Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8196
JPP 2015; 3(5): 121-125
Received: 29-10-2014
Accepted: 20-11-2014

Farhana Alam Ripa
Lecturer, Department of Pharmacy, BRAC University, 41, Pacific Tower, Mohakhali, Dhaka-1212, Bangladesh.

Pritesh Ranjan Dash
Teaching Assistant, Department of Pharmacy, BRAC University, 41, Pacific Tower, Mohakhali, Dhaka-1212, Bangladesh.

Md. Omar Faruk
Monitoring and Evaluation Officer, Safe Crop Production Project through IPM Approach, Department of Agricultural Extension, Khamarbari, Dhaka-1215.

Correspondence:
Farhana Alam Ripa
Lecturer, Department of Pharmacy, BRAC University, 41, Pacific Tower, Mohakhali, Dhaka-1212, Bangladesh.

CNS depressant, analgesic and anti-inflammatory activities of methanolic seed extract of *Calamus rotang* Linn. *fruits* in rat

Farhana Alam Ripa, Pritesh Ranjan Dash and Md. Omar Faruk

Abstract
This study was aimed to investigate CNS depressant, analgesic and anti-inflammatory activities of methanolic seed extract of *Calamus rotang* (MCRS) in rats. Hole cross and open field tests for CNS depressant effect, acetic acid-induced writhing and formalin induced pain methods for analgesic and carrageenan-induced paw edema was performed to evaluate anti-inflammatory effect of the doses of 250 and 500 mg/kg b.w. respectively. In hole cross and open field tests, maximum suppression of locomotor activity were 81.42% and 86.61% with the higher dose (500 mg/kg) of extract, whereas for the standard drug diazepam (1 mg/kg) inhibition rate were 87.14% and 91.86 % respectively. MCRS at the dose of 500 mg/kg exhibited maximum 51.27% inhibition against acetic acid-induced pain while indomethacin (10 mg/kg) displayed 58.86% inhibition. In formalin-induced test, 68.47% inhibition was produced by MCRS (500 mg/kg) and indomethacin produced 70.72% inhibition. In anti-inflammatory activity, the extract showed a significant ($p<0.01$) inhibition of paw edema after 30 min to 240 min compare to indomethacin. MCRS significantly ($P<0.01$) showed the above mentioned pharmacological effects in a dose dependent manner.

Keywords: *Calamus rotang*, CNS depressant, analgesic, anti-inflammatory activity.

1. Introduction
Utilization of medicinal herbs is terrifically mounting over a past decade as an option to develop the excellence of life and sustain an excellent health. Medicinal plants have been consumed for centuries as medications for human diseases [1, 2]. In recent times, there has been budding attention in exploiting the biological activities of flora and fauna due to their natural source, cost effectiveness and minor side effects [3, 4]. Herb-based natural ingredients can be obtained from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [5]. Since medicinal plants are believed to be an important resource of novel chemical substances with potential remedial effects [6] they are used in Bangladesh for traditional medical practice for the treatment of various diseases [7]. In fact, for centuries in many cultures all over the world plants are used in different diseases and should be considered as new sources of analgesic, CNS depressant, anti-inflammatory therapeutic agents. *Calamus rotang* Linn. a common growing shrub in Bangladesh. *Calamus rotang* (rattan palm or climbing palm) is a plant belonging to the family Arecaceae. It is a native plant of south-west Asia. The basal part of the plant grows vertically for ten meters and horizontally for about two hundred meters or more. Fruits can be consumed fresh or prepared into pickles and eaten with food. Its tender shoots are utilized as antihelminthic by tribal people [8]. Its leaf sap is used for eye problem [9]. The presence of a saponin in the stem, an alkaloid in the leaves and a flavonoid in the root of *C. rotang* is used in convulsions and cramps [10]. This previous ideas has inspired us to find CNS depressant, Analgesic and Anti-inflammatory effects of methanolic extracts of seeds of *C. rotang* in rat.

2. Materials and methods
2.1. Collection and identification of plant
In this investigation, the fresh fruits of *C. rotang* were collected from, Chittagong, Bangladesh in July, 2012. The fresh leaves and rattan fruits were identified from The National Herbarium of Bangladesh, whose voucher specimen no. is 36704 and is maintained in our laboratory for future reference. From the collected fruits seeds were separated and dried for one week and pulverized into a coarse powder with a suitable grinder. The powder was stored in an airtight container, and was kept in a cool, dark and dry place for analysis.

2.2. Preparation of extract
Seeds from Rattan fruits were collected, sun dried for seven days and ground. 500 gm dried powder was soaked in 500 ml of 95% methanol for 7 days in cold condition with occasional shaking and stirring. The whole mixture was successively filtered through a piece of clean, white cotton material and No. 1 Whatman filter paper. The methanolic portion of the seed delivered a reddish brown gummy precipitate which was designated as MCRS. The extract was transferred to a closed container for further use and fortification.

2.3. Chemicals and drugs
Acetic acid (Merck, Germany), Carrageenan (Sigma chemicals, USA), Tween-80, castor oil (BDH Chemicals, UK), formalin (CDH, India), normal saline solution (Beximco Infusion Ltd., Bangladesh), Indomethacin and Diazepam (Square Pharmaceuticals Ltd., Bangladesh), were procured and used in the experiment. All chemicals in this investigation were of analytical reagent grade.

2.4. Phytochemical analysis
The MCRS extract was subjected to qualitative chemical screening for the identification of bioactive constituents (tannins, alkaloids, flavonoids saponin etc.) using standard procedures [11].

2.5. Animals
Young Long-Evans rats of either sex weighing about 80-120 gm were used to conduct the research. The rats were procured from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRB). They were kept in standard environmental condition (at 24.0±0 °C temperature & 55-65% relative humidity and 12 hours light/dark cycle) for two weeks for acclimation and fed ICDDRB formulated rodent food and tap water ad libitum. All animals were fasted over night before tests while providing tap water ad libitum.

2.6. Ethical approval
The guidelines followed for animal experiment were accepted by the institutional animal ethical committee [12].

2.7. Oral toxicity studies
An acute oral toxicity study was followed according to "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of methanol extract. Long Evan rats (N=6, 150-200 g) overnight fasted for 18 were used for the study. Different doses of plant extracts up to 1600 mg/kg, p.o. was administered and animals were observed for the first 3 hours of administration and mortality recorded within 48 hours.

2.8. CNS depressant activity
2.8.1. Hole cross test
The most reliable behavioral change is a hyperemotional response to novel environmental. The method was adopted as described by Takagi *et al*. [13]. The aim of this study was to characterize the emotional behavior of rodents using the hole-board test. The number of passage of a rat through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of standard Diazepam (1 mg/kg) and MCRS extract at doses of 250 and 500 mg/kg body weight.

2.8.2. Open field test
This experiment also evaluates a range of anxiety-induced, locomotor activity and exploratory actions of rodents. The animals were treated as the previous stated manner and at same doses. The test was performed according to the technique described by Gupta *et al*. [14]. The number of squares visited by the animals was calculated for 3 min, at 0, 30, 60, 90 and 120 min subsequent to oral administration of the experimental crude extracts.

2.9. Analgesic activity
2.9.1. Acetic acid induced writhing method
In this method [15] acetic acid 0.7% v/v acetic acid solution is given intraperitoneally to generate pain sensation. As a positive control, indomethacin is used as standard NSAID. The plant extract was administered orally in two different doses (250 and 500 mg/kg body weight) to the tested animals after an overnight fast. Test samples, vehicle and standard were administered orally 30 minutes prior to intraperitoneal administration of acetic acid solution (0.1 ml/10 g). Each rat of all groups was observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intra-peritoneal administration of acetic acid solution. Sometimes rodents initiate writhing but do not finish and two unfinished writhing is calculated as one full writhing.

2.9.2. Formalin test
As different analgesics work in different ways in both early and late phases, the formalin test as described by Sharma *et al*. [16] was followed to elucidate the probable mechanism of an antinociceptive effect of a proposed analgesic. Control group received only 20 µl of 5% formalin whereas test groups and positive control groups received MCRS extract (250 and 500 mg/kg, p.o.) and reference drug before injection of 20 µl 5% formalin into the dorsal surface of the right hind paw. The rats were monitored for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the tenure between 15 and 30 min as the late phase. The total time spent licking or biting the wounded paw (pain manners) was checked by a stop watch.

2.10. Anti-inflammatory activity
Carrageenan induced rat paw edema [17] was followed to evaluate the an-inflammatory activity of MCRS extract. Twenty four rats were alienated into four groups (n = 6); Group I served as control received 0.9% normal saline in 3% Tween 80 suspension, while Group II was treated with indomethacin (10 mg/kg) and; Group III- IV orally received MCRS extract at 250 and 500 mg/kg body weight. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline that contained Tween 80 in the right paw of rats. The paw volume was measured at 0, 0.5, 1, 2 and 3 h after carrageenan injection using a plethysmometer. Boost in the linear diameter of the right hind paws were taken as a sign of paw edema.

2.11. Statistical analysis
All the values in the test are expressed as mean ± standard error of the mean (SEM). The data were statistically analyzed by ANOVA (Analysis of variance) and post-hoc Dunnett's tests with the Statistical Package for Social Sciences (SPSS) program (SPSS 16.0, USA). Dissimilarity between the means of the various groups were measured significant at $P < 0.01$.

3. Results

3.1. Phytochemical analysis

The extract gave positive tests for tannins, alkaloids, saponin and flavonoids.

3.2. Acute toxicity

In the acute toxicity test, the plant extract was found to be safe up to doses of 1.6 g/kg. Behavior of the animals was strictly observed for the first 3 in the next 48 h. The extracts did not affect any behavioral change or mortality on rats during 48 h inspection.

3.3. Determination of CNS depressant activity

3.3.1. Hole cross test

Rodents treated with MCRS at two doses (250 mg/kg & 500 mg/kg) showed dose dependent reduction in the locomotor activity which was comparable with standard drug diazepam. In case of control group negligible variation in number of holes crossed from one chamber to another by rat was observed from 0 to 120 min. Whereas groups treated with plant extract at the above mentioned doses showed significant decrease of movement from their primary value at 0 to 120 min (Table 1). The result was statistically significant ($p < 0.01$).

Table 1: CNS depressant activity of MCRS in hole cross test in rats

Treatment	Dose (mg/kg)	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Control	10	12.8±1.16	13±1.41	13.6±0.93	14.2±0.86	14±0.55
Diazepam	1	11.2±0.58	6±0.707* (53.84 %)	4±0.84* (70.84 %)	2.4±0.81* (83.09 %)	1.8±0.37* (87.14 %)
MCRS	250	12.6±1.54	9±0.89 (30.76 %)	6.2±0.58* (54.41 %)	4.2±0.58* (70.42 %)	2.8±0.58* (80.00 %)
MCRS	500	13.8±1.11	7±1.34* (46.15 %)	4.8±0.37* (64.70 %)	3±0.55* (78.87 %)	2.6±0.58* (81.42 %)

All values are expressed as mean±STD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.01 significant compared to control

3.3.2. Open field test

Here MCRS exhibited a perceptible decline in locomotion of test animals at 2nd observation (30 min) which continued up to 4th observation period (120 min) at both dose levels (250 and 500 mg/kg body weight). The results (Table-2) were dose dependent and statistically significant (P<0.01).

Table 2: CNS depressant activity of MCRS on open field test in rats

Treatment	Dose(mg/kg)	Number of Movements				
		0 min	30 min	60 min	90 min	120 min
Control	10	118.4±1.21	118±1.301	115.4±0.51	117.4±1.17	118±0.71
Diazepam	1	117.2±1.16	64.6±1.4* (45.25 %)	40.8±0.58* (64.64 %)	18.8±0.86* (83.98 %)	9.6±0.51* (91.86 %)
MCRS	250	119.4±0.51	92±1.38* (22.03 %)	59.2±1.02* (48.70 %)	36.2±1.74* (69.16 %)	27.8±1.65* (76.44%)
MCRS	500	120.2±1.43	67.6±2.50* (42.71 %)	42±0.84* (63.60 %)	25.4±1.72* (78.36 %)	15.8±1.68* (86.61 %)

All values are expressed as mean±STD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.01 significant compared to control

3.4. Analgesic activity of plant extracts

3.4.1. Acetic acid-induced writhing test

Table 3 shows the effects of MCRS on acetic acid-induced writhing in rats. Both doses of extract showed noteworthy reduction ($p<0.01$) of writhing provoked by the intraperitoneal administration of acetic acid in a dose dependant manner.

Table 3: Analgesic activity of MCRS by acetic acid induced writhing method in rats

Treatment	Dose(mg/kg)	No. of writhing	Percent inhibition
Control	0.1 ml/10 gm	26±.55777	
Indomethacin	10 mg/kg	10±.85635*	61.54
MCRS	250 mg/kg	14.67±.55777*	43.57
MCRS	500 mg/kg	12.33±.33333*	52.58

All values are expressed as mean±STD (n=6); One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.01 significant compared to control

3.4.2. Formalin induced licking response model

The antinociceptive property of seed extract was evaluated in Formalin test models and MCRS significantly suppressed the licking activity in either phase of pain (Table 4) in a dose dependant manner.

Table 4: Analgesic Effect of MCRS on hindpaw licking in formalin test.

Treatment	Dose (mg/kg)	Early phase (Sec)	Late phase (Sec)
Control	0.1 ml/10 gm	35.67±1.38	46±1.03
Indomethacin	10	16.83±0.91* (52.81 %)	21.83±0.70* (52.54 %)
MCRS	250	23.33±2.06* (34.59%)	22±1.34* (52.17 %)
MCRS	500	12±.26* (66.36%)	14.5±0.34* (68.47 %)

All values are expressed as mean±STD (n=6); One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.01 significant compared to control

3.5. Anti-inflammatory activity of MCRS

Carrageenan induced paw edema test was performed to evaluate the anti-inflammatory activity of the methanolic extracts of seeds *C. rotang*. There was a dose-dependent, significant reduction in carrageenan -induced rat paw edema at 250 and 500 mg/kg of extract compared to indomethacin (10 mg/kg) over a period of 240 min as shown in Table 5.

Table 5: Anti-inflammatory effect of MCRS on carrageenan – induced paw edema in rat

Treatment	Dose (mg/k)	Before Inflammation (mm)	After treatment in inflamed mice (mm)				
			0 min	30 min	60 min	120 min	240 min
Control	-	3.87±0.01	5.01±0.02	5.44±0.02*	5.25±0.02*	5.22±0.02*	5.16±0.02*
Standard	10	3.59±0.01*	4.61±0.02	4.49±0.02* (17.46 %)	4.33±0.05* (17.52 %)	4.21±0.03* (19.34%)	4.11±0.04* (20.34 %)
MCRS	250	3.88±0.01	5.06±0.03	4.89±0.03* (10.11 %)	4.75±0.02* (9.52 %)	4.64±0.03* (11.11 %)	4.58±0.03* (11.24 %)
MCRS	500	3.97±.01*	5.03±0.02	4.75±0.01* (12.68 %)	4.6±0.01* (12.38%)	4.40±0.012* (15.70 %)	4.32±.016* (16.27 %)

All values are expressed as mean±STD (n=6); One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.01 significant compared to control

4. Discussion

In the current study, we have evaluated CNS depressant, Analgesic and Anti-inflammatory, effects of different methanolic extract of seeds of *C. rotang* in rodents. To acquire evocative results regarding the effect of methanolic extract of *C. rotang* seeds on the pain management and effect on CNS in rat, a number of methods namely acetic acid induced writhing test, formalin induced pain, open field and hole cross were implemented.

The extracts showed significant CNS depressant effect in Hole cross and Open field tests in comparison to Standard drug Diazepam in dose dependent manner. 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 $GABA_A$. Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the central nervous system; which led to assume that they can act as benzodiazepine like molecules [18]. The sedation may be due to an interaction with benzodiazepines like compounds. The seeds extracts may act by potentiating GABAergic inhibition in the CNS by membrane hyperpolarization which diminish in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts [19]. Phytochemical investigations also showed the presence of alkaloids, flavonoids, saponins and steroids in the plant. So might be this phytoconstituents are responsible for its CNS depressant activity. The acetic acid induced writhing method was found efficient to assess peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect, preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [20]. The significant pain reduction of the plant extract might be due to the presence of analgesic principles acting with the prostaglandin pathways. Again formalin test is very useful for elucidating mechanism of pain and analgesia hence it was followed to check the central or peripheral mechanism of the analgesic effect of tested samples Drugs that principally act centrally such as narcotics hinder both phases of formalin-induced pain while peripherally acting drugs such as Indomethacin inhibit only the second phase [21]. As the experimented extract exerted noteworthy dose related inhibition of both phases of formalin test indicting the overall antinociceptive effect of investigated crude extracts involve central and peripheral mechanism.

Carrageenan induced paw edema test was performed to evaluate the anti-inflammatory activity of the methanolic extracts of MCRS. Carrageenan provoked edema is generally used as an investigational animal model for acute inflammation and is supposed to be biphasic, among which the first phase is initiated by the release of histamine and kinins and then prostaglandin in the later phase [22]. This study has shown that the methanolic extract of the seed of *C. rotang* possessed a noteworthy anti-oedematogenic effect on paw oedema stimulated by carrageenan. Since carrageenan-induced inflammation model is a significant extrapolative test for anti-inflammatory agents performing by the intermediaries of acute inflammation [23] we can say that MCRS can be effective in acute inflammatory anarchy.

5. Conclusion

Finally, we may say that the experimented extract of seeds of *C. rotang* fruits have potent CNS depression, analgesic (both central and peripheral), anti-inflammatory, antidiarrheal and anti-diabetic activities. However, further research is needed in

order to find out the precise mechanisms and responsible chemical constituents for the above mentioned pharmacological activities. In the near future we will conduct experiments with purified fractions of the above extracts for further pharmacological and toxicological characterization.

6. Acknowledgement
Author thanks to ICDDR, B for supplying rodents for different pharmacological tests in this research.

7. Conflict of interest statement
We proclaim that we have no conflict of interest.

8. Reference

1. Arokiyaraj S, Radha R, Martin S, Perinbam K. Phytochemical analysis and anti- diabetic activity of Cadaba fruticosa R.Br. Indian Journal of Science and Technology 2008; 1(6):1-4.
2. Gangadevi V, Yogeswari S, Kamalraj S, Rani G, Muthumary J. The antibacterial activity of *Acalypha indica* L. Indian Journal of Science and Technology 2008; 1(6):1-5.
3. Chellaram C, Edward JKP. Anti-inflammatory potential of coral reef associated gastropod, Drupa margariticola. Indian Journal of Science and Technology 2009; 2(2): 75-77.
4. Rehan A, Swayam PS, Rakesh M, Rajendran S, Arya KR, Arvind KS. Mild antihyperglycaemic activity in *Eclipta alba*, Berberis aristata, Betula utilis, Cedrus deodara, Myristica fragrans and Terminalia chebula. Indian Journal of Science and Technology 2008; 1(5):1-6.
5. Gordon DM. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. Microbiology 2001; 147:1079-1085.
6. Shekhawat N, Vijayvergia R. Investigation of anti-Inflammatory, analgesic and antipyretic Properties of *Madhuca indica* GMEL. International Journal of Molecular Medicine and Advance 2010; 6:26-30.
7. Rashid M, Hasan CM, Choudhury SAR, Begum B, Rahman S. Ethnopharmacological investigation of medicinal plants of Bangladesh. Bangladesh Journal of Physiology and Pharmacology 1997; 12:25-29.
8. Basumatary SK, Ahmed M, Deka SP. Some medicinal plant leaves used by Boro (tribal) people of Goalpara district, Assam. Natural Products Research 2004; 3(2):88-90.
9. Kagyung R, Gajurel PR, Pethy P, Singh B. Ethnobotanical studies of Dehang-Debang. Biosphere Reserve of Arunachal Pradesh with special reference to Memba tribe. Indian Journal of Traditional Knowledge 2010; 9(3):496-501.
10. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Springer-Verlag Heidelberg Publication, 2004, 110-111.
11. Trease G, Evans M. Pharmacopoeial and related drugs of biological origin. In: A Textbook of Pharmacognosy. Edn 15, London, WB Saunders, 2001.
12. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109.
13. Takagi K, Watanabe M, Saito H. Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethane. Its acylates on the central nervous system. Japanese Journal of Pharmacology 1971; 21:797-810.
14. Gupta BD, Dandiya PC, Gupta ML. A psychopharmacological analysis of behavior in rat. Japanese Journal of Pharmacology 1971; 21:293.
15. Ahmed M, Shikha HA, Sadhu SK, Rahman MT, Datta BK. Analgesic, diuretic, and anti-inflammatory principle from *Scoparia dulcis*. Pharmazie 2001; 56(8):657-660.
16. Sharma A, Bhatial S, Kharyaz MD, Gajbhiye V, Ganesh N, Namdeo AG *et al*. Anti-inflammatory and analgesic activity of different fractions of *Boswellia serrata*. International Journal of Phytomedicine 2010; 2:94-99.
17. Perez C, Pauli M, Bazevque P. An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicinal Experimentalis 1990; 15:113-115.
18. Verma A, Jana GK, Sen S, Chakraborty R, Sachan S, Mishra A. Pharmacological evaluation of *Saraca indica* Leaves for central nervous system depressant activity in mice. Journal of Pharmaceutical Sciences and Research 2010; 2(6):338-343.
19. Kolawole OT, Makinde JM, Olajide OA. Central nervous depressant activity of *Russelia equisetiformis*. Nigerian Journal of Physiological Sciences 2007; 22:59-63.
20. Ferdous M, Rouf R, Shilpi JA, Uddin SJ. Antinociceptive activity of the ethanolic extract of *Ficus racemosa* Linn. (Moraceae). Oriential Pharmacy and Experimental Medicine 2008; 8:93-96.
21. Santosm FA, Rao VSN, Silveira ER. Investigation on the antinociceptives of *Psidium guajava* leaf essential oil and its major constituents. Phytotherapy Research 1998; 12:24-27.
22. Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn. On carrageenan induced paw edema in wistar male rats. African Journal of Biotechnology 2007; 6(10):1209-1211.
23. Sawadogo WR, Boly R, Lompo M. Some N. Anti-inflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. International Journal of Pharmacology 2006; 2:435-438.