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EVALUATION OF CNS ACTIVITIES OF ETHANOL EXTRACT OF ROOTS AND RHIZOMES OF *CYPERUS ROTUNDUS* IN MICE

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Abstract: The ethanol extract of *Cyperus rotundus* (EECR) was tested for possible pharmacological effects on experimental animals. EECR significantly potentiated the sleeping time of mice induced by standard hypnotics, viz. pentobarbitone sodium, diazepam, and meprobamate in a dose dependent manner. EECR showed significant analgesic properties as evidenced by the significant reduction in the number of writhes and stretches induced in mice by 1.2 % acetic acid solution. It also potentiated analgesia induced by morphine and pethidine in mice. Pretreatment with EECR caused significant protection against strychnine and leptazol-induced convulsions. The behavioral studies on mice indicate CNS depressant activity of the ethanol extract of *C. rotundus*.

Keywords: *Cyperus rotundus*, sleeping time, general behavior, analgesic activity, anticonvulsant activity

Cyperus rotundus L., Muthaghas (Bengali), Motha (Hindi), *Nutgrass* (English); family: Cyperaceae) is a perennial sedge distributed throughout India. The roots and rhizomes of this plant are used in different diseases like chronic diarrhoea, inflammation, skin rashes and excess bleeding. It has also antiestrogenic, antimicrobial, anthelmintic, antihistaminic, antiemetic, antipyretic and antidiabetic activities (1-3). The roots and rhizomes of *C. rotundus* on preliminary chemical analysis are found to contain β-sitosterol, cyperene, cyperol, flavonoids, sesquiterpenoids, ascorbic acid and polyphenols (4-6). The ethanol extract of *C. rotundus* (EECR) showed marked CNS depressant action compared to other extracts of it in preliminary pharmacological screening. However, no work has been reported on the CNS activities of this plant. Keeping this in view, the present study has been undertaken to investigate various CNS activities such as behavioral, sedative-hypnotic, analgesic, and anticonvulsant effects of EECR in mice to substantiate the folklore claim.

EXPERIMENTAL

Fresh roots and rhizomes of *C. rotundus* L. were collected from Panua, in the district of Bankura, West Bengal, in the month of June and were authenticated by Dr H. J. Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen has been preserved in our laboratory for future reference (DPS 1).

Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40-60°C), chloroform, ethanol and distilled water using a Soxhlet extractor. The extracts were concentrated to dryness in vacuum. The yield of ethanol extract (EECR) was 12.3 %.

Chemical investigation of EECR

The ethanol extract was subjected to silica gel preparative TLC, where two compounds were isolated using chloroform : ethanol (9:1, v/v) as solvent system. Compound A (mp. 210°C, R_f value 0.59, λ_{max} 224.2 nm) having characteristic IR (Perkin Elmer, IR-297) peaks at 3350, 2930, 2850, 1715, 1690, 1640, 1600, 1450, 1380 and 1300 cm^{-1} and compound B (mp. 255°C, R_f value 0.64, λ_{max} 220 nm) having characteristic IR peaks at 2930, 2850, 1700, 1600, 1450, 1380 and 1300 cm^{-1} suggest the structural similarities with the polyphenolic type of compounds (7).

Animals and treatment

Adult Swiss albino mice of either sex (22 ± 2 g) obtained from B.N. Ghosh & Co., Kolkata, were

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acclimatized to normal laboratory conditions for one week and given pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. All experiments were performed between 8 a.m. to 12 p.m. to minimize circadian influences. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared before starting. The animals were handled according to the guidelines of the committee for the purpose of control and supervision on experimental animals (CPCSEA), New Delhi. For the pharmacological testing, the ethanol extract of C. *rotundus* (EECR) was dissolved in propylene glycol.

Toxicity study

An acute toxicity study relating to the determination of the LD_{50} value was performed with different doses of EECR into different group of mice, each containing 10 animals, according to the method described by Litchfield and Wilcoxon (8).

Effect on sleeping time

Mice were divided into 4 groups, each group containing 6 mice. The animals of group **I** served as the control (normal saline, 0.9% w/v NaCl, 5 mL/kg); groups **II**, **III**, and **IV** received EECR at a low, medium and high dose (40 mg/kg, 60 mg/kg and 80 mg/kg, respectively). Normal saline and the extracts were injected intraperitoneally 30 min prior to the administration of pentobarbitone sodium (40 mg/kg, *i.p.*), diazepam (3 mg/kg, *i.p.*) and meprobamate (100 mg/kg, *i.p.*). The sleeping time was noted by recording the interval between the losses and regaining of righting reflex (9, 10).

Analgesic properties

The analgesic activity was tested by the following methods:

Acetic acid-induced writhing (chemical stimulus) method

This method, involved intraperitoneal injection of freshly prepared 1.2% v/v acetic acid. The number of abdominal constrictions (writhing) and stretching with a jerk at the hind limbs and bending of trunk were counted between 5 and 15 min after administration of acetic acid (11-14). The analgesic effect of the drugs was calculated by the percentage inhibition of writhing episode over that of the control group. The results were compared with those of acetylsalicylic acid, (68 mg/kg), paracetamol (68 mg/kg), and morphine sulfate (1.15 mg/kg).

Thermal stimulus by Eddys hot plate method

The analgesic actions were studied using Eddys hot plate method (15). The reaction time was taken as the interval extending from the moment the mouse reached the hot plate till the animal licked its feet or jumped out of the cylinder. The reaction time was recorded at 30, 45, 60, 90, 120, 150, and 180 min after intraperitoneal injection of EECR at doses of 40, 60, and 80 mg/kg. The temperature of the hot plate was maintained at 55 ± 0.5°C. A cut off reaction time of 30 s was chosen in order to avoid the physical injury. Morphine and pethidine were used as reference drugs at doses of 5 and 10 mg/kg, *i.p.*, respectively. EECR was given individually and also 15 min prior to the administration of reference drugs to investigate the potentiation of morphine and pethidine activity (16, 17).

Anticonvulsant activities

The anticonvulsant property of EECR (25, 40, 60, and 80 mg/kg, *i.p.*) was tested against two standard drugs, strychnine (2 mg/kg, *i.p.*) and leptazol (80 mg/kg, *i.p.*). The average survival time (min) and percentage of mortality after 24 h were recorded (17-20).

Table 1. Effect of EECR on sleeping time (min) induced by pentobarbitone, diazepam and meprobamate in mice

Treatment	Sleeping time (min) induced by		
	Pentobarbitone (40 mg/kg, *i.p.*)	Meprobamate (100 mg/kg, *i.p.*)	Diazepam (3 mg,kg, *i.p.*)
Control (NS, 5 mL/kg, *i.p.*)	40.5 ± 0.94	61.8 ± 0.91	75.0 ± 0.88
EECR (40 mg/kg, *i.p.*)	81.2 ± 2.05[a]	95.5 ± 1.95[a]	138.9 ± 3.05[a]
EECR (60 mg/kg, *i.p.*)	100.5 ± 2.15[a]	117.0 ± 2.03[a]	198.7 ± 3.40[a]
EECR (80 mg/kg, *i.p.*)	125.7 ± 2.23[a]	138.6 ± 3.00[a]	242.5 ± 3.80[a]

Values are the mean ± SEM from 6 animals in each group; Statistical analysis done by ANOVA followed by *post hoc* test of significance, Dunnett's 't' test. [a] $p < 0.001$ *vs*. vehicle control. NS: normal saline, *i.p.*: intraperitoneal.

Table 2. Effect of EECR on analgesia induced by morphine and pethidine in mice (by hot plate method)

Treatment	Resting value	Average maximum reaction time (s) at min							
		15	30	45	60	90	120	150	180
Control (NS, 5 mL/kg, *i.p.*)	4.8± 0.06	10.1 ± 1.24	7.6 ± 0.06	5.2 ± 0.03	5.1 ± 1.09	4.2 ± 1.03	4.1 ± 0.73	3.7 ± 1.02	3.1 ± 0.32
EECR (40 mg/kg, *i.p.*)	4.9 ± 0.80	-	19.7 ± 1.01[a]	14.2 ± 1.16[a]	11.1 ± 0.9[a]	9.5 ± 0.83[a]	6.0 ± 0.05	5.0 ± 0.70	4.5 ± 0.66
EECR (60 mg/kg, *i.p.*)	5.1 ± 0.95	27.2 ± 1.04[a]	24.6 ± 1.32[a]	18.3 ±1.09[a]	14.1 ± 1.24[a]	12.7 ± 1.36[a]	9.0 ± 0.98[a]	6.5 ± 0.84	5.2 ± 0.72
EECR (80 mg/kg, *i.p.*)	4.6 ± 0.08	> 30[a]	> 30[a]	> 30[a]	> 30[a]	28.3 ± 1.91[a]	25.1 ± 1.40[a]	20.1 ±0.80[a]	14.1 ± 1.14[a]
Morphine (5 mg/kg, *i.p.*)	5.6 ± 0.95	> 30[a]	19.3 ± 0.43[a]	18.5 ± 1.03[a]	14.3 ± 0.96[a]	9.9 ± 1.19[a]	8.1 ± 0.83[a]	6.4 ± 0.72	5.0 ± 0.36
EECR(40 mg/kg, *i.p.*) + morphine	5.3 ± 0.88	> 30[a]	> 30[a]	27.6 ± 1.50[a]	24.5 ± 0.80[a]	21.2 ± 0.96[a]	12.2 ± 1.31[a]	9.2 ± 0.88[a]	6.6 ± 0.80
EECR (60 mg/kg, *i.p.*) + morphine	5.9 ± 0.63	> 30[a]	> 30[a]	> 30[a]	28.5 ± 1.10[a]	25.3 ± 1.71[a]	15.9 ± 2.70[a]	11.6 ± 1.24[a]	10.1 ± 0.36[a]
EECR (80 mg/kg, *i.p.*) + morphine	5.9 ± 0.96	> 30[a]	> 30[a]	> 30[a]	>30[a]	>30[a]	>30[a]	28.4 ± 2.00[a]	21.5 ± 1.88[a]
Pethidine (10 mg/kg, *i.p.*)	5.0 ± 0.85	25.1 ± 1.28[a]	23.1 ± 1.40[a]	15.6 ± 0.52[a]	11.3 ± 1.05[a]	9.5 ± 0.92[a]	6.0 ± 0.91[a]	4.4 ± 0.96	3.9 ± 0.83
EECR (40 mg/kg, *i.p.*) + pethidine	5.7± 0.8	28.4 ± 1.00[a]	27.5 ± 1.23[a]	17.7 ± 1.97[a]	16.0 ± 1.28[a]	16.0 ± 1.40[a]	11.5 ± 1.70[a]	9.3 ± 0.95[a]	6.2 ± 0.75
EECR (60 mg/kg, *i.p.*) + Pethdine	5.6 ± 1.13	> 30[a]	> 30[a]	28.2 ± 1.48[a]	21.3 ± 1.07[a]	17.5 ± 1.60[a]	14.3 ± 1.18[a]	11.7 ± 1.12[a]	9.1 ± 0.76[a]
EECR (80 /kg, *i.p.*) + pethidine	5.8 ± 0.96	> 30[a]	> 30[a]	> 30[a]	> 30[a]	>30[a]	28.8 ± 1.57[a]	23.5 ± 2.00[a]	18.5 ± 1.31[a]

Values are the mean ± SEM from 6 animals in each group. Statistical analysis done by ANOVA followed by *post hoc* test of significance, Dunnett's 't' test, [a] $p < 0.05$ *vs*. resting value (average reaction time before treatment). Results of (EECR + morphine) and (EECR + pethidine) were significant ($p < 0.05$) *vs*. EECR. NS: Normal saline; > 30: animals fail to react within 30 s (30 s – response latency). *i.p.*: intraperitoneal.

Table 3. Effect of EECR on average survival time on strychnine- and leptazol-induced convulsions in mice.

Treatment	Survival time (min) after treatment of	
	strychnine (2 mg/kg, *i.p.*)	leptazol (80 mg/kg, *i.p.*)
Control (NS, 5 mL/kg, *i.p.*)	6.2 ± 0.95	12.5 ± 1.19
EECR (25 mg/kg, *i.p.*)	120.5 ± 1.10[a]	131.6 ± 1.05[a]
EECR (40 mg/kg, *i.p.*)	150.5 ± 1.14[a]	165.7 ± 1.25[a]
EECR (60 mg/kg, *i.p.*)	193.8 ± 1.76[a]	208.6 ± 1.97[a]
EECR (80 mg/kg, *i.p.*)	275.8 ± 2.30[a]	319.0 ± 2.96[a]

Values are the mean ± SEM from 10 animals in each group; Statistical analysis done by ANOVA followed by *post hoc* test of significance, Dunnett's 't'test. [a] $p < 0.001$ *vs.* control. NS: normal saline, *i.p.*: intraperitoneal.

Table 4. Effect of EECR on behavioral profiles in mice

Treatment	Awareness Response	Touch response	Pain response	Righting reflex	Pinna reflex	Corneal reflex	Grip strength
Control (NS, 5 mL/kg, *i.p.*)	0	0	0	0	0	0	+
Chlorpromazine (5 mg/kg, *i.p.*)	4+	4+	4+	4+	4+	4+	4+
EECR (40 mg/kg, *i.p.*)	2+	3+	3+	2+	2+	3+	2+
EECR (60 mg/kg, *i.p.*)	3+	4+	4+	3+	3+	4+	3+
EECR (80 mg/kg, *i.p.*)	4+	4+	4+	4+	4+	4+	4+

Key for scoring: 0, no effect (normal); +, slight depression; 2+, moderate depression; 3+, strong depression; 4+, very strong depression. *i.p.*: intraperitoneal. NS: Normal saline. Number of animals used for each group (n = 6). EECR values were significant ($p < 0.05$) *vs.* control.

Behavioral effects

The effects of EECR (40, 60, and 80 mg/kg, *i.p.*) on righting reflex, pinna reflex, corneal reflex, awareness, grip strength, touch and pain responses on mice were observed by conventional methods. Chlorpromazine (5 mg/kg, *i.p.*) was used as a reference drug (21-23).

Statistical analysis

Results are expressed as the mean ± SEM. ANOVA followed by Dunnett's "*t*" test was performed as a *post hoc* test of significance taking vehicle treated animals as a control; p value of < 0.05 was considered as statistically significant.

RESULTS

Acute toxicity tests in mice established the LD_{50} of EECR to be 240 mg/kg, *i.p.* Three doses of EECR (40, 60, and 80 mg/kg) potentiated the sleeping time induced by standard hypnotics *viz.* pentobarbitone (100.5%, 148.2%, and 210.4%, respectively), diazepam (85.2%, 164.9%, and 223.3%, respectively) and meprobamate (54.5%, 89.3%, and 124.3%, respectively) (Table 1).

EECR exhibited a dose dependent and significant analgesic activity in the acetic acid induced writhing test. As can be seen in Figure 1, EECR with a dose of 40 mg/kg, *i.p.* exhibited percentage of protection of 43%. This dose dependent effect reached 82% with a dose of 80 mg/kg, *i.p.* Analgesic compounds: acetyl salicylic acid (68 mg/kg, *i.p.*), morphine sulfate (1.15 mg/kg, *i.p.*), and paracetamol (68 mg/kg, *i.p.*) gave 60%, 70%, and 61% protection, respectively. From Table 2, it is also found that EECR not only produced analgesia in mice but also potentiated the analgesic action of morphine and pethidine.

Strychnine and leptazol at the doses of 2 mg/kg, *i.p.* and 80 mg/kg, *i.p.,* respectively, induced in mice tonic type of convulsions with clonus. The degrees of convulsions were measured visually. Table 3 and Figure 2 show that EECR increased the average survival time and decreased the percentage mortality in a dose dependent manner against strychnine- and leptazol-induced convulsions. It was

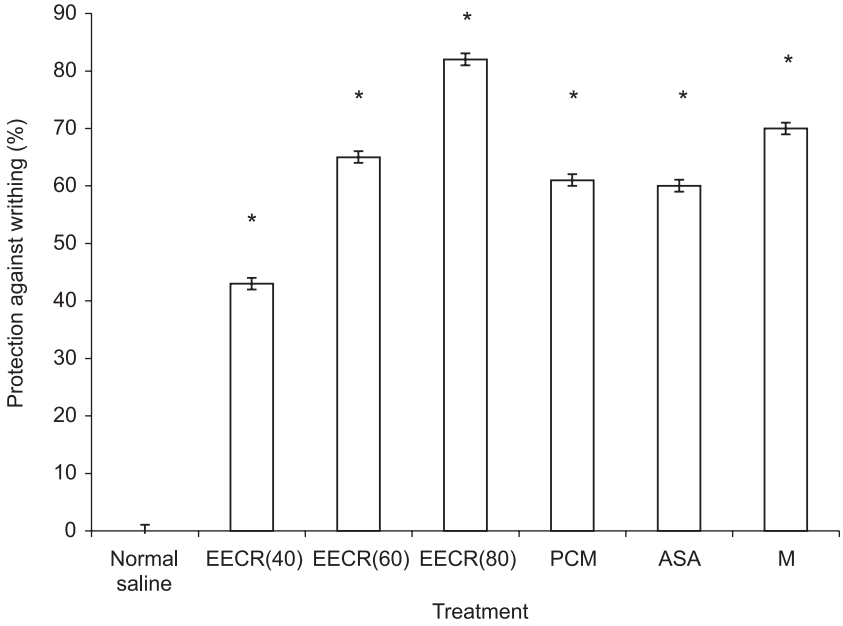

Figure 1. Influence of EECR (40, 60, 80 mg/kg, *i.p.*) on the writhing and stretching induced in mice by 1.2 % v/v acetic acid (writhing test).The activity was compared with paracetamol (PCM) (68 mg/kg, *i.p.*), acetyl salicylic acid (ASA) (68 mg/kg, *i.p.*), and morphine sulfate (M) (1.15 mg/kg, *i.p.*). Values are the mean ± SEM from 6 animals in each group. * $p < 0.001$ *vs.* control (ANOVA followed by Dunnett's 't' test).

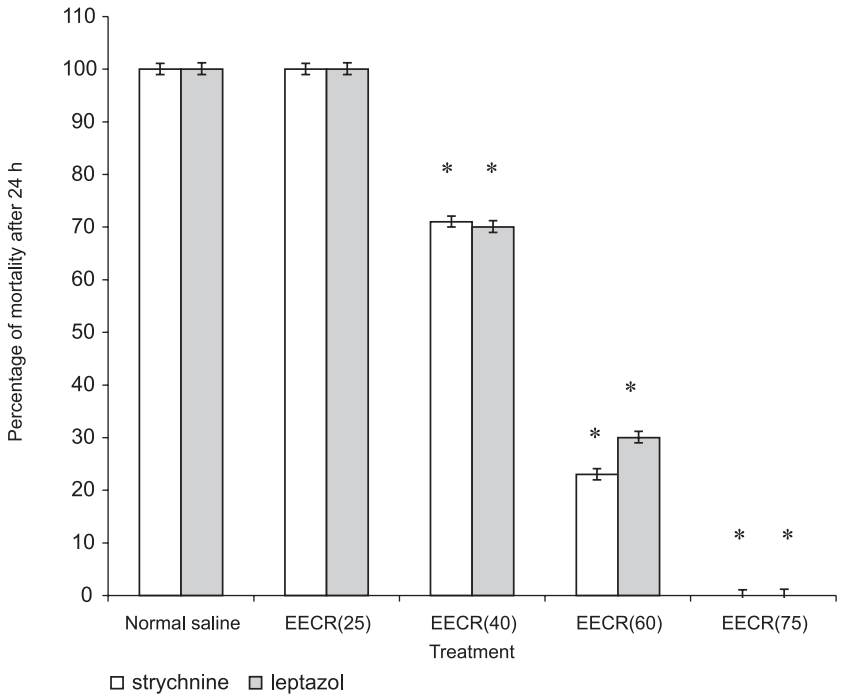

Figure 2. Anticonvulsant effect of EECR on strychnine (2 mg/kg, *i.p.*) and leptazol (80 mg/kg, *i.p.*)-induced convulsions in mice. Results are expressed in percentage of mortality. Respective doses of the extracts (mg/kg, b.w.) are in the parenthesis. Values are the mean ± SEM from 10 animals in each group. * $p < 0.001$ *vs.* control (ANOVA followed by Dunnett's 't' test).

observed that different combinations of strychnine or leptazol with EECR did not show any significant protective action against convulsions.

The results obtained from general behavioral profiles are shown in Table 4. It was noted that EECR depressed awareness and alertness, touch and pain responses, grip strength, altered righting, pinna and corneal reflexes when compared to the control (normal saline 0.9% w/v, 5 mL/kg). However, chlorpromazine hydrochloride (standard) produced a significant depression of these responses in comparison with EECR.

DISCUSSION AND CONCLUSION

Pentobarbitone, diazepam and meprobamate were used to induce sleep in this study. Benzodiazepines are believed to act at specific binding sites that are closely linked to gamma-aminobutyric acid (GABA) receptors, the binding of benzodiazepines enhancing GABA-ergic transmission. Although the cause of prolongation of diazepam-induced sleeping time is not known, the enhancement of GABA-ergic transmission might be related to its sedative activity. Prolongation of pentobarbitone-induced sleeping time might be due to tranquilizing action as well as CNS depressant action. Although the exact mechanism responsible for the sedation action of meprobamate is not clear, it might be due to CNS depressant action or due to enhancement of GABA-ergic transmission (12, 16, 17, 24). EECR potentiated significantly the duration of pentobarbitone-, diazepam- and meprobamate-induced sleep in mice, suggesting probable tranquilizing action as well as CNS depressant action (13, 22).

Pal et al., found that analgesic activity of *Celsia coromandeliane* is probably mediated by inhibition of a post synaptic specific sensitive mechanism either by depleting endogenous levels of nor epinephrine *via* dopamine-β-hydroxylase inhibition or by blocking norepinephrine effects at the receptor level (25). Analgesic and anticonvulsant activities can also be mediated by other mechanisms. The increase of brain serotonin and GABA level is responsible for analgesic and anticonvulsant activities (16, 17, 20, 25-27). It was found that EECR increased the brain serotonin and GABA level in mice (unpublished data). Therefore, analgesic and anticonvulsant activities produced by EECR may be related to the increased brain serotonin and GABA level in mice (25).

Gupta et al. established that inhibition of the touch response, righting reflex, and grip strength is probably produced due to a pronounced CNS depressant action (19). Reduction of pinna reflex and awareness may be due to synapses block of the afferent pathway or due to the overall CNS depressant action (28, 29). In this study, the mechanism whereby EECR depressed awareness, touch and pain responses, righting reflex, pinna reflex, corneal reflex, and grip strength may also be due to synapses block of the efferent pathway or by overall CNS depressant action.

EECR enhanced sleeping time, analgesic, and anticonvulsant activities and reduced different behavioral reflexes. It can be concluded from the present discussion that the ethanol extract of *C. rotundus* exhibited strong CNS depressant action.

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