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ACUTE TOXICITY STUDY AND EVALUATION OF ANTI-INFLAMMATORY & CNS DEPRESSANT ACTIVITIES OF *RICHARDIA SCABRA*

Aziz, M.A.*; Sarkar, K.K.; Roy, D.N.

Department of Pharmacy, Jessore University of Science & Technology, Bangladesh.

*debusubju@gmail.com

Abstract

Methanolic extract of Richardia scabra (MRS) was evaluated for its safety as well as anti-inflammatory & CNS depressant activities by using OECD guidelines, carrageenan induced paw edema in mice, open field and hole cross test. Mortality, sign of any toxicity or behavioral changes were not observed up to the dose as high as 4000mg/kg. In carrageenan induced paw edema model, MRS at 200 mg/kg revealed significant ($p < 0.05$, vs.control) & highest percentage inhibition (116.00±3.67) of paw edema. Moreover, in open field test, significant ($p < 0.05$, vs.control) depressant effect was found by MRS at 200 & 400 mg/kg during 60 min & 60 min, 120 min, 180 min correspondingly. Gradual decrease (30 min to 120 min) of movement was found by MRS 400 mg/kg but it showed significant ($p < 0.05$, vs.control) increase of movement during 180 min through hole cross test. The results obtained in the present study point out that MRS can be a possible source of anti-inflammatory and CNS depressant agents.

Key Words: Acute toxicity, anti-inflammatory, CNS depressant, Richardia Scabra.

Introduction

Richardia scabra also called Florida Pusley of Rubiaceae family is locally known as 'Riim-raaz' in Bangladesh. It is a branched plant that possesses distinctive characteristics because of its hairy stems and leaves. It can grow annually up to 80 cm but is frequently prostrate. As a forage plant, green manure and soil covering it is grown Southern North America. The whole plant is used as tonic and emetic, along with its activity against asthma and dermatitis. The root of this plant possesses diaphoretic property [1, 2, 3, 4]. So far some survey was carried out locally and internationally on some medicianal plants which disclosed some valuable information of *Richardia scabra* [1,4,5]. Hence, the current study was performed to evaluate the anti-inflammatory and CNS depressant activities of the methanolic extract of R. *scabra* whole plant (MRS).

Materials and Methods

Collection and Identification of The Plant

R. scabra (whole plant) was collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in November, 2012. Species identification was verified by Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium. A dried specimen was deposited in the herbarium for future reference.

Extraction

Methanol extraction was prepared on 200 g of powdered whole plant of *R. scabra*. Plant was rinsed 3–4 times successively with running water and once with sterile distilled water. Washed plant was then dried in the shade for a period of 7 d. The dried plant was then ground by using a laboratory grinding mill (Model 2000 LAB Eriez®) and passed through a 40-mesh sieve to get fine powders. Powdered whole plant of *R. scabra* (200 g) was extracted in 2 L of methanol, using a soxhlet apparatus and a hot extraction procedure. Whatman No.1 filter papers were used to filter the liquid extracts. The filtrates were then dried in a hot air oven at 400C. The extraction yield was 1.50% (w/w). Extract was stored at 40C for additional studies.

Experimental Animals

Sixty Swiss albino mice of either sex, 6–7 weeks old, weighting 25–30 g were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. These animals were kept under standard environmental conditions, having relative humidity 55%–65%, 12 h light/12 h dark cycle and (27.0±1.0) 0C temperature. Proper supply of foods and water *ad libitum* were ensured. Before the experiment, animals were adapted to the laboratory conditions for 1 week. The Institutional Animal Ethical Committee of Jahangirnagar University, Savar, Dhaka, Bangladesh approved all the protocols used in the experiments conducted with these animals.

Acute Oral Toxicity Study

Adverse effects that result either from a single exposure or from multiple exposures over a short time (normally less than 24 h) are known as acute toxicity. To find the half lethal dose (LD_{50}) of the experimental samples, the acute toxicity study was carried out following the Organization of Economic Cooperation and Development (OECD) guidelines [6]. Ten mice were divided into two groups: control group and test group (MRS), with five animals per group. The experimental sample (MRS) was administered orally at different concentrations (100, 250, 500, 1 000, 2 000, 3 000 and 4 000 mg/kg body weight). After that the animals were observed every 1 h for next 5–6 h for mortality, behavioral pattern changes such as weakness, aggressiveness, food or water refusal, diarrhea, salivation, discharge from eyes and ears, noisy breathing, changes in locomotor activity, convulsion, coma, injury, pain or any signs of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted [6].

Anti-Inflammatory Activity

Carrageenan Induced Edema In Mice Paw

Carrageen-induced paw edema test was performed acccording to winter et al. and Thambi et al [7,8]. Tweenty mice were divided into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Indomethacin, 10 mg/kg, i.p.) and test groups (MRS at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. 30 min later of the respective treatment, 0.1 mL, 1% w/v carrageenan suspension was injected into the subplantar tissue of the right hind paw. Paw thickness was measured just before and at hourly interval for 4 h using vernier caliper. Increase in paw thickness was calculated using the formula Pt-Po, where, Po is the initial paw thickness at time to and Pt is the thickness at time t. Percentage inhibition of the increase in paw thickness was calculated by the

formula, (1-Pt/Pc) x 100, where Pt is the increase in paw thickness of treated and Pc is that of carrageenan induced control.

CNS Depressant Study

Open Field Test

The test was completed according to Hawiset et al [9]. Tweenty mice were divided into control group (distilled water, 10 mL/kg, p.o.), positive control or standard group (Diazepam, 1mg/kg, p.o.) and test groups (MRS at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. Through this test, the CNS depression activity can be evaluated. A series of alternating white and black squares made the open field having 40 cm height. The number of movement of the test animals i.e., total number of squares that every group of animals visited was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min.

Hole Cross Test

Hole cross test was performed following the method of Takagi et al [10] by using a cage having a size of 30×20×14 cm and in the middle of this cage a steel partition was attached. Here, the grouping of mice was done according to open field test. The diameter of the hole was 3 cm, which was made at the center of the cage at a height of 7.5 cm. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 120 and 180 min after respective treatment.

Statistical Analysis

All the results were expressed as mean ± S.E. (Standard Error). Statistical analyses for anti-inflammatory and neuropharmacological studies were performed by one-way ANOVA following Dunnet's test through the SPSS software (version 20; IBM Corporation, New York, USA). ($P < 0.05$, *vs.*control) was considered statistically significant.

Results

Acute Oral Toxicity Study

After acute toxicity study, no mortality was observed up to the dose as high as 4000mg/kg for MRS or control group. Sign of any toxicity or behavioral changes were not observed up to the dose as high as 4000mg/kg for MRS (test group) or control group, before or after their administration in any animal, which lived up to 14 days. This apparently indicated that the test group does not show acute oral toxicity.

Anti-Inflammatory Activity

Carrageenan Induced Paw Edema In Mice

In carrageenan induced paw edema model, MRS showed significant ($p < 0.05$, *vs.*control) percentage inhibition of paw edema in 1st hr, 2nd hr, 3rd hr and 4th hr at 200 mg/kg and 400 mg/kg doses respectively. At the same time, indomethacin gradually increased percentage inhibition of paw edema (6.67±21.47% to 83.33±16.67) from 1st to 4th hr. Besides, MRS at 200 mg/kg revealed highest percentage inhibition (116.00±3.67) of paw edema [Table 1 (a)]**.** In addition, significant ($p < 0.05$, *vs.*control) reduction of paw thickness was found by MRS at both doses with increasing time [Table 1 (b)].

CNS Depressant Study

Open Field Test

Open field tests showed that the depressing action of the extract was noticeable from the 2nd & 3rd observation period in the test animals at the dose of 200 & 400 mg/kg body weight respectively. In this case, significant ($p < 0.05$, *vs.*control) depressant effect was found by MRS at 200 & 400 mg/kg during 60 min & 60 min, 120 min, 180 min correspondingly. The maximum depressant effect was observed from the 2nd (30 min) to 5th (180 min) period at 200mg/kg body weight [Table 2].

Hole Cross Test

CNS depressant property of MRS was carried out by hole cross test. In case of MRS 200 mg/kg, fluctuation of movement was noticed, whereas maximum & minimum movement was observed during 30 min & 60 min respectively. Moreover, gradual decrease (30 min to 120 min) of movement was found by MRS 400 mg/kg but it showed significant ($p < 0.05$, *vs.*control) increase of movement during 180 min [Table 3]**.**

Discussion

Although many plant-derived products are in use in systems of traditional medicine, scientifically rigorous toxicity studies have been conducted on very few. Hence, acute oral toxicity studies are extremely important to determine the proper range of doses for subsequent usage and to identify the potential adverse effects of the materials under examination. During the investigation of therapeutic index of drugs and xenobiotics, acute oral toxicity study becomes a suitable factor[6]. LD50 of the plant extract could not be obtained, as no mortality was observed up to the dose as high as 4000 mg/kg and the extract was found to be safe with a broad therapeutic range. Therefore, two comparatively high doses (200 and 400 mg/kg) for MRS were used for *in-vivo* doses.

Anti-edematous effect is evaluated normally by carrageenan induced paw edema test which is applied as acute inflammatory test. After the administration of carrageenan, edema is formed as biphasic episode. Initial phase that occurs 0-2 hr after the administration of carrageenan is characterized by the release of serotonin, bradykinin and histamine on vascular permeability [11]. The second phase initiates after the completion of first phase and continues for at least 5 hr. Different phase, like- lysosome, bradykinin and prostaglandin intervenes second phase of edema. Nearly all of the currently existed NSAIDs act on late phase of inflammation. Carrageenan-induced edema is occurred by Nitrous oxide (NO) which is a potent vasodilator having the capability of changing local blood flow which enhances vascular permeability and edema [12]. Indomethacin is a well known cyclooxygenase inhibitor [13]. Although inflammatory process is caused either by lipoxygenase or cyclooxygenase pathway, carrageenan-induced inflammation is markedly blocked by cyclooxygenase inhibitors [12]. From table 1(a) it is noticed that MRS showed significant percentage inhibition of paw edema in first phase rather than second phase, which may be due to the inhibiton of serotonin, bradykinin and histamine release. MRS at 200 mg/kg revealed highest percentage inhibition of paw edema than that of 400 mg/kg, which might be due to the competitive interaction of MRS 400 mg/kg with its receptor. In addition, weak binding may occur between MRS 400 mg/kg with its receptor. Moreover, physiological problems might be present in the mice of MRS 400 mg/kg that can permit poor absorption from the gastro-intestinal tract.

By observing the locomotor activities of animals, CNS activity of any drug can be evaluated. The locomotor activity of animal can be defined as the measurement of the level of excitability of the CNS. Sedation that is originated from CNS depression has a close relationship with reduced locomotor activity [14]. GABAA receptor is involved for the action of CNS depressant drugs [15]. The extract significantly decreased the locomotor activity as shown by the results of open field test. The locomotor activity lowering effect was evident in the 2nd observation period (30 min) and continued up to a 5th observation period (180 min) at the doses of 200 & 400 mg/kg body weight by MRS [Table 3]. MRS at 200 mg/kg exposed highest depressant activity than that of 400 mg/kg, which reasons may be similar that was mentioned in the discussion of carrageenan induced paw edema test. Analyzing the above result, it may be suggested that, MRS had CNS depressant activity. MRS at 200 mg/kg showed CNS depressant activity during 60 min, whereas MRS 400 mg/kg exhibited that activity during 60 min and 120 min. This kind of CNS depressant activity may be due to binding of MRS with GABAA receptor [15]. Further extensive neuropharmacological studies are needed to confirm the CNS depressant activity of MRS.

Conclusion

The present results showed that methanolic extract of *Richardia scabra* may have anti-inflammatory and neuropharmacological activities. Further investigations are required to find the active component of the extract in order to confirm the mechanism of action in the development of anti-inflammatory and CNS depressant agents. Beisdes, genotoxicity study of this extract may be a promising area for the researcher.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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Table 1 (a). Anti-inflammatory effect of MRS against Carrageenan induced paw edema in mice.

Group	Doses (mg/kg)	Initial paw thickness (cm)	Paw Thickness (cm) after 1hr	% of inhibition	Paw Thickness (cm) after 2 hr	% of inhibition	Paw Thickness (cm) after3 hr	% of inhibition	Paw Thickness (cm) after 4hr	% of inhibition
Control	10	0.24±0.02	0.52±0.02	-	0.50±0.00	-	0.47±.005	-	0.41±0.005	-
Standard	10	0.24± 0.02	0.46±0.02	-6.67±21.47	0.39±0.00*	18.67±13.85	0.30±0.00*	50.64±12.43*	0.24±0.02*	83.33±16.67*
MRS	200	0.30±0.00	0.40±0.0*0	63.33±9.71*	0.38±0.01*	116.00±3.67*	0.32±0.01*	94.59±12.46*	0.30±0.00*	110.00±10.00*
MRS	400	0.30±0.00	0.42±0.02*	50.00±5.27*	0.40±0.00*	112.00±2.00*	0.30±0.00*	100.00±0.00*	0.30±0.00*	100.00±0.00*

Values are presented as mean±S.E., (n=5 animals); ($p < 0.05$, *vs.*control).

Table 1 (b). Anti-inflammatory effect of MRS against Carrageenan induced paw edema in mice.

Group	Doses(mg/kg)	Increase in paw thickness			
		1st hr	2nd hr	3rd hr	4th hr
Control	10 ml/kg	0.24±0.02	0.22±0.02	0.19±0.02	0.13±0.02
Standard	10 mg/kg	0.24±0.04	0.17±0.02	0.08±0.02*	0.02±0.02*
MRS	200 mg/kg	0.08±0.02*	0.06±0.02*	0.00±0.03*	-0.02±0.02*
MRS	400 mg/kg	0.12±0.02*	0.10±0.00*	0.00±0.00*	0.00±0.00*

Values are presented as mean±S.E., (n=5 animals); ($p < 0.05$, *vs.*control).

Table 2. Effect of MRS on open field test.

Group	Doses(mg/kg)	Number of movement				
		0 min	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 ml/kg	154±1.77	135.6±1.81	27.8±0.86	38.2±1.16	27.2±1.39
Standard	1 mg/kg	119±1.58	114.4±1.5	107.4±0.93*	84.4±1.57	72.6±1.81
MRS	200 mg/kg	183.6±6.5	144.2±8.66	94±4.09*	73.4±11.81	67.2±11.44
MRS	400 mg/kg	176.6±4.17	178.2±16.11	140.6±3.19*	104.6±2.54*	87.6±1.03*

Values are presented as mean±S.E., (n=5 animals); (p <0.05, *vs.*control).

Table 3. Effect of MRS on hole cross test.

Group	Doses(mg/kg)	Number of movement				
		0 min	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 ml/kg	3.2±0.2	1.8±0.2	1±0.32	0.2±0.2	0.8±0.37
Standard	1 mg/kg	0.2±0.2*	0.8±0.2	1.2±0.49	0.8±0.2	0.6±0.4
MRS	200 mg/kg	2±0.71	3.6±1.37	1.2±0.37	1.4±0.25	2.2±0.58
MRS	400 mg/kg	1.2±0.37	3.4±1.03	1.6±0.25	1.2±0.37	4±0.71*

Values are presented as mean±S.E., (n=5 animals); (p <0.05, *vs.*control).