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ORIGINAL ARTICLE

Analgesic, Anti-Inflammatory, and Central Nervous System Depressant Activities of *Monochoria hastate* (L.) Solms. in Animal Models

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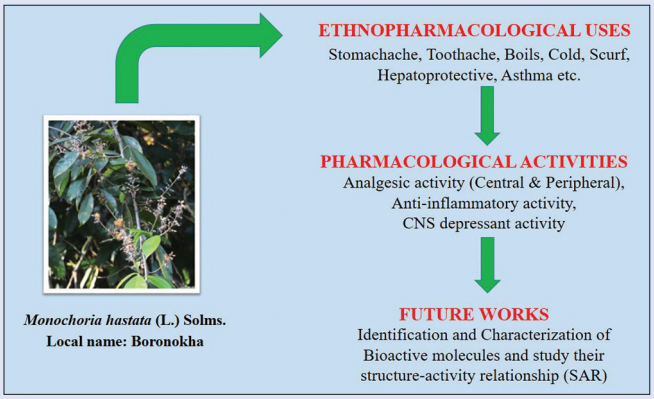
ABSTRACT

Background: *Monochoria hastate* (L.) Solms. is extensively used as traditional medicine despite the fact that no scientific data has yet been published revealing its biological activity *in vivo*. **Objectives:** The objective of the study was to investigate the pharmacological effect of crude leaf extracts of *M. hastate* in mice model, justifying its medicinal use and evaluating its safety and efficacy as a crude drug. **Materials and Methods:** Crude extracts were prepared using methanol and ethyl acetate as solvent. Analgesic activity was evaluated by writhing and formalin-induced licking bioassay. Anti-inflammatory activity was examined by detecting its effect on carrageenan-induced edema. Effect on the central nervous system (CNS) was tested by monitoring movements of test animals by open-field and hole-cross assay. **Results:** Oral administration (400 mg/kg) of methanol and ethyl acetate extract resulted in 93% and 92% reduction of writhing ($P < 0.001$) compared to the 87% by diclofenac, whereas ethyl acetate extract caused maximum inhibition (91%) of licking response. Both the extracts manifested marked ($P < 0.05$) reduction in edema diameter, but the effects were less significant than the standard. Open-field and hole-cross test demonstrated significant ($P < 0.001$) suppression of motor activity in the treated group. The resulting movements 120 min after oral administration of methanol and ethyl acetate extract (400 mg/kg) and diazepam (1 mg/kg) were 205.8 ± 33, 1.4 ± 0.5, 217 ± 28, 1.0 ± 0.3, 191.2 ± 25, and 2.0 ± 0.7, respectively. **Conclusion:** These findings provide substantial evidence that leaf extracts of *M. hastate* possess analgesic, anti-inflammatory, and CNS depressant activity in mice explaining some of its use in traditional medicine.

Key words: Analgesic, anti-inflammatory, central nervous system, depressant, *in vivo*, *Monochoria hastate*

SUMMARY

- *Monochoria hastate* has been a popular medicinal plant used by local folk practitioners to treat stomachache, toothache, cold, scurf, and asthma, and no *in vivo* study has been conducted to assess its biological activity. This study reveals that crude methanol and ethyl acetate extracts from the leaf of *M. hastate* possess significant analgesic (central and peripheral), anti-inflammatory activity possibly mediated through the inhibition of prostaglandin synthesis (similar to diclofenac). The extracts also showed significant central nervous system depressant activity comparable to the effects of diazepam likely to be mediated through GABAergic pathway.



Abbreviations used: MEMH: Methanol extract of *Monochoria hastate* leaves; EAMH: Ethyl acetate extract of *Monochoria hastate* leaves; CNS: Central nervous system; SEM: Standard error of mean; Std: Standard.

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INTRODUCTION

Monochoria hastate (L.) Solms. (Family Pontederiaceae), locally known as Boronokha, is an aquatic plant widely distributed in Bangladesh and other regions of India, Australia, and Southeast Asia.[1] The plant usually grows in the shallow water bodies such as swamps, ponds, and paddy fields and has been listed as a federal noxious weed.[2] Regional folk medicine in Southeast Asia employs various parts of the plant abundantly for treating cases of diarrhea and dysentery and also as tonic during cold. Rhizomes made into powder and mixed with charcoal have been mentioned to be used against scurf. Leaves are applied over matured boils after they have burst and its juice to alleviate cough and asthmatic conditions. Juice from its root is used to treat stomachache and toothache and also has been reported to possess hepatoprotective and anti-lipoxygenase activity. The extract from the aerial parts has exhibited antibacterial property against various gastrointestinal and topical pathogens.[2-5] A preliminary phytochemical screening has

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revealed the presence of glycosides, alkaloids, flavonoids, and tannin, while an *in vitro* study disclosing its notable antioxidant activity and cytotoxicity brine shrimp.[6,7] Despite its numerous applications as a medicinal plant and marked *in vitro* biological properties, there has not yet been any scientific investigation conducted on the plant determining its pharmacological property in the animal model, hence justifying and providing scientific basis supporting its traditional use. Therefore, the aim of this investigation was to conduct various *in vivo* biological investigations using the crude methanol and ethyl acetate extracts of *M. hastate* to identify and evaluate its pharmacological property as well as verifying its safety and efficacy as a folk medicine.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *M. hastate* were collected from the Barisal district (Rupatoli village), Bangladesh, and the species was taxonomically confirmed by a principal scientific officer from the Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen (accession number: DACB-42529) was deposited in the library for preservation and future referencing purposes.

Preparation of crude extract

The leaves were dried using indirect sunlight for a week and pulverized into coarse powder before soaking it with methanol and ethyl acetate separately in two amber glass bottles for 2 consecutive weeks. The whole mixtures were filtered first using a cotton plug and followed by Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The retained solvents were gradually evaporated off via a vacuum water bath, finally leaving a gummy and dark concentrate which was used as the two crude extracts.[8]

Animal experiments

Swiss albino mice (male), weighing 30–40 g, were obtained from the animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. The mice were kept under ambient temperature with light (12 h) and followed by a dark (12 h) cycle. All experimentations associated with living subjects were performed according to the institutional guidelines for animal experimentation of the Department of Pharmacy, State University of Bangladesh. The Federation of European Laboratory Animal Science Associations guidelines and recommendations were also followed to reduce the pain and stress of the experimental animal. In case of *in vivo* bioassays, the mice were divided into six groups (Group I to VI) with five individuals in each group. Group I and II served as the control and positive control, respectively, whereas Group III, IV, V, and VI received crude methanol and ethyl acetate extract orally at 200 and 400 mg/kg body weight, respectively.[8]

Data analysis

All the results were recorded in triplicates and expressed as mean ± standard error of mean. During statistical analysis, one-way ANOVA and *post hoc* Dunnett's tests were performed using SPSS version 25.0 where $P < 0.05$ between groups was considered to be statistically significant.

Evaluation of analgesic activity: Acetic acid induced writhing assay

The acetic acid-induced "writhing" assay performed by Winter *et al.* 1962 was followed to evaluate analgesic activity.[9] Six groups of mice ($n = 5$) received an oral dose of 1% TWEEN® 80 solution, diclofenac (10 mg/kg. bd.wt.), Methanol extract of *Monochoria hastate* leaves (MEMH), and Ethyl acetate extract of M*onochoria hastate* leaves (EAMH) at doses 200 and 400 mg/kg body weight, respectively. Thirty minutes later, acetic acid was injected intraperitoneally to every individual mouse to induce the "writhing" response. After a delay of 5 min, the mice were observed for the next 25 min and the total writhing responses were counted. In case, where the writhing was not fully complete, two half writhing movements were considered as one.

The percentage inhibition of writhing (%) was calculated using the formula:

$= ([A–B]/A) \times 100$

Where,

A = Average number of writhing of the control group

B = Average number of writhing of the test group.

Evaluation of analgesic activity: Formalin induced paw licking and biting test

The antinociceptive activity was determined using the formalin-induced "paw licking" test.[10] Group I and II received 1% TWEEN® 80 and ibuprofen (10 mg/kg) orally. The other four groups received the MEMH and EAMH at 200 and 400 mg/kg, (p.o.). Thirty minutes after the oral administration of the sample extract and standard, 20 µL of 2.5% formalin was injected into the dorsal surface of the right hind paw of each individual mice in all the groups. The mice were observed for the next 30 min after the injection of formalin, and the time spent in licking and biting (pain-induced behavior) of the injected hind paw was recorded. The first 5 min post formalin injection was referred to as the early phase and the period between 15 and 30 min as the late phase.[11]

Evaluation of anti-inflammatory activity: Carrageenan-induced paw edema method

Edema in the hind paw of the test animals was induced by 0.1 mL subcutaneous injection of 1% carrageenan; 30 min prior, the animals were treated with the sample extract and standard. The volume of the paw was recorded (diameter of edema) using a Vernier caliper at 1 h interval for the next 3 h after the oral administration.[12]

Evaluation of central nervous system depressant activity: Open-field test

After treating the control, positive control, and the four test groups with 1% TWEEN® 80, standard diazepam (1 mg/kg), and MEMH and EAMH 200 and 400 mg/kg, respectively, every mice from all the groups were placed in a special wooden box (60 cm × 60 cm × 30 cm) whose floor was marked and divided into 16 whole squares with dimension 15 cm × 15 cm. The number of boxes covered by each mouse was counted for a period of 5 min at 0, 30, 60, 90, and 120 min after injecting diazepam.[13]

Evaluation of central nervous system depressant activity: Hole-cross test

In this bioassay after administering the animal groups with the saline solution, standard, and sample extracts, sedation was induced by injecting diazepam (1 mg/kg). Each individual mouse was then placed in a wooden box (30 cm × 20 cm × 14 cm) containing a partition in the middle, in which a 3 cm hole has been made so that the mouse can pass through the gap and cover either side of the box. The number of times each mouse passed through the hole for a period of 5 min was recorded for the next 2 h at 30 min interval after the diazepam injection.[14]

RESULTS

Evaluation of analgesic activity: Acetic acid-induced writhing assay

The MEMH and EAMH resulted in significant ($P < 0.001$) inhibition of writhing movement in a dose-dependent manner when compared to the control group. The MEMH and EAMH at dose 400 mg/kg caused 92.98% and 91.57% inhibition of writhing compared to the 87.36% caused by the standard diclofenac sodium [Figure 1].

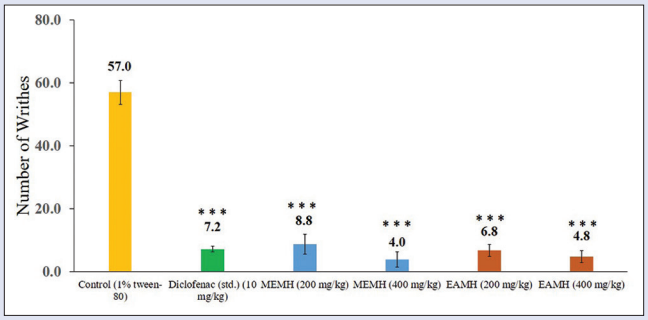

Figure 1: Evaluation of analgesic activity of methanol and ethyl acetate extract of *Monochoria hastate* in acetic acid-induced writhing in mice. Values are means ± SEM of five mice ($n = 5$). Here, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ when compared with control group (Dunett's test). SEM: Standard error of mean; MEMH: Methanol extract of *Monochoria hastate* leaves; EAMH: Ethyl acetate extract of *Monochoria hastate* leaves; *n*: Sample size; Std: Standard

Evaluation of analgesic activity: Formalin-induced paw licking and biting test

Significant ($P < 0.001$) inhibition of licking response was recorded in groups treated with the crude extracts in both the early and late phases of the experiment. Both the methanol and ethyl acetate extracts at 400 mg/kg (p.o.) reduced the formalin-induced licking response by 63.76% and 75.84%, respectively, in the early phase of the experiment, whereas the standard diclofenac sodium caused 70.53% reduction. The percentage of inhibition increased to 83.18% and 91.15% in the late phase compared to the 83.19% of diclofenac [Figure 2].

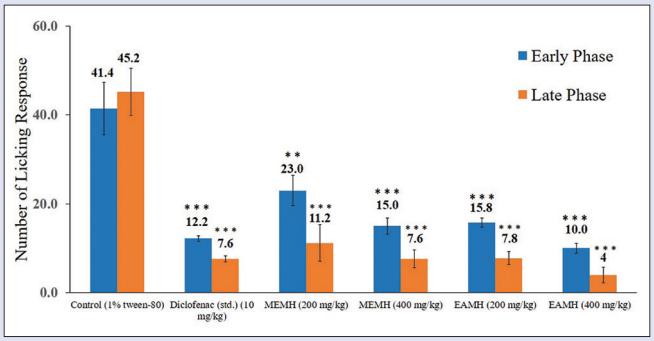

Figure 2: Evaluation of analgesic activity of methanol and ethyl acetate extract of *Monochoria hastate* in formalin-induced licking test in mice. Values are means ± SEM of five mice ($n = 5$). Here, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ when compared with control group (Dunett's test). SEM: Standard error of mean; MEMH: Methanol extract of *Monochoria hastate* leaves; EAMH: Ethyl acetate extract of *Monochoria hastate* leaves; *n*: Sample size; Std: Standard

Evaluation of anti-inflammatory activity: Carrageenan-induced paw edema method

The 400 mg/kg oral dose of MEMH and EAMH significantly ($P < 0.05$) lowered the diameter of carrageenan-induced edema by 0.92 ± 0.9 and 0.72 ± 0.1 mm, respectively, 3 h after the oral intake. However, both these values were lower compared to the standard diclofenac (10 mg/kg) which reduced the edema diameter by 1.42 ± 0.8 mg ($P < 0.001$) in the same time period of 3 h [Figure 3].

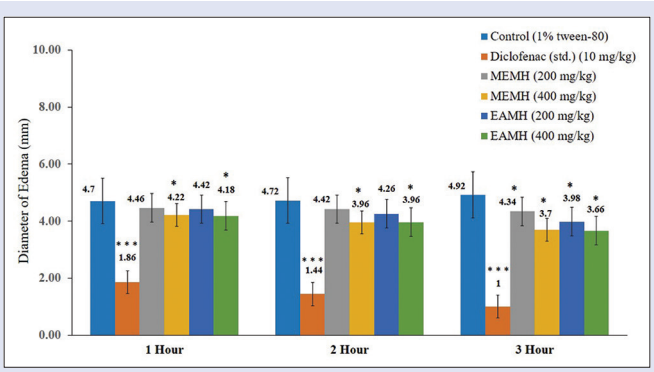

Figure 3: Evaluation of anti-inflammatory activity of methanol and ethyl acetate extract of *Monochoria hastate* in carrageenan-induced paw edema test in mice. Values are means ± SEM of five mice ($n = 5$). Here, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ when compared with control group (Dunett's test). SEM: Standard error of mean; MEMH: Methanol extract of *Monochoria hastate* leaves; EAMH: Ethyl acetate extract of *Monochoria hastate* leaves; *n*: Sample size; Std: Standard

Evaluation of central nervous system depressant activity: Open-field test

Both the crude extracts at two different doses (200 and 400 mg/kg) caused significant ($P < 0.001$) reduction of movement in the test animals in the open-field assay comparable to the sedative effect of diazepam. The untreated control group receiving oral saline only showed the considerable movement of 242.2 ± 23.4. However, intraperitoneal injection of diazepam at 1 mg/kg body weight reduced the number of movement to 51.0 ± 1.6, while the oral administration of MEMH and EAMH lowered the movement of the test animals by 36.4 ± 9.4 and 25.2 ± 4.6, respectively. The maximum sedative effect among the mice was noted 30 min after the groups (except the group received MEMH) were treated with diazepam and crude extracts, and the effect lasted till 120 min after the oral administration of crude extracts [Table 1].

Evaluation of central nervous system depressant activity: Hole cross test

Significant inhibition of movement ($P < 0.001$) was recorded in the groups receiving the standard diazepam and the crude extracts at two different doses. The MEMH and EAMH at 400 mg/kg reduced the movement of mice from 7.8 ± 0.6 to 1.4 ± 0.5 and 7.2 ± 1.2 to 1.0 ± 0.3, respectively, whereas the maximum effect was observed 30 min after the oral administration of the crude extracts. The standard diclofenac however caused maximum inhibition starting after 60 min of its administration, reducing the movement by 6.6 ± 0.5 to 2.0 ± 0.7 and the effect lasting till 120 min after the oral treatment in the positive control and the test groups [Table 2].

Table 1: Effect of oral administration of MESF and EASF of *Monochoria hastate* on the central nervous system of mice in open-field test

Test groups	*n*	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Control (1% TWEEN® 80 in water)	5	224.4±32.3	232.2±31.3	235.4±57.1	230.6±28.24	242.2±23.4
Diazepam (standard) (1 mg/kg)	5	89.0±1.5**	87.4±0.8***	70.0±1.6***	65.0±1.41***	51.0±1.6***
MEMH (200 mg/kg)	5	176.0±19.1	145.6±7.9*	106.4±12.7***	73.0±9.2***	59.8±8.4***
MEMH (400 mg/kg)	5	143.0±15.9	122.0±15.7**	93.6±14.0***	62.2±13.3***	36.4±9.4***
EAMH (200 mg/kg)	5	185.4±20.6	164.2±15.7*	131.8±21.8**	97.0±17.0***	86.0±24.4***
EAMH (400 mg/kg)	5	166.0±23.1	80.2±11.3***	61.0±24.3***	40.0±7.6***	25.2±4.6***

Values are expressed as mean±SEM of five mice (n=5). *P<0.05, **P<0.01 and ***P<0.001 when compared with control group (Dunett's test). SEM: Standard error of mean; MEMH: Methanol extract of *Monochoria hastate* leaves; EAMH: Ethyl acetate extract of *Monochoria hastate* leaves; *n*: Sample size

Table 2: Effect of oral administration of MESF and EASF of *Monochoria hastate* on the central nervous system of mice in hole-cross test

Test groups	*n*	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Control (1% TWEEN® 80 in water)	5	15.2±2.2	15.2±2.3	16±1.7	14.8±1.4	14.2±1.5
Diazepam (standard) (1 mg/kg)	5	6.6±0.5**	5.4±0.5**	3.8±0.4***	3.2±1.0***	2.0±0.7***
MEMH (200 mg/kg)	5	6.6±2.2**	4.8±1.3***	3.4±1.4***	3.0±1.3***	1.6±0.4***
MEMH (400 mg/kg)	5	7.8±0.6*	5.2±1.1***	3.0±0.8***	2.4±0.7***	1.4±0.5***
EAMH (200 mg/kg)	5	8.8±2.0*	4.4±0.9***	3.2±0.6***	1.6±0.4***	1.2±0.4***
EAMH (400 mg/kg)	5	7.2±1.2**	3.6±0.6***	2.4±1.0***	1.2±0.4***	1.0±0.3***

Values are expressed as mean±SEM of five mice (n=5). *P<0.05, **P<0.01 and ***P<0.001 when compared with control group (Dunett's test). SEM: Standard error of mean; MEMH: Methanol extract of *Monochoria hastate* leaves; EAMH: Ethyl acetate extract of *Monochoria hastate* leaves; *n*: Sample size

DISCUSSION

The writhing response initiated by the acetic acid indicates that it may cause the release of inflammatory mediators such as prostaglandins and prostacyclines which are responsible for the sensitization of the nerve fibers resulting in pain sensations.[15] Phytochemicals such as flavonoids, steroids, and triterpenes possess analgesic and anti-nociceptive properties. These classes of compound have been reported to be present abundantly in *M. hastate* which could be responsible for the analgesic activity of *M. hastate* leaf extracts.[16] The formalin-induced licking and biting response can be subdivided into the early neurogenic and the late inflammatory pain phases.[17] Formalin generates the licking response by stimulating the primary afferent neurons by increasing the influx of calcium ion through TRPA1 cation channels.[18] Both the MEMH and EAMH demonstrated significant ($P < 0.001$) dose-dependent reduction of the licking response prominently in the late phase, indicating the presence of phytochemicals capable of inducing analgesic activity both centrally and peripherally by preventing the increase of intracellular influx of calcium ions inside the neurons. These results could explain its use in various illnesses such as stomachache and toothache where it can aid in pain suppression. Carrageenan triggers an inflammatory response by increasing the production of pro-inflammatory mediators such as serotonin, histamine, prostaglandins, tumor necrosis factor-alpha, leukotrienes, and bradykinins, followed by further stimulating their subsequent release.[19,20] Most of these mediators are produced by the cyclooxygenase enzymes using the arachidonic acid released from the ruptured cell membranes. Reduction of tissue edema by the crude extracts of *M. hastate* could be an indication that the extracts contain chemicals capable of inhibiting the cyclooxygenase enzymes, thus preventing the production of major inflammatory mediators. Decreased motor activity is caused by the sedative and anxiolytic activity of various agents such as barbiturates and benzodiazepines. Most of these agents exhibit their activity by binding with the GABA ion channels facilitating the influx of chloride ion and depressing the generation of the action potential in the neurons. Both the crude extracts of *M. hastate* showed significant ($P < 0.001$) sedative effect in mice, comparable to the effect of diazepam, thereby reducing the movement of test animals in the open-field and hole-cross tests. This central nervous system (CNS) depressant activity could be produced by the GABAergic inhibition of CNS causing fewer generation and propagation of action potential in the neurons. This sedative property *M. hastate* could attenuate sensation of pain (central and peripheral) making it popular as a folk medicine in treating various acute and chronic diseases.

CONCLUSION

Our current study for the first time provides comprehensive evidence that the crude methanol and ethyl acetate extracts from the leaves of *M. hastate* possess notable central and peripheral analgesic, anti-inflammatory, and CNS depressant activity in Swiss albino mice. These pharmacological properties could be the reason behind its popularity as a folk medicine. These results also indicate that *M. hastate* could be potential source of new chemical agents with promising biological activities. Isolation, characterization, and future studies with these active agents could aid in the discovery of new drug entities with better activity and lesser side effects.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Vagelos PR. Are prescription drug prices high? Science 1991;252:1080-4.
2. Rahman MH. Evaluation of phytochemical screening and cytotoxic activity of different extracts of *Monochoria hastata* leaves 2018.
3. Das NJ, Saikia SP, Sarkar S, Devi K. Medicinal plants of North-Kamrup district of Assam used in primary healthcare system. Indian J Tradit Knowl 2006;5:489-93.

4. Nair DD, Gupta AK, Das HB, Chakraborti A. Medicinal Plants of Tripura: A Photo Descriptive Field Manual of 203 Medicinal Species. 2009.
5. Misra D, Mandal M, Ghosh NN, Mandal V. Pharmacognostic standardization of an ethnomedicinal aquatic herb, *Monochoria hastata* (l.) solms for its antibacterial potentiality. Pharmacogn J 2018;10:533-40.
6. Bai N, Ilango K. High performance thin layer chromatography-automated multiple development bioautography of phytoconstituents and quantification of stigmasterol in *Monochoria vaginalis* and *Monochoria hastata* with antioxidant potential. Pharmacogn Mag 2018;14:S45-51.
7. Anh N, Yen T, Hang N, DA-VJ of, 2019 U. Lignan compounds from *Stixis suaveolens*. Wiley Online Libr 2019;57:304-10.
8. Nesa ML, Karim SM, Api K, Sarker MM, Islam MM, Kabir A, *et al.* Screening of *Baccaurea ramiflora* (Lour.) extracts for cytotoxic, analgesic, anti-inflammatory, neuropharmacological and antidiarrheal activities. BMC Complement Altern Med 2018;18:35.
9. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med 1962;111544-7.
10. Loganga OA, Vercruysse AF. Contribution to the ethanobotanical, Phytochemical and pharmacology studies of traditionally used medicinal plant in the treatment of dysentery and diarrhoeal in Lomela area, Democratic Republic of Congo (DRC). J Ethnopharmacol 2000;71:423.
11. Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, *et al.* Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia 2008;79:557-61.
12. Koo HJ, Lim KH, Jung HJ, Park EH. Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. J Ethnopharmacol 2006;103:496-500.
13. Nyeem MA, Alam MA, Awal MA, Mostofa M, Uddin SJ, Islam N, *et al.* CNS depressant effect of the crude ethanolic extract of the flowering tops of *Rosa damascena*. Iran J Pharmacol Ther 2006;5:171-4.
14. Vhuiyan MM, Biva IJ, Saha MR, Islam MS. Anti-diarrhoeal and CNS depressant activity of methanolic extract of *Saccharum spontaneum* Linn. Stamford J Pharm Sci 1970;1:63-8.
15. Lourens AC, Reddy D, Başer KH, Viljoen AM, Van Vuuren SF. *In vitro* biological activity and essential oil composition of four indigenous South African Helichrysum species. J Ethnopharmacol 2004;95:253-8.
16. Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH, *et al.* Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol 2000;387:111-8.
17. Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory plant flavonoids and cellular action mechanisms. J Pharmacol Sci 2004;96:229-45.
18. Voilley N, de Weille J, Mamet J, Lazdunski M. Nonsteroid anti-inflammatory drugs inhibit both the activity and the inflammation-induced expression of acid-sensing ion channels in nociceptors. J Neurosci 2001;21:8026-33.
19. Srinivasan K, Muruganandan S, Lal J, Chandra S, Tandan SK, Raviprakash V, *et al.* Antinociceptive and antipyretic activities of *Pongamia pinnata* leaves. Phytother Res 2003;17:259-64.
20. Yaksh TL. Spinal systems and pain processing: Development of novel analgesic drugs with mechanistically defined models. Trends Pharmacol Sci 1999;20:329-37.