ejpmr, 2016,3(3), 106-111

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

PHARMACOLOGICAL SCREENING FOR ANTI-INFLAMMATORY, ANALGESIC, AND CNS DEPRESSANT ACTIVITIES OF THE METHANOL AND ETHYL ACETATE FRACTIONS OF *STEREOSPERMUM PERSONATUM* (HASSK.) CHATTERJEE LEAVES IN MICE

Luthfun Nesa[1*], Md. Akherul Islam[1], Mohammad Amzad Hossain[1], Md. Monirul Islam[1], Asma Kabir[1] and Mohammad Sirajum Munir[2]

[1]Atish Dipankar University of Science and Technology, Department of Pharmacy.
[2]Beximco Pharmaceutical LTD.

***Author for Correspondence: Luthfun Nesa**
Atish Dipankar University of Science and Technology, Department of Pharmacy.

Article Received on 13/01/2016 **Article Revised on 02/02/2016** **Article Accepted on 23/02/2016**

ABSTRACT

Aims: The study was carried out to assess anti-inflammatory, analgesic and CNS depressant activities of methanol & ethyl acetate fractions of *Stereospermum personatum* leaves. The methanol and ethyl acetate fractions (200mg/kg and 400mg/kg dose) were ingested to determine the acetic acid-induced writhing, formalin induced licking and biting, carrageenan induced hind paw edema and CNS depressant activity by open field and hole cross test. The both fractions (400mg/kg) exhibited moderate analgesic activity (57.55% and 56.1%) against acetic acid induced pain compared to standard (83.02%). Though the both fractions (400mg/kg) showed same analgesic effect (54.57% and 56.43%) at early phase but only ethyl acetate fractions (400mg/kg) is effective (58.21%) at late phase of formalin induced compared to standard (59.70%). The both fractions (200mg/kg and 400mg/kg) exhibited significant anti-inflammatory activity at 1st h to 4th hours. The two fractions (200mg/kg and 400mg/kg) showed significant (*$p<0.05$) depressant activity at 30min to 120min in the hole cross and open field test. This study observed that the effect of methanol and ethyl acetate fractions of *Stereospermum personatum* leaves (400mg/kg) were almost similar but only 400mg/kg dose has shown moderate analgesic, significant CNS depressant action and mild anti-inflammatory activity.

KEYWORDS: *Stereospermum personatum*, Analgesic, acetic acid, Formalin, anti-inflammatory, CNS Depressant.

INTRODUCTION

The utilization of medicinal plants in curing diseases is as ancient as human being. To increasing the interest of the public in the use of medicinal plants and their products in the treatment of various disorders, The World Health Organization (WHO) has long recognized and grown the awareness of many countries. These plants which are found in our environment enjoy wide acceptability through the population and serve as cheaper alternatives to traditional medicine.[1] *Stereospermum personatum* is a recognized medicinal tree. It belongs to the family of Bignoniaceae which locally known by various names (English: Trumpet Flower; Tamil: Poopadiri; Paadhalaamaram Hindi: Patiri; Malayalam: Karingkruna; Flora of Nilgiri Biosphere). It is commonly found in India, Tropical Himalaya, Sri Lanka, Burma, Thailand, Indo-China, Malaysia. In Bangladesh, it is also found in the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, Gazipur and Tangail.[2] It is a medium sized medicinal tree.[3] frequently used in ayurvedic system of medicine. The fruits of *Stereospermum personatum* are used to cure migraine and bark is useful in the management of piles. It is one of the ingredients used in the preparation of an ayurvedic tonic. It has antimicrobial, antiprotozoal and anti inflammatory properties.[4] Other species of *Stereospermum* are popularly known for their medicinal uses. Their leaves, roots, bark, flowers and fruits all have potential medical applications. They are commonly used as a diuretic, Lithotropic, expectorant, cardio tonic, and anti inflammatory, anti bacterial, febrifuge, anti pyretic, rheumatalgia, malarial fever, wound, asthma and cough. Cardioglycosides, Flavonoid, Quinones, Terpenoids, Alkaloids and Steroids such like bioactive compounds are present in high concentration in Stereospermum species.[5] Free radical scavenging and xantine oxidase inhibitory constituents have been obtained from extracts of both stem and bark of *Stereospermum* personatum,[6] which is of high therapeutic value. In nature, its regeneration occurs through seeds. Since there is no specific results about the analgesic, anti-inflammatory and CNS depressant properties of the various types fraction of *Stereospermum personatum* leaves. So our target was to assess the analgesic, anti-inflammatory, and

CNS depressant activities of the Methanolic & ethyl acetate fractions of *Stereospermum personatum* leaves (MFSPL and EAFSPL) respectively.

MATERIALS AND METHOD

Plant material

The *Stereospermum personatum leaves* were collected from the area of National Botanical garden, Dhaka, Bangladesh and were identified by a taxonomist of Bangladesh National Herbarium, Dhaka. The leaves of that plant was dried for a week under sunlight and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until use for analysis.

Preparation of extract

In a Soxhlet extractor, 300 gm of the powdered leaves was extracted with 500 ml of methanol at room temperature until all the extractable components were exhausted. The methanol extract was concentrated and kept in a dessicator. Twenty gram (20 g) of the methanol extract was partitioned by ethyl acetate and yield 30.2% ethyl acetate fraction (EAF). The ethyl acetate sub-fraction was evaporated under reduced pressure at 40°C, yielding dry residue, considered as the fraction. The both fractions were stored in a dessicator until required.

Animal

Swiss albino mice weighing about 25-35 gm were used for the experiment. The mice were purchased from the animal research branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B) were used for the evaluation of analgesic, anti-inflammatory and CNS depressant activities. The animals were housed under standard laboratory conditions (relative humidity 55–65%, room temperature 23.0±2ºC and 12-h light, 12-h dark cycle). The animals were fed with a standard diet and water adlibitum in all animal experiments; the guidelines of the Animal Experimentation Ethics Committee, ICDDR, B were followed. Each group consists of five mice and the animals are divided into four groups. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee.[7]

Chemicals

Diclofenac sodium and Diazepam were obtained from Beximco Pharmaceuticals Ltd., Bangladesh; Acetic acid was purchased from Merck, Germany. Normal saline water (0.9% Sodium Chloride), a product of Beximco Infusion Company Ltd., Bangladesh was purchased from local market. BDH Chemicals Ltd kindly provided tween-80, formalin, Carrageenan and all other chemicals were of analytical grade.

ANALGESIC ACTIVITY

Acetic acid-induced writhing method

The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice.[8] Test samples (200 and 400 mg/kg body weight), vehicle (1% tween 80 in water) and Diclofenac sodium (10mg/kg) were administered orally 30 min before intraperitoneal administration of 0.1% acetic acid. Then the mice were observed for specific contraction of the body referred to as 'writhing' for the next 30 min.[8] Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half writhing. Accordingly two half-writhing were taken as one full writhing. The number of writhing in each treated group was compared to that of a control group while. Diclofenac sodium (10mg/kg) was used as a reference drug (positive control).The percent inhibition (% analgesic activity) was calculated by

% inhibition = {(A-B) /A} X100.

Where, A= Average number of writhing of the control group; B= Average number of Writhing of the test group.

Formalin induced liking and biting

The antinociceptive activity of the fractions were determined using the formalin test.[8] The control group received 2.5% formalin. 20 µl of 2.5% formalin was injected into the dorsal surface of the right hind paw 30 min after the administration of MFSPL , EAFSPL (200mg/kg, 400 mg/kg, p.o.) and Diclofenac sodium (10 mg/kg, p.o.). The mice were observed for 30 min after the injection of formalin, and the licking of the injected hind paw was recorded. The first 5 min of formalin injection was referred to as the early phase and the period between 15 and 30 min as late phase. The total time spent of licking and biting of the injured paw (pain behavior) was measured with a stop watch.

ANTI-INFLAMMATORY ACTIVITY

Carrageenan-induced paw edema method

The mice were divided into six groups containing 5 mice. Acute inflammation was induced by injecting 0.1 ml of (1%) carrageenan into the plantar surface of the mice hind paw.[9] The MFSPL and EAFSPL (200 and 400 mg/kg), normal saline (1 ml/kg) and Diclofenac (10 mg/kg, p.o.) as the referral agents were administered 30 min before carrageenan injection. The paw volume was measured at 0, 1, 2, 3, and 4 h using a vernier caliper to determine the diameter of edema. The difference between the readings at time 1 h and different time interval was taken as the thickness of edema.

CNS DEPRESSANT ACTIVITY

Hole cross test

The method was carried out as described by Takagi *et al.*[10] A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm3. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. Thirty animals were divided into six groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 ml/kg, p.o.), animals of Group II received diazepam at 1 mg/kg body weight (p.o.) while Group III, Group IV, Group V and Group VI were treated with MFSPL and EAFSPL (200 and 400

mg/kg body weight p.o.). The number of passages of mice through the hole from one chamber to another was counted for a period of 3 min on 0, 30, 60, 90, and 120 min after oral administration of test drugs.

Open field test

The animals were treated as discussed above. The experiment was carried out according to the methods described by Gupta *et al*.[11] The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had 40 cm height a wall. The number of squares visited by the animals was counted for 3 min at 0min, 30min, 60min, 90min, and 120min after oral administration of the MFSPL and EAFSPL (200 and 400 mg/kg body weight p.o.).

RESULT

ANALGESIC ACTIVITY

Acetic acid induced writhing in mice

The effect of MFSPL and EAFSPL was investigated against acetic acid induced writhing in mice (Table 1). About 83.02% inhibition of writhing was found in group-II (Diclofenac sodium, 10 mg/kg). The group-III, IV, V and VI (200mg/kg and 400mg/kg) significantly reduced the acetic acid induced abdominal constrictions and stretching compared to control and standard (group-I, group-II) at a significant ($p<0.05$) with a dose dependent manner.

Table 1. Effects of methanol and ethyl acetate fractions of the *Stereospermum personatum* leaves on acetic acid induced writhing in mice.

Groups	Dose (mg/kg)	No. of writhing	% inhibition
Group I (control)	Vehicle	42.4±3.011	-
Group II(Standard)	10	7.2±0.914*	83.02
Group III	200	25.4±1.067*	40.1
Group IV	400	18±1.257*	57.55
Group V	200	26±1.800*	38.68
Group VI	400	18.4±1.791*	56.1

Values are mean ± SEM=standard error of mean (n = 5); *$p<0.05$ Dunnet test as compared to control (one way ANOVA followed by Dunnet's test). Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Sodium 10 mg/kg body weight, Group III, IV, V and VI were treated with 200 and 400 mg/kg (p/o) methanol and ethyl acetate extract of *Stereospermum personatum* leaves respectively.

Formalin induced hind paw licking in mice

The experiment was carried out to test whether the extract of *Stereospermum personatum* leaves had any effect on formalin induced hind paw licking in mice. Though the MFSPL and EAFSPL have shown almost same effect but the ethyl acetate (400 mg/kg body weight) has given 58.21% protection which is equal to standard (Diclofenac sodium at 10 mg/kg dose, Table 2).

Table 2. Effects of methanol and ethyl acetate fractions of the *Stereospermum personatum* leaves on hind paw licking in the formalin test in mice.

Groups	Dose(mg/kg)	Early phase	% of inhibition	Late phase	% of protection
Group I (control)	Vehicle	28± 2.29	-	13.40±1.07	-
Group II (Standard)	10	12.2±1.14*	56.43	7.60± 1.29*	59.70
Group III	200	17.2± 1.57*	38.57	9.00±1*	32.84
Group IV	400	12.8 ± 1.37*	54.27	8 ± 0.84*	40.30
Group V	200	17.6±2.33*	37.14	9± 0.84*	32.84
Group VI	400	12.2±1.60*	56.43	5.60±1.52*	58.21

Values are mean (n = 5) ± SEM (standard error mean) *$p<0.05$ compared with vehicle control (one way ANOVA followed by Dunnet'stest. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Na 10 mg/kg body weight, Group III, IV, V and Group VI were treated with 200 and 400 mg/kg (p.o) methanol and ethyl acetate fractions of *Stereospermum personatum* leaves respectively.

ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced paw edema in mice

The *Stereospermum personatum* (200 and 400 mg/kg) exerted a significant (*$p<0.05$) anti-inflammatory effect at 1^{st} h to 4^{th} hours which was comparable to that of the control group (Table 3).

Table 3. Effect of *Stereospermum personatum* methanol and ethyl acetate leaves extract on carrageenan induced paw edema in mice.

Groups		Oedema diameter(mm)					Inhibition (%)				
Group	Dose (mg/kg)	0min	1hr	2hr	3hr	4hr	0min	1hr	2hr	3hr	4hr
Group I	Vehicle	4.48±0.54	3.78±.06	3.58±0.29	3.38±0.21	3.32±0.36	-	-	-	-	-
Group II	10	2.42±0.4*	1.86±0.6*	1.44±0.3*	1±0.32*	0.98±0.29*	45.99	50.79	59.77	70.41	71.00
GroupIII	200	3.76±0.30	3.12±0.21*	2.78±0.28*	2.48±0.28*	2.34±0.38*	16.07	17.46	22.34	26.62	30.76
GroupIV	400	3.50±0.29	2.84±0.29*	2.58±0.28*	2.3±026*	2.2±0.26*	21.88	24.86	27.93	31.95	34.91
Group V	200	3.74±0.34	3.06±0.23*	2.62±0.33*	2.32±0.28*	2.18±0.33*	16.51	19.04	26.81	31.36	35.50
Group VI	400	3.48±0.29	3.66±0.33*	2.42±0.28*	2.14±0.33*	2.06±0.29*	22.32	29.62	32.40	36.68	39.05

Probability values (calculated as compared with control using one way ANOVA followed by Dunnet's test): * $p<0.05$. All values are mean (n=5), ± indicates standard error mean. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac sodium (10 mg/kg body) weight, Group III, IV, V VI, V and VI were treated with 200 and 400 mg/kg (p.o) methanol and ethyl acetate fractions of ***Stereospermum personatum*** leaves respectively.

CNS DEPRESSANT ACTIVITY

Hole-cross test

The Table 4 shows hole-cross test of ***Stereospermum personatum***. The both dose of methanol and ethyl acetate fractions were statistically significant (* $p<0.05$) at 30min to 120min and followed a dose-dependent response.

Table 4: Effect of methanol and ethyl acetate extract of the *Stereospermum personatum* leaves on hole cross test in mice.

Group	Dose(mg/kg)	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Group I	Vehicle	9.40.±1.06	8.20±0.92	7.20±0.92	5.80±0.92	4.80± 0.92
Group II	1 mg	6.60±1.068	5.40±1.068*	3.80±.915*	3.80±1.472*	3.00±1.257*
Group III	200mg	7.80±0.915	5.80±0.915*	4.80±0.915*	3.80±0.915*	2.80±0.915*
Group IV	400mg	6.20±1.107	4.60±0.740*	3.80±0.915*	2.60±0.740*	1.80±0.915*
Group V	200mg	7.80±0.915	5.80±0.915*	4.40±0.740*	3.60±0.946	2.80±0.915*
Group VI	400mg	6.00±1.107	4.40±0.740*	3.60±0.740*	2.60±0.946*	1.20±0.915*

Values are mean ± SEM=Standard error mean. (n = 5); * $p<0.05$, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III, IV, V and VI were treated with 200 and 400 mg/kg methanol and ethyl acetate fractions (p.o) of ***Stereospermum personatum*** leaves respectively.

Open-field test

The ***Stereospermum personatum*** fractions exhibited a decrease in the movements of the test animals at all dose levels. The results of Standard and extract (200 and 400 mg/kg) were statistically significant (*$p<0.05$) at 0 to 120min followed a dose-dependent response (Table 5).

Table 5. Effect of methanol and ethyl acetate extract of the *Stereospermum personatum* leaves on open field test in mice.

Group	Dose(mg/kg)	Number of movements				
		0 min	30 min	60 min	90 min	120min
Group I	Vehicle	280.00±6.28	227.40±4.74	175.00+4.54	153.80±3.99	122±3.60
Group II	1	89.00±1.84 *	87.40±1.347*	70.00±1.916*	65.00±1.778*	51+1.880*
Group III	200	233.60±3.68	182.00±5.18*	144.80±5.01*	136.00±5.65*	117.00±4.65*
Group IV	400	198.00±3.66	154.00±4.91*	127.40±4.41*	115.20±4.94*	86.00±3.29*
Group V	200	193.00±3.47	180.40±3.93*	162.00±3.01	131.80±2.723	105.20±3.11*
Group VI	400	176.20±2.60*	155.80±5.57*	142.40±3.42*	111.20±4.25*	80.40±3.94*

Values are mean ±Standard error mean (n = 5). * $p<0.05$, Dunnet test compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III , IV, V and VI were treated with 200 and 400 mg/kg methanol and ethyl acetate fractions of ***Stereospermum personatum*** leaves respectively.

DISCUSSION

Stereospermum personatum is highly regarded as a universal panacea in the herbal medicine with diverse spectrum of pharmacological activity. Acetic acid induces pain by enhancing levels of PGE2 and PGF2α[12] at the receptors of peritoneal cavity[13,14] which mean the acetic acid acts indirectly by increasing the release of endogenous mediators, leading to stimulation of the nociceptive neurons which are sensitive to most of the non-steroidal anti-inflammatory drugs. Though the MFSPL and EAFSPL (200 mg/kg body weight) showed mild percent of inhibition (40.1% and 38.68%) but 400mg/kg of both fractions have given same analgesic action and which was moderate action compared to standard (Diclofenac Na10 mg/kg body weight). This activity suggests that the taking part of peripheral mechanisms of analgesia.

The formalin test is another important model of analgesic which is better related to clinical pain.[15,16] This method elucidates central and peripheral activities. Formalin-induced nociception is biphasic in which first phase involves direct stimulation of sensory nerve fibers representing neuropathic pain and second phase involves inflammatory pain mediated by prostaglandin, serotonin, histamine, bradikinin and cytokines such as IL-1β, IL-6, TNF-α, eicosanoids and NO.[17,18,19,20,21] At early phase MFSPL and EAFSPL (400 mg/kg) have shown same significiant (*p<0.05) effect compared to standard but at late phase only EAFSPL (400mg/kg) showed 58.21% percent of protection which is same as standard, Diclofenac Sodium (59.70%, Table. 2). The suppression of neurogenic and inflammatory pains of the extracts indicates it contains active analgesic principles of both centrally and peripherally acting.

Carageenan –induced paw edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic in which the early phase (1-2) of the carageenan model is mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings and the late phase is sustained by prostaglandin release mediated by bradikinin, leukotrienenes, polymorphonuclear cells, and prostaglandins produced by tissue macrophase.[22,23] However, in this study the crude extract (methanol and ethyl acetate) of *Stereospermum personatum* leaves (200 and 400 mg/kg) exhibited significiant (*P<0.05) inhibition of paw edema at 1h to 4th hours (Table 3) compared to standard (Diclofenac sodium). The MFSPL and EAFSPL (400mg/kg) result is higher than (200mg/kg) but ethyl acetate action was little bit higher than methanol. The possible mechanism of the observed anti-inflammatory activity might be its ability to reduce the release of histamine, serotonin or kinin like substances or biosynthesis of prostaglandins which is consistent with the test of analgesic activity.

Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect.[24] Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through GABA, therefore it is possible that extracts of *Stereospermum personatum* leaves may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts.[25] Many research presented that plant containing flavonoids, saponins and tannins are useful in many CNS disorders.[26] Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system; which led to assume that they can act as benzodiazepine like molecules.[27] In the hole cross test, the both fractions (200mg/kg and 400mg/kg) has significant (*P<0.05, Table 4) depressant activity. On the other hand in case of open field test, the MFSPL (200mg/kg and 400mg/kg) has depressant action at 30 to 120min but only 400mg/kg of EAFSPL has shown significant (*P<0.05) depressant effect at 0 min to 120 min (Table 5.) according to dose dependant manner. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system; which led to assume that they can act as benzodiazepine like molecules.

CONCLUSION

From our research we observed that the effect of MFSPL and EAFSPL (400mg/kg) were almost similar but only 400mg/kg dose has shown moderate analgesic, significant CNS depressant action and mild anti-inflammatory activity compared to control and standard respectively. The present work was a preliminary effort which will require further detailed investigation, including characterization of active compounds and requires preformulation studies for development of a potential dosage form

ACKNOWLEDGEMENT

Authors thanks Md. Akherul Islam, Mohammad Amzad Hossain, Dr. Monirul Islam, Asma Kabir and Mohammad Sirajum Munir for their kind co-operation in this research work and the Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh for providing laboratory facilities to carry out the entire experiment.

REFERENCES

1. Akah PA and Nwambie AI. Evaluation of Nigerian traditional medicines Plants used for rheumatic (inflammatory) disorders. J Ethnopharmacol, 1994; 42: 179-82.

2. http://www.mpbd.info/, MEDICINAL PLANTS OF BANGLADESH.
3. Ambasta SP. The useful plants of India. Publication and Information Directorate, Council for Scientific and Industrial Research, New Delhi, 1986; 602.
4. Binutu OA, Adesogan KE, Okogul JI. The natural napthoquinone sterekunthal-B. Planta Med, 1996; 62: 352–353.
5. Florida M, and Sekar T, Phytochemical investigation of tropical medicinal plants -Stereospermumcolais L. And Barringtoniaacutangula L. Journal of research in plant sciences, 2012; 1(2): 109-115.
6. Kumar US, Tiwari AK, Reddy SV, Aparna P, Rao RJ, Ali AZ, Rao JM. Free radical scavenging and xanthine oxidase inhibitory constituents from Stereospermum personatum. J Nat Prod, 2005; 68: 1615–1621.
7. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 1983; 16: 109.
8. Sharma A, S. Bhatial MD. Kharyaz V, Gajbhiye, Ganesh N, Namdeo AG and Mahadik KR. Antiinflammatory and analgesic activity of different fractions of *Boswellia serrata*. Int. J. Phytomed, 2010; 2: 94-99.
9. Winter CA, Risley EA and Nuss GW. Carrageenan induced oedema in hind paws of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med, 1962; 111: 544-557.
10. Takagi K, Watanabe M, Saito H. Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethane, its acylesters on the centralnervous system. Jpn J Pharmacol, 1971; 21: 797-810.
11. Gupta BD, Dandiya PC, Gupta ML. A psychopharmacological analysis of behavior inrat. Japan J of Pharmacol, 1971; 21: 293.
12. Delevalcee F, Falhout M. Release of prostaglandin E and F in an algogenic reactionand its inhibition. Eur J pharmacol, 1980; 61: 17-24.
13. Bently GA, Newton SH, Star J. Studies on the antinociceptive action of alpha agonistdrugs and their interaction with opioid mechanisms. Br J Pharmacol, 1983; 79: 125-134.
14. Lee K, Choi E. Analgesic and anti-inflammatory effects of *Ligulariafischeri*leaves in experimental animals. J Ethnopharmacol, 2008; 12: 103-107.
15. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain, 1992; 51(1): 5-17.
16. Ghanadi A, Hajhasemi V, Jafarabadi H. An investigation of analgesic and anti-inflammatory effects of Nigella sativa seed polyphenols. J of medicinal food, 2005; 8: 488-493.
17. Murrey CW, Porreca F, Cowan A. Methodological refinements to the mouse paw formalin test: an animal model of tonic pain. J Pharmacol Toxicol Methods, 1988; 20: 175-186.
18. Watkins LR, Martin D, Ulrich P, Tracey KJ, Maier SF. Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the raty. Pain, 1997; 71: 225-235.
19. Chichorro JG, Lorenzetti BB, Zampronio AR. Involvement of bradykinin, cytokines, sympathetics amines and prostaglandins in formalin induced orofacial nociception inrats. BR J pharmacol, 2004; 14: 84-117.
20. Hunskar S, Hole K. The formalin test in mice: dissociation between inflammatory andnon inflammatory Pain. Pain, 1987; 30: 103-114.
21. Moore PK, Oluyomi AO, Baddedge, Wallace P, Hart SL. L-NG-nitro arginine methylester exhibits antinociceptive activity in the mouse. Br J Pharmacol, 1991; 102: 198-202.
22. Gupta M, Mazumder UK, Gomathi P. Selvan. Anti-inflammatory evaluation of leaves of Plumer acuminate. BMC complementary and alternative Medi, 2006; 6: 36.
23. Gupta S, Ali M, Pillai KK, Alam MS. Evaluation of anti-inflammatory activity of some constituents of *Lawsonia inermis*. Fitoterapia, 1993; 64: 365-366.
24. Kolawole OT, Makinde J, Mand OA, Olajide OA. Central nervous depressant activity of Russelia equisetiformis. Niger J Physiol Sci, 2007; 22: 59-63.
25. Bhattacharya SK, Satyan KS. Experimental methods for evaluation of psychotropicagentsinrodents: I-Anti-anxiety agents. Indian J Exp Biol, 1997; 35: 565-575.
26. Verma A, Jana GK, Sen S, Chakraborty R, Sachan S, Mishra A. Pharmacological evaluation of<I>Saracaindica</I>Leaves for central nervous system depressant activity in mice. J Pharm Sci Res, 2010; 2(6): 338-343.
27. Ghanshyam Y, Garg VK, Thakur N and Khare P. Locomotor Activity of Methanolic Extract of Saracaindica Bark. Adv Bio Res, 2013; 7(1): 01-03.