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ACUTE TOXICITY AND NEUROPHARMACOLOGICAL STUDIES OF *MICROCOS PANICULATA* & *RICHARDIA SCABRA*

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Abstract

The present study was designed to evaluate the safety of the methanolic extract of *Microcos paniculata* roots (RME), as well as neuropharmacological activity of the methanolic extracts of *M. paniculata* fruits (FME), RME and *Richardia scabra* (whole plant) (MRS) by using OECD guidelines, Y-maze and Elevated plus-maze test respectively. Mortality, sign of any toxicity or behavioral changes were not observed up to the dose as high as 4000mg/kg. In Y-maze test, anti-depressive and depressive activities of FME 200 mg/kg and 400 mg/kg, RME 200 mg/kg and 400 mg/kg, MRS 200 mg/kg and 400 mg/kg were noticed. Again, the Elevated plus maze test revealed that every extract including both 200 and 400 mg/kg doses demonstrated anxiolytic and depressive activities. The results obtained in the present study point out that FME, RME and MRS can be the possible sources of CNS depressant, anti- depressant and anxiolytic agents. But further investigation is required for the confirmation of their activities.

Key Words: Acute toxicity, neuropharmacological studies, *Microcos paniculata*, *Richardia scabra*.

Introduction

Microcos paniculata L. of Tiliaceae family is locally known as 'Kathgua' or 'Fattashi' in Bangladesh. It has the growth form of a shrub or small tree, grows wildly and is cultivated throughout Bangladesh. Traditionally the plant is used to treat fever, diarrhea, dyspepsia, heat stroke, colds, hepatitis, wounds, for its activity in the digestive system and to kill insects. A review of the literature showed that *M. paniculata* has been found to have a wide range of activities, including neuropharmacological, larvicidal, insecticidal, free radical scavenging, antimicrobial, brine shrimp lethality, antidiarrheal, analgesic, anti-inflammatory, antipyretic, α-glucosidase inhibition, cytotoxic and nicotinic receptor antagonistic activities, as well as preventative effects for coronary heart disease and angina pectoris. Moreover, acute toxicity study of the methanolic extract of *M. paniculata* fruits were conducted also [1, 2]. *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 in 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. Analysis of the literature showed that acute toxicity study, anti-inflammatory and CNS depressant activities of *Richardia scabra* were performed. However, some surveys were carried out locally and internationally on few medicinal plants which disclosed several valuable information of *Richardia scabra* [3]. Therefore, the present study was designed to evaluate the neuropharmacological activity of the methanolic extracts of *M. paniculata* fruits (FME) and roots (RME), along with *Richardia scabra* (whole plant) (MRS).

Materials and Methods

Collection and Identification of the Plant

Fruits and roots of *M. paniculata* and *R. scabra* (whole plant) were 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. Dried specimens were deposited in the herbarium for future references.

Extraction

Methanol extraction was carried out on 200 g of powdered fruits and roots of *M. paniculata,* along with *R. scabra* (whole plant). Plant parts and whole plant were rinsed 3–4 times successively with running water and once with sterile distilled water that were then dried in the shade for a period of 7 d. The dried plant parts and plant were then ground by using a laboratory grinding mill (Model 2000 LAB Eriez®) and passed through a 40-mesh sieve to get fine powders. Powdered fruits and roots of *M. paniculata* and whole plant of *R. scabra* (200 g) were extracted individually 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 40^0C. The extraction yield of fruits and roots of *M. paniculata* and whole plant of *R. scabra* were 11.08% (w/w), 1.56% (w/w) and 1.79% (w/w) respectively. Extracts were stored at 4^0C for additional studies.

Experimental Animals

Ninety 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 (27.00±1.00) ^{0}C 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 [2]. Ten mice were divided into two groups: control group and test group (RME), with five animals per group. The experimental sample (RME) 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 sign of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted [2].

Neuropharmacological Study

Y-maze Test

Y-maze test was completed according to Mandal *et al.,* 2001; Rushton *et al.,* 1961 and Ma *et al.,* 2007 [4,5,6]. Fourty 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 (FME, RME and MRS at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. Three wooden arms with an angle of 120° between each of the two arms made the Y-maze apparatus, where dimensions of the arms were 30 cm x 8 cm x 15 cm (length x width x height). Each mouse was placed in the centre of a Y – shaped runway. The number of entry of the test animals into the closed and open arms was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min. Arm entry was defined as the entry of all four paws into one arm

Elevated Plus-maze Test (EPM)

Elevated plus-maze test was performed following the method of Lister [7]. Mice grouping and administration were performed as mentioned before. The apparatus was made of two opposing closed arms (50 x 10 x 30 cm) (length x width x height) and two opposing open arms (50 x 10 cm) (length x width) that was placed at 70 cm high from the floor level. Each mouse was placed in the centre of elevated plus-maze apparatus. The number of entry of the test animals into the closed and open arms was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min. Arm entry was defined as the entry of all four paws into one arm.

Statistical Analysis

All the results were expressed as mean ± S.E. (Standard Error). Statistical analyses for 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 4000 mg/kg for RME or control group. Sign of any toxicity or behavioral changes were not observed up to the dose as high as 4000 mg/kg for RME (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.

Neuropharmacological Study

Y-maze Test

The result of the table-1 showed that standard drug diazepam revealed depressive activity with time. Moreover, FME 200 mg/kg exhibited significant ($p <0.05$, *vs.*control) anti-depressive activity with time. In case of FME 400 mg/kg, the number of entries into the closed arm was reduced during second observation. After that, depressive effect was noticed at 60 min, 120 min and 180 min respectively, whereas, third and fourth observations were significant ($p <0.05$, *vs.*control). RME 200 mg/kg and 400 mg/kg exposed anti-depressive activity at second and third observations. Again, fourth and fifth observations of them showed depressive and anti-depressive activities respectively. Furthermore, MRS 200 mg/kg disclosed depressive effect during second observation. But, anti-depressive activity was found at 60 min, 120 min and 180 min respectively. Besides, second, third and fourth observations were significant ($p <0.05$, *vs.*control). Again, anti-depressive effect was marked by MRS 400 mg/kg at second, third, fourth and fifth observations, in which second and third observations were significant ($p <0.05$, *vs.*control). From table-2, it was clear that standard drug diazepam revealed depressive activity with time. Moreover, FME 200 mg/kg and FME 400 mg/kg exhibited significant ($p <0.05$, *vs.*control) fluctuating effects including both depressive and anti-depressive activities. Therefore, RME 200 mg/kg expressed depressive activity with time except fourth observation. In addition to, RME 400 mg/kg showed both anti-depressive and depressive activities during different observations. Besides, MRS 200 mg/kg elicited anti-depressive activity during second observation and depressive activity at 60 min, 120 min and 180 min respectively. Later, MRS 400 mg/kg disclosed fluctuating effects including both depressive and anti-depressive activities.

Elevated Plus-maze Test (EPM)

The result of the table-3 exhibited that standard drug

diazepam revealed depressive activity with time. In addition to, FME 200 mg/kg elicited both depressive and anti-depressive activities at 30 min and 60 min, 120 min respectively. Besides, FME 400 mg/kg and RME 200 mg/kg showed anti-depressive activity during all observations except fourth observation. Furthermore, fluctuating effects including both depressive and anti-depressive activities were found by RME 400 mg/kg and MRS 200 mg/kg respectively. Similar result was noticed by MRS 400 mg/kg as like as FME 200 mg/kg. Again, both high and low doses of FME and RME exposed significant ($p < 0.05$, *vs.*control) depressive and anti-depressive activities during second observation. From table-4, it was obvious that standard drug diazepam exhibited depressive activity with time. Moreover, FME 200 mg/kg, MRS 200 mg/kg and MRS 400 mg/kg expressed fluctuating effects including both anxiolytic and depressive activities during second and third, fourth, fifth observations respectively. In addition to, FME 200 mg/kg also exposed significant ($p < 0.05$, *vs.*control) effects. Besides, fluctuating effects including both depressive and anxiolytic activities were noticed by FME 400 mg/kg and RME 400 mg/kg respectively. Furthermore, RME 200 mg/kg revealed both depressive and , 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 sign of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted [2].

Neuropharmacological Study

Y-maze Test

Y-maze test was completed according to Mandal *et al.,* 2001; Rushton *et al.,* 1961 and Ma *et al.,* 2007 [4,5,6]. Fourty 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 (FME, RME and MRS at 200 and 400 mg/kg body weight, p.o.), containing five mice in each group. Three wooden arms with an angle of 120° between each of the two arms made the Y-maze apparatus, where dimensions of the arms were 30 cm x 8 cm x 15 cm (length x width x height). Each mouse was placed in the centre of a Y – shaped runway. The number of entry of the test animals into the closed and open arms was counted at 0, 30, 60, 120 and 180 min after respective treatment and the every counting was continued for 3 min. Arm entry was defined as the entry of all four paws into one arm anxiolytic activities at 30 min and 60 min, 120 min, 180 min respectively.

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 [2]. LD_{50} 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 RME were used for *in-vivo* doses.

In the current study, the effect of methanolic extracts of fruits and roots of *Microcos paniculata,* along with *Richardia scabra* (whole plant) was evaluated for CNS activities. By observing the locomotor activities of animals, CNS activity of any drug can be assessed. 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 [3]. Locomotor activity was determined by observing the number of arm entries of Y-maze and Elevated plus-maze test apparatus [8]. $GABA_A$ receptor is involved for the action of CNS depressant drugs [3]. Increasing concentration of $GABA_A$ receptor in brain is responsible for CNS depressant activity [9]. $GABA_A$ receptor can be divided into various subtypes and at least 17 subunits like $\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$ and others (single ε, θ, π and δ) make diverse arrangements of its subtypes. Binding of benzodiazepines with α_2 and α_1 containing subunits of $GABA_A$ receptor initiates anxiolytic and sedative, amnesic effects respectively. There is a relationship between the anxiolytic effects of benzodiazepines with the secondary suppression of serotonergic and/or nonadrenergic and other excitatory systems [10, 11]. Assessment of learning, memory function and exploratory behaviors in rodents are broadly conducted by Y-maze test [12]. Anxiety-like behaviour is evaluated in rodents by well known Elevated plus maze model where elevated and open place entry is averted [13, 14]. Promotion of the incidence of escape-related behavior and overturning of immobility position are come about

by several antidepressant medications, such as tricyclic antidepressants (TCA), N-methyl-D-aspartate (NMDA) receptor antagonists or selective serotonin reuptake inhibitors (SSRIs). Instability in some neurotransmitter's role mainly noradrenalin, dopamine and serotonin is responsible for generating depression. One of the most vital etiological features, depletion of serotonin causes depression. Accessibility of extracellular serotonin is boosted up by SSRIs [12]. Uptake of 5-HT and/or noradrenaline are hindered by SSRIs and TCA, which result antidepressant action [15]. Decline in the metabolism of MAO (monoamine oxidase) enzyme system by MAO inhibitors trigger the increment of some endogenous amines like serotonin, catecholamines etc. which exerts antidepressant action [16]. In case of Y-maze test, FME 200 mg/kg and MRS 400 mg/kg exhibited significant ($p < 0.05$, *vs.*control) anti-depressive activity with time which mechanism of action may be as like as TCA, MAO inhibitors, SSRIs or atypical antidepressants whose mode of action is not clear. Moreover, FME 400 mg/kg showed anti-depressive activity as like as FME 200 mg/kg and depressive activity by biding with $GABA_A$ receptor. In addition to, the mode of action of RME 200 mg/kg, RME 400 mg/kg and MRS 200 mg/kg may be similar to FME 400 mg/kg (table-1). In table-2, the mechanism of anti-depressive and depressive activities of FME 200 mg/kg and 400 mg/kg, RME 200 mg/kg and 400 mg/kg, MRS 200 mg/kg and 400 mg/kg may follow FME 400 mg/kg of table-1. Again, from table-3, it can be understood that all the extracts including both low and high doses exhibited anti-depressive and depressive activities which mechanism of action may be as like as FME 400 mg/kg of table-1. Therefore, every extract including both 200 and 400 mg/kg doses demonstrated anxiolytic and depressive activities which mode of action may be due to binding with $GABA_A$ receptor. Generally α_2 subunit of $GABA_A$ receptor is responsible for anxiolytic activity (table-4).

Conclusion

The current outcomes revealed that all of the extracts of *Microcos paniculata and Richardia scabra* might have several neuropharmacological properties, such as depressive, anti-depressive and anxiolytic properties. But further investigations are needed to confirm which neuropharmacological property become eminent and to find out the active components of the extracts for discovering the mechanism of actions in the improvement of neuropharmacological agents. Beisdes, genotoxicity study of these extracts may be a promising area for the researchers.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Table 1. Effect of test groups on Y-maze apparatus after entrance into closed arm.

Group	Doses (mg/kg)	Number of entries into the closed arm				
		0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	3.80±0.37	2.00±0.55	1.00±0.32	1.00±0.32	1.40±0.51
Standard	1 mg/kg	1.60±0.40*	2.20±0.37	2.40±0.24	2.80±0.37	0.40±0.24
FME	200 mg/kg	7.80±0.20*	6.00±0.45*	5.40±0.40*	4.20±0.86*	2.60±0.51
FME	400 mg/kg	4.40±0.60	3.20±0.37	3.60±0.24*	3.80±0.97*	2.00±0.55
RME	200 mg/kg	3.60±0.60	1.00±0.55	0.40±0.24	0.80±0.20	0.40±0.40
RME	400 mg/kg	3.40±0.75	2.20±0.37	1.80±0.49	2.20±0.37	1.20±0.20
MRS	200 mg/kg	4.20±0.58	4.60±0.51*	4.40±0.93*	3.80±0.80*	3.00±0.84
MRS	400 mg/kg	5.80±0.49	3.80±0.20*	3.40±1.17*	2.80±0.86	1.75±0.48

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

Table 2. Effect of test groups on Y-maze apparatus after entrance into open arms.

Group	Doses (mg/kg)	Number of entries into the open arms				
		0 min	30 min	60 min	120 min	180 min
Control	10 mL/kg	4.20±0.49	2.20±0.80	2.80±0.97	1.60±0.40	2.40±0.93
Standard	1 mg/kg	2.20±0.80	1.80±0.37	1.00±0.00	0.80±0.37	0.60±0.24
FME	200 mg/kg	9.80±0.73*	5.20±1.39	6.00±0.84*	3.80±1.02*	5.60±0.81*
FME	400 mg/kg	6.80±0.73*	6.20±0.20*	3.40±0.60	4.80±0.73*	3.60±0.75
RME	200 mg/kg	4.00±0.55	2.00±1.10	0.80±0.37	1.60±0.40	0.80±0.80
RME	400 mg/kg	4.00±0.55	5.20±0.92	2.60±0.40	3.60±0.40	2.80±0.66
MRS	200 mg/kg	0.60±0.24*	2.60±0.40	1.20±0.20	0.40±0.24	0.00±0.00
MRS	400 mg/kg	0.20±0.20*	1.00±0.45	0.80±0.37	0.80±0.20	1.00±0.41

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

Table 3. Effect of test groups on Elevated plus-maze apparatus after entrance into closed arms.

Group	Doses (mg/kg)	Number of entries into the closed arms				
		0 min	30 min	60 min	120 min	180 min
Control	10 ml/kg	1.60±0.40	1.80±0.37	1.80±0.37	1.20±0.20	1.20±0.37
Standard	1 mg/kg	0.20±0.20	1.00±0.32	2.40±0.51	3.00±0.55	0.40±0.24
FME	200 mg/kg	6.20±1.96*	6.40±0.24*	5.40±1.29*	2.80±2.31	2.80±0.10
FME	400 mg/kg	8.00±0.84*	7.80±0.49*	4.40±0.98	4.60±0.68	3.00±1.00
RME	200 mg/kg	7.40±0.24*	4.40±0.24*	3.40±0.68	4.60±0.60	3.60±0.60
RME	400 mg/kg	6.40±0.93*	5.00±0.45*	3.20±0.73	3.80±0.73	4.00±0.55*
MRS	200 mg/kg	1.80±1.80	3.40±0.75	3.40±0.68	1.00±0.00	0.60±0.24
MRS	400 mg/kg	1.20±0.49	2.40±0.40	2.00±0.55	1.00±0.45	1.25±0.25

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

Table 4. Effect of test groups on Elevated plus-maze apparatus after entrance into open arms.

Group	Doses (mg/kg)	Number of entries into the open arms				
		0 min	30 min	60 min	120 min	180 min
Control	10 ml/kg	0.40±0.24	0.60±0.40	0.40±0.40	0.20±0.20	0.40±0.24
Standard	1 mg/kg	1.20±0.73	0.60±0.24	0.40±0.40	0.20±0.20	0.00±0.00
FME	200 mg/kg	1.80±0.20	7.60±1.66*	5.80±1.50*	1.80±0.80*	1.60±0.40
FME	400 mg/kg	2.40±0.93	2.00±0.32	1.60±0.68	0.80±0.20	2.00±0.89
RME	200 mg/kg	1.20±0.73	0.00±0.00	0.40±0.24	0.40±0.24	2.40±0.68
RME	400 mg/kg	5.60±1.66*	0.40±0.24	0.40±0.24	0.40±0.24	0.80±0.37
MRS	200 mg/kg	0.60±0.24	2.60±0.40	1.20±0.20	0.40±0.24	0.00±0.00
MRS	400 mg/kg	0.20±0.20	1.00±0.45	0.80±0.37	0.80±0.20	0.80±0.37

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