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Y-MAZE, ELEVATED PLUS MAZE AND HOLE CROSS TESTS OF *RICHARDIA SCABRA* WHOLE PLANT

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Abstract

The present study was designed to evaluate the neuropharmacological activity of the petroleum ether extract of *Richardia scabra* (whole plant) (PRS) by using y-maze, elevated plus-maze and hole cross test respectively. In Y-maze test, anti-depressive and depressive activities of PRS 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. In the hole cross test, both extracts (PRS 200 mg/kg and 400 mg/kg) showed increasing and decreasing of movement at different observations. The results obtained in the present study point out that PRS can be the possible sources of CNS depressant, anti-depressant and anxiolytic agents. But further investigation is required for the confirmation of their activities.

Keywords: Y-maze, elevated plus maze, hole cross, *Richardia scabra*.

Introduction

At first, the ancient people discovered the CNS acting drugs among various groups of pharmacological agents. Till now, this group of drugs are highly used therapeutic agents rather than others. This category of drugs possess precise physiological as well as psychological effects, which made them as precious therapeutic agents. However, numerous plants showed activities against CNS disorders and eventually act as very useful remedies to relieve human sufferings [1].

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 [2].

Therefore, the present study was designed to evaluate the neuropharmacological activity of the petroleum ether extract of *Richardia scabra* (whole plant) (PRS).

Materials and Methods

Collection and Identification of the Plant materials

The whole plants of Richardia scabra were collected from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh in November, 2012. Sarder Nasir Uddin, Principal Scientific Officer at the Bangladesh National Herbarium verified the identification of the species. In the herbarium the dried specimens were preserved for the utilization as future references.

Extraction

200 g of powdered whole plants of R. scabra were used for the petroleum ether extraction. Plant parts were then washed with running water successively for 3–4 times. Before drying in the shade for a period of 7 d, the plant parts were also rinsed with sterile distilled water. The grinding of the dried parts was then carried out through a laboratory grinding mill (Model 2000 LAB Eriez®) and then passed through a 40-mesh sieve to obtain fine powders. Through a hot extraction procedure accompanied by a Soxhlet apparatus powdered whole plants of R. scabra were extracted in 2 L of petroleum ether. The liquid extract was filtered by using Whatman No.1 filter papers. Then the filtrate was kept in a hot air oven at 40^0C for drying. The petroleum ether extraction yield of R. scabra (whole plants) was 7.2% (w/w). Extract was stored at 4^0C for accessory studies.

Experimental Animals

Sixty Swiss albino mice of both sex having weight of about 25–30 g with 6–7 weeks of old were purchased from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Therefore, the animals were kept under optimum environmental conditions along with relative humidity 55%–65%, 12 h light/12 h dark cycle and (27.00±1.00) ^{0}C temperature. Suitable food supply together with water ad libitum was ensured. Adaptation of the animals to the laboratory conditions for a period of seven days was completed prior to the experiment. All the protocols used in the experiments were approved by the Institutional Animal Ethical Committee of Jessore University of science and technology, Jessore, Bangladesh and were then performed with these animals.

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 [3,4,5]. Twenty 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 (PRS 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)

The method of Lister was utilized to carry out elevated plus-maze test [6]. Here grouping of the mice and sample administration were carried out as like as *y-maze test. The apparatus consisting 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) was kept at a height of 70 cm from the floor level. Each mouse was placed in the centre of elevated plus-maze apparatus. Then the animals were kept under observation for counting the number of entry into the closed and open arms at 0, 30, 60, 120 and 180 min after respective treatment and every counting was continued for 3 min. The entry of all four paws into one arm was termed as arm entry.

Hole Cross Test

According to the method of Takagi et al. [7], the hole cross test was conducted. The apparatus was made of a cage with a size of 30×20×14 cm (length x width x height) where a steel partition was attached in the middle of this cage. Here grouping of the mice and sample administration were also carried out as like as y- maze and elevated Plus-maze Test (EPM). A hole was made at the center of the cage at a height of 7.5 cm which diameter was 3 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). One-way ANOVA following Dunnet's test ($P < 0.05$, vs. control) and Post-hoc Bonferroni test ($P < 0.05$, vs. standard/extract) through the SPSS software (version 20; IBM Corporation, New York, USA). was utilized for statistical analyses of the neuropharmacological studies which was considered statistically significant.

Results

Neuropharmacological Study

Y-maze Test

The result of the table-1 showed that standard drug diazepam revealed depressive activity with time. Moreover, PRS 200 mg/kg exhibited fluctuating effects including both anti-depressive and depressive activity during different observations. Again, depressive effects were disclosed by PRS 400 mg/kg at 30 min, 60 min and 180 min respectively but it showed anti-depressive effect at 3rd observation. From table-2, it was noticeable that standard drug diazepam exerted depressive activity with time. In case of PRS 200 mg/kg, the number of entries into the open arms was gradually decreased with time at 30 min, 60 min, 120 min and 180 min respectively that indicated the depressive effects. Besides, PRS 400 mg/kg exhibited both depressive and anti-depressive effects at 30 min, 180 min and 60 min, 120 min respectively.

Elevated Plus-Maze Test (EPM)

The result of the table-3 showed that standard drug diazepam revealed depressive activity with time. Moreover, PRS 200 mg/kg demonstrated both depressive and anxiolytic effects at different observations. Again, anxiolytic effects were showed by PRS 400 mg/kg at all observations except 4th observation. From table-4, it was obvious that standard drug diazepam exhibited depressive activity with time. Moreover, PRS 200 mg/kg exerted anxiolytic effect at 30 min but from 2nd observation it started to reveal depressive effects. Again, depressive effects were showed by PRS 400 mg/kg during all observations except 3rd observation.

Hole Cross Test

Hole cross test was utilized for the evaluation of CNS depressant property of PRS. In case of PRS 200 mg/kg, gradual decrease of movement was noticed during different observations but it exerted increase of movement during 60 min. Besides, PRS 400 mg/kg demonstrated fluctuating effects in movement during different observations. Again it showed maximum number of movement at 2nd observation (Table-5).

Discussion

Plant-derived products play a significant role in traditional system of medicine. In the present study, the effect of chloroform extract of Microcos paniculata bark was evaluated for neuropharmacological activity. The assessment of CNS activity of any drug depends on the locomotor activities of animals. The estimation of the level of excitability of the CNS refers to the locomotor activity of animal. An increase in alertness is regarded as locomotor activity and reduction in locomotor activity is an indication of sedative effect [8]. There is a close relationship between reduced locomotor activity and sedation which is derived from CNS depression [9]. The number of arm entries of Y-maze and Elevated plus-maze test apparatus was observed for the determination of locomotor activity [10]. Generally CNS depressant drugs expose their action through GABAA receptor [9]. CNS depressant activity can be exerted due to the higher concentration of GABAA receptor in brain [11].

$GABA_A$ receptor contains various subtypes and at least 17 subunits including $\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$ and others (single ε, θ, π and δ) are responsible for the diverse

arrangements of its subtypes. Benzodiazepines bind with $GABA_A$ receptor containing α_2 and α_1 subunits and elicit anxiolytic and sedative, amnesic effects respectively. Anxiolytic effects of benzodiazepines are related to the secondary suppression of serotonergic and/or nonadrenergic and other excitatory systems [12, 13]. Assessment of learning, memory function and exploratory behaviors in rodents are broadly conducted by Y-maze test [14]. At present it has been reported that numerous plants elicit their anxiolytic effects by animal models of anxiety [15, 16]. EPM is one of them utilized for the evaluation of anxiolytic effect of drugs [17, 18]. Besides, anxiolytic agents enhance the frequency of entries as well as time spent in open arms of the EPM [17]. Anxiolytic compounds not only decrease the natural animal phobia to the open arms but also improve exploration in the elevated-plus maze test. Otherwise, the forced or voluntary passages of the animal into the closed arms of the EPM along with hormonal and behavioral changes are the indication of increased anxiety [19, 20]. It was reported by Montgomery (1955) that when placed in mazes consisting of both open and closed arms the rodents spend more time in closed arms. Abstaining open arm indicates an exposure of fear and anxiety. On the basis of these statements, the elevated plus maze test has been a tool for the identification of selective anxiolytic effect of drugs [21]. Handley & Mithani (1984) further reported that rodents avoid the open arms and also demonstrated that diazepam, an anxiolytic agent decreased the aversion of open arm [22].

Elevated plus maze, a well known model is utilized for the assessment of anxiety-like behaviour in rodents in which elevated and open place entry is avoided [23, 24]. Different antidepressant drugs including TCA, NMDA receptor antagonists or SSRIs enhance the occurrence of escape-related behavior and overtune the immobility position. Depression is originated due to the oscillation of the role of neurotransmitters mainly serotonin, noradrenalin and dopamine. Lack of serotonin results in depression which is one of the potential causative features. SSRIs boost up the accessibility of extracellular serotonin [14]. SSRIs and TCA elicit antidepressant action through the inhibition of uptake of 5-HT and/or noradrenaline [25]. MAO inhibitors exert their antidepressant action through the reduction of metabolism of MAO enzyme system as well as the enhancement of some endogenous amines like serotonin, catecholamines etc. [26]. In case of Y-maze test, PRS 200 mg/kg and 400 mg/kg exhibited depressive activity by binding with $GABA_A$ receptor. Moreover, both of the extract (PRS 200 mg/kg and 400 mg/kg) exerted anti-depressive activity which mechanism of action may be as like as TCA, MAO inhibitors, SSRIs or atypical antidepressants whose mode of action is not clear (table-1). In table-2, the mechanism of depressive activity of PRS 200 mg/kg may be due to binding with $GABA_A$ receptor. Again, higher dose of PRS showed both depressive and anti-depressive activities which mechanism of action may be as like as table-1. In elevated plus-maze test, every extract of PRS showed depressive activity which mechanism of action may be as like as y maze test (table-3). In table 4, PRS 200 mg/kg and 400 mg/kg demonstrated anxiolytic activity which mechanism of action may be due to binding with α_2 subunit of $GABA_A$ receptor. In hole cross test, both decrease and increase of movements were noticed which mechanism of action may be as like as table-1(table-5).

Conclusion

The current results demonstrated that petroleum ether extract of *R. scabra* (whole plant) might possess several neuropharmacological activities including depressive, anti-depressive and anxiolytic activities. But further studies are required to identify exact bioactive compounds responsible for the neuropharmacological effects as well as to fully elucidate the underlying mechanism of action for the development of neuropharmacological agents. Beisdes, genotoxicity study of this extract may be a prominent area for the researchers.

Conflict of interests

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

List of abbreviations

PRS = petroleum ether extract of *Richardia scabra*
CNS = Central Nervous System
P.O. = Per Oral
EPM = Elevated Plus-Maze
GABA = Gamma Amino Butyric Acid
TCA = Tricyclic Antidepressant
NMDA = N- methyl-D-aspartate
SSRIs = Selective Serotonin Reuptake Inhibitors
MAO = Monoamine Oxidase

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

No. of movement in closed arm						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	4.38±0.45	4.34±0.32	4.34±0.40	4.38±0.51	4.30±0.13
Standard	1 mg/kg	1.20±0.37 *	1.80±0.22 *	2.42±0.31 *	2.70±0.42 *	3.00±0.49
PRS	200 mg/kg	9.80±0.65 * □	5.20±0.33 □	6.00±0.51 * □	3.80±0.37	5.40±0.44 □
PRS	400 mg/kg	6.80±0.48 * □ ■	6.20±0.66 * □	3.40±0.33 ■	4.80±0.46 □	3.20±0.58 ■

Number of movement in closed arms is presented as mean ± standard error. *P<0.05, *vs* control (Dunnett's *t* test); □P ＜0.05, *vs* standard; ■P ＜0.05, *vs* PRS 200 mg/kg (pair-wise comparison by post-hoc Bonferroni test).

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

No. of movement in open arms						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	3.96±0.37	3.94±0.55	3.98±0.32	3.98±0.32	3.99±0.51
Standard	1 mg/kg	3.60±0.26	3.24±0.38	2.78±0.12	2.16±0.09 *	1.80±0.20 *
PRS	200 mg/kg	7.80±0.66 * □	6.00±0.52 * □	5.40±0.71□	4.20±0.59 □	2.60±0.52
PRS	400 mg/kg	4.40±0.23 ■	3.20±0.26 ■	3.60±0.24 ■	3.80±0.34 □	2.00±0.40 *

Number of movement in open arms is presented as mean ± standard error. *P<0.05, *vs* control (Dunnett's *t* test); □P ＜0.05, *vs* standard; ■P ＜0.05, *vs* PRS 200 group (pair-wise comparison by post-hoc Bonferroni test).

Table 3. Effect of PRS on elevated plus maze apparatus after entrance into closed arms.

No. of movement in closed arms						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	1.30±0.39	1.36±0.19	1.32±0.25	1.36±0.36	1.30±0.48
Standard	1 mg/kg	0.60±0.19	1.20±0.54	1.80±0.83	2.30±0.97	3.00±0.61
PRS	200 mg/kg	1.80±0.14 □	5.80±0.55 * □	5.80±0.65 * □	1.80±0.28	1.60±0.21
PRS	400 mg/kg	2.40±0.32 * □	2.00±0.39 ■	1.60±0.39 ■	0.80±0.30	2.00±0.59

Number of movement in closed arms is presented as mean ± standard error. *P<0.05, *vs* control (Dunnett's *t* test); □P ＜0.05, *vs* standard; ■P ＜0.05, *vs* PRS 200 mg/kg (pair-wise comparison by post-hoc Bonferroni test).

Table 4. Effect of PRS on elevated plus maze apparatus after entrance into open arms.

No. of movement in open arms						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	1.60±0.30	1.80±0.34	1.80±0.53	1.70±0.40	1.58±0.44
Standard	1 mg/kg	3.00±0.40	2.40±0.54	1.00±0.30	0.40±0.14	0.20±0.05
PRS	200 mg/kg	6.20±0.32 * □	6.40±0.25 * □	5.40±0.57 * □	2.80±0.44 □	2.80±0.76 □
PRS	400 mg/kg	8.00±0.74 * □	7.80±0.70 * □	4.40±0.66 * □	4.60±0.73 *□	3.00±0.29 □

Number of movement in open arms is presented as mean ± standard error. *P<0.05, *vs* control (Dunnett's *t* test); □P ＜0.05, *vs* standard (pair-wise comparison by post-hoc Bonferroni test).

Table 5. Effect of PRS on hole cross test.

No. of movement						
Group	**Dose**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**
Control	10 mL/kg	3.20±0.35	3.24±0.31	3.22±0.49	3.22±0.25	3.26±0.27
Standard	1 mg/kg	3.00±0.32	1.00±0.29 *	0.80±0.20 *	0.20±0.03 *	0.10±0.03 *
PRS	200 mg/kg	3.40±0.32	1.80±0.39 *	2.20±0.48	1.40±0.31 * □	1.40±0.50 *
PRS	400 mg/kg	3.40±0.60	1.40±0.42 *	2.60±0.47	1.40±0.37 * □	1.60±0.69 *

Number of movement is presented as mean ± standard error. *P<0.05, *vs* control (Dunnett's *t* test); □P ＜0.05, *vs* standard (pair-wise comparison by post-hoc Bonferroni test).