.66	-
Indomethacin (20mg/kg)	4.63	5.72	5.46	5.42 *	5.23 *	5.14 4*	5.02 **	4.91** *	0.32	80.89
RELM200(mg/kg)	4.41	5.65	5.75	5.60	6.22	5.95	5.75	5.32*	0.91	45.43
RELM400(mg/kg)	5.37	6.06	6.21	6.72	6.77	6.53	6.03	5.93*	0.56	66.47

Values are expressed as mean ± SEM. (n=6), significantly different at * p<0.05, ** p<0.01, *** p<0.001 when compared to control. RELM - *R. ellipticus* leaf methanol

Croton oil induced ear edema

The similar result was obtained from croton-oil induced ear inflammation in mice; the standard drug Indomethacin 10mg/kg significantly (p<0.001) decreased the weight of inflamed ear. Table 2 shows percent reduction of the inflammatory response following topical application of croton oil. The topical application of croton oil-induced edema on the ears of mice caused a significant increase in the ear plug weight of the right ear compared with the vehicle-treated left ear after 6 hrs. A dose-dependent edema inhibition was

observed in the methanol extract treated groups in comparison with the non-steroidal anti-inflammatory drug indomethacin; a stronger and effective anti-inflammatory agent. The mouse ear plug weight was reduced by 76.52% after indomethacin treatment. However, *R. ellipticus* leaf methanol extract reduced the edematous response by 36.66% for 200 mg/kg and by 45.78% for 400 mg/kg., respectively.

Table 2: Shows Effect of *R. ellipticus* leaf methanol extract in croton oil induced ear edema

Groups	Difference in thickness(mm) between left and right ear after 6 hrs	Difference in weight(mg) mean±SEM between left and right ear after 6 hrs	% Inhibition
Control	0.48±0.08	118.4±2.9	-
Indomethacin (10mg/kg)	0.11±0.17***	27.8±2.01***	76.52
RELM200 (mg/kg)	0.36±0.08*	75±1.29**	36.66
RELM400 (mg/kg)	0.33±0.14*	64.2±2.01**	45.78

Values are expressed as mean ± SEM. (n=6), significantly different at * p<0.05, ** p<0.01, *** p<0.001 when compared to control. RELM - *R. ellipticus* leaf methanol

Analgesic activity

Acetic acid induced writhing test

The results presented in Table 3 shows that the methanolic extract at the doses 200 and 400 mg/kg exhibited significant (p<0.01 and p<0.001) analgesic activity (19.4% and 32.84% inhibition respectively) compared to the control and also to that of aspirin, 100 mg/kg (73.13%). Significant protection against writhing was observed in animals treated with aspirin, 200 and 400 mg/kg extract; where number of writhes after treatment were 18, 54 and 45 respectively compared to 67 in the control group.

Table 3: Shows Effect of *R. ellipticus* leaf methanol extract on acetic acid induced writhing

Treatment groups	No. of writhes (per 15min)	% Inhibition
Control	67± 2.81	-
Aspirin100 (mg/kg)	18±3.05***	73.13
RELM 200 (mg/kg)	54±2.89**	19.40
RELM 400 (mg/kg)	45±3.46***	32.84

Values are expressed as mean ± SEM. (n=6), significantly different at * p<0.05, ** p<0.01, *** p<0.001 when compared to control. RELM - *R. ellipticus* leaf methanol

Eddy's hot plate mediated pain reaction

As shown in Table 4, the methanolic extract produced significant analgesic activity in a dose-dependent manner. In this model, the higher dose (400 mg/kg) prolonged significantly the reaction time of animal with relatively extended duration of stimulation. At the high dose level the animals could withstand on the hot plate for 11.2, 13.6 and 7.7 seconds at 30, 60 and 120 min reaction time which was the highest and comparable with that of the reference drug morphine10 mg/kg (7.8, 9.6 and 12.4 sec.). The basal reaction time of the higher dose and standard drug were 6 and 5.8 seconds.

Table 4: Shows Effect of *R. ellipticus* leaf methanol extract on hot plate mediated pain reaction

Groups	Basal reaction time 15 min cut off (after drug administration)	Reaction time 15 min cut off (after drug administration)		
		30 min	60 min	120 min
Control	7.2±0.23	9.4±0.43	8.8±0.53	9.8±0.6
Morphine (10mg/kg)	5.8±0.87	7.8±0.34***	9.6±0.8***	12.4±0.1***
RELM200 (mg/kg)	5.4±0.01	8.4±0.1***	9.4±0.1***	6.6±0.2**
RELM400 (mg/kg)	6±0.5	11.2±0.23***	13.6±0.1***	7.7±0.4**

Values are expressed as mean ± SEM. (n=6), significantly different at * p<0.05, ** p<0.01, *** p<0.001 when compared to control. RELM - *R. ellipticus* leaf methanol

Antipyretic activity

Yeast induced pyrexia in rats

Effect of *R. ellipticus* leaf methanol extract on yeast induced hyperpyrexia is shown in table 5. Subcutaneous injection of yeast suspension markedly elevated the rectal temperature of rats after 18 h of administration and the treatment with the methanolic leaves extract at 200 and 400 mg/kg significantly decreased the rectal temperature in a dose-dependent manner. The result obtained from both the standard (paracetamol) and methanolic leaves extract treated rats were compared with that of control and a significant reduction in the yeast induced elevated rectal temperature was observed from 3rd to 7th h after the treatment.

Table 5: Shows Antipyretic activity of *R. ellipticus* leaf methanol extract

Groups	Rectal temperature in ºC							
	Before treatment	After treatment						
	0th Hr	18th Hr	19th Hr	20th hr	21st Hr	22nd Hr	23rd Hr	24th Hr
Control	37.1±0.45	39.02±0.34	39.06±0.21	38.94±0.36	39.08±0.29	39.22±0.39	38.86±0.52	38.84±0.48
Paracetamol 100(mg/kg)	36.96±0.46	38.58±0.18	38.16±0.79	37.4±0.35**	37.5±0.25***	37.4±0.19***	37.28±0.22***	37.06±0.18***
RELM200 (mg/kg)	36.88±0.32	38.6±0.25	38.52±0.41	38.9±0.42	39.12±0.41	38.92±0.45*	38.7±0.41**	38.32±0.48**
RELM400 (mg/kg)	37.16±0.26	38.6±0.25	38.74±0.15	38.7±0.61*	39.02±0.43	38.82±0.37**	38.58±0.35**	37.98±0.36**

Values are expressed as mean ± SEM. (n=6), significantly different at * p<0.05, ** p<0.01, *** p<0.001 when compared to control. RELM - *R. ellipticus* leaf methanol

DISCUSSION

The previously studied antioxidant activity and traditional use of *R. ellipticus* encouraged us to extend our evaluation using *in vivo* anti-inflammatory, analgesic and antipyretic models. *Rubus* species is well known for its pharmacological properties; *R. niveus* is a related species which exhibited strong anti-inflammatory, analgesic and antipyretic activities [19]. It is well known that all the pharmaceutical companies are now interested in developing more effective drugs to treat inflammatory disorders.

Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and devoid of apparent systemic effect [20]. Carrageenan-induced hind paw edema has been widely used as an experimental model of acute inflammation which is used as primary test for the screening of new anti-inflammatory agents and is believed to be biphasic i.e. the early phase (up to 2 h) and late phase (1-6h) [21]. The early phase was associated with significantly severe inflammation, whereas the late phase was observed to have slow increase in volume of paw edema. The early phase has been attributed to the action of mediators such as histamine, serotonin, and bradykinin on vascular permeability [22]. The late phase edema has been shown to be a result of over production of prostaglandins [23]. The data obtained revealed that the 400 mg/kg leaf methanol extract of *R. ellipticus* possess potent anti-inflammatory effect in the carrageenan-induced inflammation.

The test of erythema in mouse ears induced by croton oil is commonly used for the evaluation of the effect of new anti-inflammatory drugs, topical steroids or non-steroidal anti-inflammatory agents. [24]. Dermatitis induced by croton oil represents a model of acute inflammatory response. The edema is mediated by cyclooxygenase metabolism of arachidonic acid [25, 26]. Regarding the croton-oil induced edema, groups treated with 200 and 400 mg/kg doses of *R. ellipticus* leaf methanol extract showed significant results when compared to control, which were also statistically significant to that of indomethacin (10mg/kg).

Acetic acid-induced writhing is a non-specific pain model and many compounds belonging to diverse pharmacological categories including opioids, non-steroidal anti-inflammatory drugs, calcium channel blockers, anticholinergics, antihistamines, and corticosteroids show analgesic activity in this test [14]. Acetic acid test is a visceral pain model produces a painful reaction and acute inflammation in the peritoneal area. Release of arachidonic acid and biosynthesis of prostaglandin via cyclooxygenase pathway plays a role in the nociceptive mechanism of this test [27]. The analgesic effect of the tested compounds may be mediated through inhibition of cyclooxygenase and/or lipooxygenase (and other inflammatory mediators) [14]. Aspirin offers relief from inflammatory pain by suppressing the formation of pain mediators in the peripheral tissues, where prostaglandins and bradykinins were suggested to play an important role in the pain process. Prostaglandins elicit pain by the direct stimulation of sensory nerve endings [28]. It is evident from the study that *R. ellipticus* exhibits significant peripheral analgesic effect in mice comparable with standard.

The classic hot plate model was followed to evaluate the analgesic activity of *R. ellipticus* leaf methanol extract. The hot plate model has been found suitable to investigate central antinociceptive activity because of several advantages, particularly the sensitivity to antinociceptives and limited tissue damage [29]. Proinflammatory mediators like prostaglandins and bradykinins were suggested to play an important role in analgesia [30]. The obtained results confirmed that leaf methanol extract at the dose 200 and 400 mg/kg has a central analgesic effect, which was compared with reference drug (Aspirin100mg/kg). The analgesic effect of *R. ellipticus* might be attributed to the inhibition of the synthesis of some pro-inflammatory mediators, such as prostaglandins and cytokines. The anti-inflammatory effect may also be due to inhibition of either vascular event or cellular events or due to both in experimental rats [31].

Antipyretics are drugs, which reduces the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and a drug like paracetamol does not influence body temperature when it is elevated by factors such as exercise or increases in ambient temperature [32]. Fever is thought to be produced by several endogenous substances such as interleukins, tumor necrosis factor-α (TNF-α), macrophages, prostaglandins etc. [33]. An antipyretic drug reduces fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These agents suppress peripheral production of pyrogenic cytokines such as TNF-α and interleukin-1β, while lowering the thermoregulatory set point by blocking central cyclooxygenase production of prostaglandin E2 (PGE2) [34]. The present results showed that *R. ellipticus* leaf methanol extract at two doses possessed a significant antipyretic effect in yeast-induced elevation of body temperature in rats and its effect is comparable to that of paracetamol (100mg/kg).

CONCLUSION

In conclusion, the results of the present study revealed the acute anti-inflammatory, central and peripheral analgesic and antipyretic activity of the leaf methanol extract of *R. ellipticus*. The data reported in this study confirms the traditional use of *R. ellipticus* to treat various disorders. This study is an eye opener to the researchers to find out the mechanism and the responsible compound behind these pharmacological properties; since inflammation and cancer are interrelated this study will also lead to the development of anticancer agents from *R. ellipticus.*

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REFERENCES

1. Ballabh B, Chaurasia OP, Amed Z, Signh SB. Traditional medicinal plants of cold desert Ladakh-Used against kidney and urinary disorders. Food Chem 2008; 118: 331-339.
2. Porta C, Larghi P, Rimoldi M, Totaro MG, Allavena P. Cellular and molecular pathways linking inflammation and cancer. Immunobiol 2009; 214: 761–777.
3. Cuzick J, Otto F, Baron JA, Brown PH, Burn J. Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. Lancet Oncol 2009; 10: 501–7.
4. Finn CE. *Rubus* spp., blackberry. In: Janick, J., Paull, R.E. (Eds.), The Encyclopedia of Fruits and Nuts. 2008 (pp. 348–351).CABI, Cambridge, MA.
5. Patel AV, Rojas-Vera J, Dacke CG. Therapeutic constituents and actions of *Rubus* species. Cur Med Chem 2004; 11: 1501-1512.
6. Pradhan BK, Badola HK. Ethnomedicinal plant use by Lepcha tribe of Dzongu valley, bordering Khangchendzonga Biosphere Reserve, in North Sikkim, India. J Ethnobiol and Ethnomed 2008; 4:22 doi: 10.1186/1746-4269-4-22.
7. Maity D, Pradhan N, Chauhan AS. Folk uses of some medicinal plants from north Sikkim. IJTK 2004; 3: 66-71.
8. Uprety Y, Ram C, Asselin PH, Boon E. Plant biodiversity and ethnobotany inside the projected impact area of the Upper Seti Hydropower Project, Western Nepal. Environ Dev Sustain. 2011; 13:463–492.
9. Pfoze NL, Kumar Y, Myrboh B. Survey and assessment of ethnomedicinal plants used in Senapati District of Manipur State, Northeast India. Phytopharmacol 2012; 2: 285-311.
10. OECD. Guidelines for testing of chemicals, acute oral toxicity - acute toxic class method. Paris: OECD; 2001. Available from:http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL423.pdf.
11. Galani VJ, Patel BG. Analgesic and anti-inflammatory activity of Argyreia *speciosa* and *Sphearanthus indicus* in the experimental animals. Global J Pharmacol 2011; 5: 54-59,
12. Wang SY, Lan XY, Xiao JH, Yang JC, Kao YT, Chang ST. Anti-inflammatory activity of *Lindera erythrocarpa* fruit. Phytother Res 2007; doi:10.1002/ptr.2289.

13. Lin CT, Chen CJ, Lin TY, Tung JC, Wang SY. Anti-inflammation activity of fruit essential oil from *Cinnamomum insularimontanum* Hayata. Bioresource Technol 2008; 99: 8783–8787
14. Vogel GH, Vogel WH. "Drug Discovery and Evaluation" Pharmacological assays. 1st ed. Springer –Berlag Berlin Heidelberg, Germany 1997; 759-769.
15. Vyas S, Agrawal PR, Solanki P, Trivedi P. Analgesic and anti-inflammatory activities of *Trigonella foenum*-graecum (seed) extract. Acta Poloniae Pharmaceutica 2008; 65: 473-476.
16. Eddy NB, Leimback D. Synthetic analgesics: II. Dithyienylbutenylamines and dithyienylbutylamines. J Pharmacol Exp Ther 1953; 3: 544–547.
17. Awaad AS, El-meligy RM, Qenawy SA, Atta AH, Soliman GA. Anti-inflammatory, antinociceptive and antipyretic effects of some desert plants. J Saud Chem Soc 2011; 15: 367–373.
18. Loux JJ, De Palma PD,Yankell SL. Antipyretic testing of aspirin in rats. Toxicol Appl Pharmacol 1972; 2: 672–675.
19. George BP, Parimelazhagan T, Saravanan S, Chandran R. Anti-inflammatory, analgesic and antipyretic properties of *Rubus niveus* Thunb. root acetone extract. Pharmacologia 2013; 4(3): 228-235.
20. Firdous S, Raju K. *In vivo* and *in vitro* antiinflammatory activity of leaves of *Ipomoea staphylina*. Int J of Pharm and Pharm Sci 2012; 4: 339-343.
21. Saha A, Masud MA, Bachar SC, Kundu JK, Datta BK, Nahar L. The analgesic and anti-inflammatory activities of the extracts of *phyllanthus reticulatus.* Pharmaceut Biol 2007; 45: 335-359.
22. Niazi J, Singh P, Bansal Y, Goel RK. Anti-inflammatory, analgesic and antipyretic activity of aqueous extract of fresh leaves of *Coccinia indica*. Inflammopharmacol 2009; 17: 239-244.
23. Gurrero PC, Herrera MD, Ortiz R de Sotomayor MA, Fernandez MA. A pharmacological study of *Cecropia obtusifolia* Betrol aqueous extract. J Ethnopharmacol 2001; 76: 279-284.
24. Falodun A, Owolabi OJ, Osahon O. Physicochemical, Antimicrobial and anti-inflammatory evaluation of fixed oil from boa constrictor. Acta Poloniae Pharmaceutica- Drug Research 2008; 65: 477-480.
25. Galey GI, Ziboh VA, Marcelo CI, Voorhees JJ. Modulation of phospholipid metabolism in murine keratinocytes by tumor promoter, 12-O-tetradecanoyl-13-acetate. J Investigative Dermatol 1985; 85: 319-323.
26. Inoue H, Saito K, Koshihara Y, Muroto S. Inhibitory effect of glycyrrhetinic acid derivatives on lipoxygenase and prostaglandin synthetase. Chem Pharm Bull 1986; 34: 897-901.
27. Franzotti EM, Santos CV, Rodrigues HM, Mourao RH, Andrade MR, Antoniolli AR. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia L.* (Malva-branca). J Ethnopharmacol 2000; 72: 273-277.
28. Das BN, Ahmed M. Analgesic activity of the fruit Extract of *averrhoa carambola.* Intl J life sci Biotechnol pharma res 2012; ISSN 2250-3137 Vol. 1, no. 3.
29. Kou J, Ni Y, Li N, Wang J, Liu L, Jiang ZH. Analgesic and anti-inflammatory activities of total extract and individual fractions of Chinese medicinal plant *Polyrhachis lamellidens*. Biol Pharm Bull 2005; 28: 176–180.
30. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan edema in rats. J Pharmacol Exp Ther 1969; 166: 96–103.
31. Khan A, Noorulla SMD, Muqtader M, Roshan S, Ali S. Anti inflammatory activity of a novel herbal combination. Int J of Pharm and Pharm Sci 2013; 5 [1]: 33-34
32. Goodman G. The Pharmacological Basis of the Therapeutics, 19th ed. Mc Graw-Hill, New York. pp. 1996; 959–975.
33. Kluger MJ. Fever; Role of pyrogens and cryogens. Physiol Rev 1991; 71: 93- 127.
34. Aronoff DM, Neilson EG. Antipyretics: Mechanism of Action and Clinical Use in Fever Suppression. Am J Med 2001; 111: 304-315.