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Evaluation of Sedative and Hypnotic Activities of Ethanolic Extract of Leaves of *Cleome Rutidosperma* DC. (Capparidaceae) in Mice

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Abstract

Objectives: *Cleome rutidosperma* DC. (Capparidaceae) traditionally used medicine for insomnia and depression management. The aim of the recent study conducted to investigate the sedative and hypnotic activities of ethanolic extract from the leaves of *C. rutidosperma* (EECR) in Swiss Albino mice.

Methods: Neuropharmacological test on mice employed to this study sedative (open field, hole cross, hole-board and rota-rod tests) and hypnotic (thiopental sodium-induce sleeping test) activities of EECR in mice model. The acute toxicity study and phytochemical analysis of EECR also carried out.

Results: EECR exhibits significant ($p < 0.001$) sedative effect by decreasing the number of (square and hole) crossed by mice in open field and hole cross tests as a dose-dependent (100-400 mg/kg) manner. Hole-board test significantly ($p < 0.001$) decreased the number of head dips and showed the similar effect as diazepam (1 mg/kg) in a dose-dependent manner. Rota-rod test also explicit significant ($p < 0.001$) decreased the performance time as well as increased number of falls in a dose-dependent manner. Thiopental sodium-induce sleeping test expressed that EECR significantly ($p < 0.001$) produce shortening latency period and prolonging the sleeping time in a dose-dependent (100- 400 mg/kg) manner.

Conclusion: The experimental result indicates, *C. rutidosperma* containing phytochemicals that possess sedative and hypnotic activity which traditionally used as insomnia and depression management.

Keywords: *Cleome rutidosperma*, Capparidaceae, Sedative, Hypnotic, Phytochemicals

Abbreviations: EECR: Ethanolic Extract of *Cleome Rutidosperma*; ICDDR, B: International Center for Diarrheal Disease and Research, Bangladesh; CNS: Central Nervous System; GABA: Gamma Amino butyric Acid; TS: Thiopental Sodium

Introduction

Natural products found from plants have been conveying a vital role among human being since ancient

times. An enormous number of scientific reports evidenced of using the medicinal plants as natural remedies. Nowadays, the medicinal plant using as an alternative to synthetic drugs [1]. *Cleome rutidosperma* DC

is purple color flower regionally known as Fringed Spider. Cleome 33 genus of the family Capparidaceae which is a native plant of Tropical Africa. However, *C.rutidosperma* has also found in different parts of tropical America and Southeast Asia as well as the naturalized plant [2]. In Bangladesh, it is locally known as Beguni Hurhuria Ful. It is a ubiquitous weed of lawns. *C.rutidosperma* has been well-studied by different researchers. Traditionally, the ancient people used various parts of Cleome genus like roots, leaves, and seeds as antiscorbutic, anthelmintic, rubefacient, vesicant and carminative [3]. People of some regions of Malaysia planted *C.rutidosperma* at the edges of the field to protect crop which considers as insect repeller [4]. *C.rutidosperma* has several types of pharmacological evidence as Antiplasmodial [5], diuretic, laxative, analgesic, anti-inflammatory, antipyretic. *C.rutidosperma* root exhibits the significant association on the hypoglycemia and has an anthelmintic effect [6]. *C.rutidosperma* was traditionally popular among ancient people as the treatment of paralysis, epilepsy, convulsions, spasm, and earache, different kind of pain and skin disease [4]. Although *C.rutidosperma* is the ethnomedicinal plant, the phytochemical analysis reveals the CNS depressant activity in animal models [7]. However, no scientific literature reported about sedative and hypnotic activity of *C.rutidosperma*. Due to the lack of knowledge and traditional use, we are convinced to design our recent study explores *C.rutidosperma* leaves sedative and hypnotic activity in the mice models.

Methods

Plant collection and extraction

C.rutidosperma leaves collected from beribadh, Mirpur, Dhaka, Bangladesh. The time of collection was 1st July 2013. The specific identification and authentication of *C.rutidosperma* plant for the present study authenticated by the Botanist Bushra Khan the Scientific Officer of Bangladesh National Herbarium which situated at Mirpur in Dhaka, Bangladesh. The verified voucher specimen Code (DACB: 38625) deposited at Herbarium center for further references. The dried *C.rutidosperma* leaves (250 g) soaked into 900mL ethanol. During the extraction process that soaked leaves stirring with glass rod every 18 h interval. Further, raw solvent extraction filtered with sterile cotton set inside Buchner funnel give the final yield of 5.6 % (14 g) crude extracts after solvent evaporation using a rotary evaporator. The crude extract analyzed for the sedative and hypnotic activity in the mice model.

Experimental animals

We selected Swiss Albino mice (20-25 g) for our experiments. We collected those mice from the Animal Research Institution of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were nursing standard environmental conditions. Additionally, we maintained a particular temperature: $25 \pm 2^{\circ}\text{C}$; relative humidity: 55-65% in 12h light/ dark cycle where mice kept for the experimental purpose. ICDDR, B food pellets were given to mice every time and fresh water *ad libitum* during habituation with environments. The animal's habituation with laboratory environments for 14 days before the experiments. Before the experiment, we selected ten mice of each group as the control, Positive control and three EECR test groups such as 100,200, and 400 mg/kg. Experimental mice kept overnight without food before starting the pharmacological activity test. We followed Ethical Principles and Guidelines for mice observations which provided by Scientific Experiments on Animals (1995). This guideline created by Swiss Academy of Medical Sciences and Swiss Academy of Science. Our research program approved by the Institutional Animal Ethical Committee (SUB/IAEC/ 13.07) of Stamford University Bangladesh.

Treatments

The mice distributed to five groups containing ten animals each for control, positive control, and EECR for every experiment. We dissolved EECR with pharmaceutical grade 0.9% saline water. We administered the EECR at 100, 200, and 400 mg/kg doses considered to body weight 30 min before the experimental observation. Contrary, the standard drug diazepam (1 mg/kg) employed in all tests animal as intraperitoneal (i.p.) routes considered before 15 min of pharmacological observation whereas, thiopental sodium (40 mg/kg) in sleeping time measurement test administered 15 min after treatment with diazepam and 30 min of 0.9% saline water or EECR. The animals of the control group provided 0.9% saline water (0.1 mL\mouse) 30 min before, during the experiments.

Phytochemical screening

The phytochemicals of ethanolic extract of *C.rutidosperma* qualitatively analysis followed by the standard procedure for the presence of alkaloids, flavonoids, tannins, glycosides, reducing sugar, saponins, and steroids [8].

Acute toxicity test

Experimental mice divided into control, positive control, and test groups, each group contains ten mice ($n = 10$) during experiments. The oral administration range of EECR at 200-3000 mg/kg doses of the body weight in the test animals. The mice were given access to food and water during the observation periods. Meanwhile, we observed allergic manifestations and mortality rates of mice for next 72h [9].

Open field test

The apparatus made of wood containing black and white area printed half square meter of a sequence [10]. The wall of open field cage height about 50 cm. During experiments, treated mice with diazepam and EECR placed in the center of the open field apparatus. The black and white area travelled by each mouse noticed during 3 minutes observation period (30, 60, 90 and 120 min) intervals (after the treatments).

Hole cross test

The hole cross apparatus made of a wooden box (30cm×20cm×14cm) which attached with a partition in the center contains a hole (3cm diameter) [11]. The animals treated with either 0.9% saline water or drug or EECR and performed to cross the hole from one compartment to another. Mice observed for 3 minutes and the number of passages recorded at the different time (30, 60, 90, and 120 minutes).

Hole-board test

The hole board apparatus made of a wooden square box (40cm×40cm×25cm) with uniformed sixteen holes at a particular diameter (3 cm) on the ground [12]. The apparatus ground placed 15 cm above from the floor separated into 10cm×10cm squares with a water-resistant marker. During observation treated mouse with diazepam and EECR positioned at the center of hole-board and recorded the head dips with both eyes for 5 minutes.

Rota-rod test

Motor coordination and performance of each mouse evaluated 30 minutes after oral treatment of EECR in a rota-rod apparatus. Experimental mice divided into five groups ($n = 10$) and allowed each mouse to 32 mm horizontal wooden rod to performing 3 minutes at 20 rpm. The performance time of mice observed (in second) contrary, the number of falls once considered passing

the test [13].

Thiopental sodium-induced sleeping time test

Sleeping time test evaluation done after 30 minutes post-administration of EECR and 0.9% saline water whereas, 15 minutes of diazepam administration. Thiopental sodium (TS) injected each mouse as intraperitoneally (40 mg/kg). Mice observed until losing their reflex, immediately after post-administration of thiopental sodium injection (latent period) as well as the duration of sleeping (time between the loss and recovery of reflex) induced by TS [14].

Statistical Analysis

The results presented as mean $\pm$ SEM. The statistical analysis performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test as appropriate using SPSS 20 software whereas open field and hole cross tests analysed by ANOVA with repeated measures. Differences between groups were considered significant at a level of $p < 0.001$, $p < 0.01$ and $p < 0.05$.

Results

Preliminary phytochemical screening

The quantitative phytochemical analysis of EECR expressed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, steroids and reducing sugar (Table 1).

Open field test

The number of squares crossed by the mice decreased significantly ($p < 0.001$) and dose-dependent manner demonstrated for the second (30 min) to fifth (120 min) observation period at 100, 200 and 400 mg/kg doses when compared with control. It is important to mention that sedative used in the positive control (diazepam 1 mg/kg), induced similar effects observed by EECR (Table 2).

Hole cross test

The hole crossed tendency reduced significantly ($p < 0.001$) and dose-dependent manner from the second (30 min) to fifth (120 min) observation periods at 100, 200 and 400 compared with the control. Moreover, the positive control (diazepam 1 mg/kg) decrement a similar modification observed by EECR (Table 3).

Hole-board test

In respect to hole-board observation, the tendency of

head dips significantly ($p < 0.001$) decrease with a dose-dependent manner at 100, 200 and 400 mg/kg (after 30 min of oral administration) while comparing with control. The similar effect found in mice treated with diazepam (1 mg/kg) (Table 4).

Table 1: Preliminary qualitative phytochemical screening of EECR

Phytochemical constituents	Tests	Inferences
Alkaloids	Mayer's test	+
	Hager's reagent test	+
	Wagner's reagent test	+
	Dragendorff's test	+
Glycosides	Modified borntreger's test	+
Flavonoids	Lead acetate test	+
	Molisch's test	+
Tannins	Potassium Dichromate Test	+
	Ferric Chloride Test	+
Saponins	Frothing test	+
	Foam test	+
Sterols	Libermann-burchard's test	+
Reducing sugar	Benedict's test	+
	Fehling's Test	+
Key: + present		

Rota-rod test

In rota-rod observations, the performance time significantly ($p < 0.001$) decreased when the number of falls increased in a dose-dependent manner at 100, 200 and 400 mg/kg doses, compared with control (Table 5).

Thiopental sodium-induced sleeping time test

The onset of sleep significantly ($p < 0.001$) decreased observed in TS- sleeping test in a dose-dependent manner. Additionally, significant ($p < 0.001$) increase total sleeping time duration follow in mice treated with EECR at 100, 200 and 400 mg/kg doses when compared with control. Diazepam 1 mg/kg as a positive control also produce a similar effect as well as EECR (Table 6).

Discussion

Our experimental findings explored the neuropharmacological activity of ethanolic leaves extracts

of *C.rutidosperma*. The results represented that EECR shows the sedative and hypnotic effects of enhancing the response gamma amino butyric acid (GABA) receptor on central nervous system (CNS). Additionally, it found that EECR administration at 2000 mg/kg doses did not show any allergic manifestations and mortality rates of mice during 72 h observation. Thus, our evidence endow that EECR has no toxicity to our experimental dose (2000 mg/kg).

Our preliminary conveyed alkaloids, glycosides, flavonoids, tannins, saponins, steroids, and reducing sugar presence (Table 1). Experimental evidence proof that the alkaloids, glycosides, steroids, and flavonoids abundant plant and plant extracts possess sedative and hypnotic properties [15] mediated through their affinity (in vitro) with benzodiazepine site of the GABAergic complex system modulators of this receptor [16]. Correspondingly, non-specific CNS depression associated with tannin [17]. Therefore, it seems that the phytochemicals mentioned above present in the EECR may exist the sedative and hypnotic effects on the CNS.

Although our investigation started with the open field and hole cross test, the sedative effects of EECR which recording by the spontaneous locomotor activity of mice. In these behavioral tests, sedative properties of diazepam decreased the number of movements as well as expound on the new environment (Figure 1). Hence, the oral administration of EECR successively exhibits the sedative activity by those tests.

Figure 1: The sedative effect of EECR on the open field test (number of square crossed at 30mins, 60mins, 90mins and 120mins) in mice.

Locomotion effects remarkably decreased during first observation (30 min) and the fourth observation (120 min) after oral administration of EECR. Moreover, the similar type of responses investigated in hole cross models. The

hole cross number decreased at first (30 min) observation and continued to fourth (120 min) observation period (Figure 2). This efficacy of EECR convinced us about the CNS depressant activity which abolished locomotion capacity.

Figure 2: The sedative effect of EECR on the hole cross test (number of hole crossed at 30mins, 60mins, 90mins and 120mins) in mice.

Sedative and hypnotic effects investigated by hole board test which is one of the most popular methods for determination of exploratory behavior in mice. This method is acceptable for its methodological advantages. Additionally, different behavioral responses of mice observed in unfamiliar environments. It endorsed that the head dipping behavior and emotional state entirely related to mice [18]. This inspection suggests that the emotional assertion of the anxiolytic condition as head dipping activity usually increase in mice. Contrary, the behavior of head dips decrease associated with depressant effects [19]. Our results revealed that the EECR diminished number of head dips in a dose-dependent manner in mice which proposed that EECR exhibits sedative activity rather than anxiolytic effects. The sedative effects obtained from the treated groups markedly significant ($p < 0.001$) than the control (Figure 3).

Rota-rod test reliable method to investigate muscle relaxation effects in mice model. Our results demonstrated that treatment with EECR at 100, 200, and 400 mg/kg doses notably increases ($p < 0.001$) the number of decreased the performing time from the rotating rod (Figure 4). It observed that like diazepam cause muscle weakness [20], the reduce of ambulatory activity, and sedation, thus

diminishing activity in the rota-rod [21]. As expected, we also found that diazepam at 1 mg/kg dose caused muscle relaxation of the animals and increment of falling time in rota-rod. This similar effect swayed us to conceive that EECR induced sedative effect by affecting the general activity and motor coordination of mice as like diazepam.

Figure 3: The sedative effect of EECR on hole board test (number of head dips) in mice.

Moreover, our above findings suggested us the result obtained from the thiopental sodium-induced sleeping time determination test. This behavioral pharmacology method considers as suitable to determine the sedative and hypnotic properties. This recent study, treatment with 100, 200, and 400 mg/kg doses of EECR, TS injection significantly modified the time of latency to induce sleep before 30 min while the prolonged hypnosis increased by Thiopental sodium (Figure 5). The almost same pattern of outcome also inspected with the diazepam (1 mg/kg). The observation revealed that the Thiopental sodium, attached to the specific barbiturate allosteric attachment site on GABA_A receptor and potentiate postsynaptically GABA_A-mediated hyperpolarization in the brain. Present experimental data suggest that there may be a similarity between the sedative action originated by EECR and diazepam sedation induced capability. Therefore, the possibility of the GABA_A subunit is permitted to take part in the EECR-induced intensification of TS response.

Figure 4: The sedative effect of EECR on the rota rod test (performance time as seconds and number of falls) in mice.

Figure 5: The hypnotic effect of EECR on the thiopental sodium induce sleeping test (Latency time and sleeping time) in mice.

Table 2: The sedative effect of EECR on the open field test in mice.

Treatment	Dose (mg/kg)	Number of squares crossed			
		30mins.	60mins.	90mins.	120mins.
Control	0.1ml/mouse	102±2.92	105.80±2.41	101.00±2.30	95.40±2.04
Diazepam	1	27.60±1.91***	22.00±1.00***	10.60±0.51***	5.20±0.66***
EECR	100	94.60±1.88*	90.60±1.96***	72.00±2.25***	8.60±1.83***
EECR	200	72.00±1.22***	63.40±1.20***	35.80±1.15***	15.60±2.31***
EECR	400	45.00±1.41***	33.40±1.47***	22.00±0.89***	8.80±0.97***

Each value is presented as the mean ± SEM (n = 10), EECR = Ethanolic extract of *Cleome rutidosperma* leaves. ***p < 0.001; **p < 0.01; *p < 0.05 compared with the control group (Dunnett's Test)

Table 3: The sedative effect of EECR on the hole cross test in mice.

Treatment	Dose	Number of holes crossed			
	(mg/kg)	30mins.	60mins.	90mins.	120mins.
Control	0.1ml/mouse	29.40±2.52	21.80±0.97	20.00±1.30	18.40±0.51
Diazepam	1	11.20±0.58***	6.40±0.24***	5.00±0.44***	1.40±0.74***
EECR	100	24.00±1.61	14.60±0.98***	12.20±0.80***	11.00±0.89***
EECR	200	19.00±1.97*	11.40±0.98***	8.00±0.63***	5.80±0.58***
EECR	400	17.60±1.43**	8.60±0.24***	6.60±0.60***	3.60±0.74***

Each value is presented as the mean ± SEM (n = 10), EECR = Ethanolic extract of *Cleome rutidosperma* leaves.
 ***p < 0.001; **p < 0.01; *p < 0.05 compared with the control group (Dunnett's Test)

Table 4: The sedative effect of EECR on hole-board test in mice.

Treatment	Dose (mg/kg)	Number of head dips
Control	0.1ml/mouse	51.20±2.57
Diazepam	1	13.00±0.89***
EECR	100	33.00±2.19***
EECR	200	23.20±1.46***
EECR	400	16.40±0.87***

Each value is presented as the mean ± SEM (n = 10), EECR = Ethanolic extract of *Cleome rutidosperma* leaves.
 ***p < 0.001; **p < 0.01; *p < 0.05 compared with the control group (Dunnett's Test)

Table 5: The sedative effect of EECR on rota-rod test in mice.

Treatment	Dose (mg/kg)	Performance time(s)	Number of falls
Control	0.1ml/mouse	176.00±1.89	1.40±0.74
Diazepam	1	113.40±2.65***	17.40±1.28***
MEPH	100	163.40±1.88**	10.00±1.41*
MEPH	200	141.60±1.91***	13.80±2.28***
MEPH	400	118.60±0.92***	16.20±1.56***

Each value is presented as the mean ± SEM (n = 10), EECR = Ethanolic extract of *Cleome rutidosperma* leaves.
 ***p < 0.001; **p < 0.01; *p < 0.05 compared with the control group (Dunnett's Test).

Table 6: The hypnotic effect of EECR on thiopental sodium-induced sleeping time test in mice.

Treatment	Dose (mg/kg)	Onset of action (min)	Duration of sleeping time (min)
Control	0.1ml/mouse	15.05±1.04	89.00±2.09
Diazepam	1	6.82±0.06***	188.40±1.88***
EECR	100	10.12±0.29***	132.00±1.26***
EECR	200	8.40±0.29***	164.00±1.18***
EECR	400	7.24±0.11***	176.60±1.32***

Each value is presented as the mean ± SEM (n = 10), EECR = Ethanolic extract of *Cleome rutidosperma* leaves.
 ***p < 0.001; **p < 0.01; *p < 0.05 compared with the control group (Dunnett's Test).

Conclusions

The present experimental findings in our study show that *C.rutidosperma* possesses strong sedative and hypnotic activities. Sedative and hypnotic principally mediated by the GABA_A receptor complex in the CNS, which also associated with neurological function and disorders. However, further studies necessary to isolate the phytochemical components and the precise mechanisms of action responsible for the pharmacological activities of the plant.

Declarations

Ethics approval and consent to participate

All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/IAEC/13.07) of Stamford University Bangladesh approved all experimental rules.

Availability of data and material

Not applicable

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Competing interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

Authors' contributions

Md. Shahed-Al-Mahmud coordinated all laboratory experiments, analyzed and interpreted results. Hasib Al Hasan conducted acute toxicity test. Md Jalal Ahmed Shawon did phytochemical analysis, open field test, hole cross test, hole board test, rota-rod test, thiopental sodium sleeping test and interpreted all experiments. Assistant professor Rashidur Rahman supervised our research. Md. Shahed-Al-Mahmud did statistical analysis and drafted the manuscript. All authors read and approved the manuscript.

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