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Neuropharmacological Effects of Ethanolic Extract of *Portulaca quadrifida* Linn. In Mice

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ABSTRACT: In the present study, some neuropharmacological effects of ethanolic extract of *Portulaca quadrifida Linn.* were studied in mice using various models. The effect of ethanolic extract of *Portulaca quadrifida Linn.* on central and peripheral nervous system were studied by using spontaneous motor activity, antinociceptive activity, *in vivo* muscle relaxant activity(Grip strength) and anticonvulsant activity. Preliminary phytochemical evaluation of extract was also carried out. The extract (400 and 800mg./kg i.p.) showed a significant reduction in spontaneous motor activity, antinociceptive activity and also showed reduction in time to recover from the electrically induced convulsions.The effect of extract on grip strength was found non significant. Preliminary phytochemical analysis of the extract revealed the presence of alkaloids, saponins, flavonoids, triterpenoids/steroids, tannins and glycosides. Results from the present study indicate that *Portulaca quadrifida Linn.* has significant effect on central nervous system but not on peripheral nervous system.

Key words : *Portulaca quadrifida Linn.*, Spontaneous motor activity, Analgesic activity, Muscle relaxant activity, Anticonvulsant activity

INTRODUCTION

Portulaca quadrifida Linn. belongs to the family portulacaceae. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is used as a vegetable and also used for various curative purposes. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids[1].

Portulaca quadrifida Linn. has been reported to possess antifungal activity against *Aspergillus fumigates* and *Candida* albicans[2]. Although the plant is widely used for various medicinal purposes, its CNS potential has not been explored yet. Therefore, in the present study an attempt has been made to investigate the effect of ethanolic extract of *Portulaca quadrifida Linn.* (EEPQ) on central and peripheral nervous system. It could be the first documentation of *Portulaca quadrifida Linn.* for its neuropharmacological properties.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL : Fresh whole plants of *Portulaca quadrifida Linn.* were collected from the local fields of Gulbarga. The plant specimen was identified and authenticated by Prof. Y.N. Seetaram Dept. of Botany, Gulbarga University, Gulbarga. A voucher specimen (No. HGUG -906) is preserved in the herbarium of Dept. of Botany, Gulbarga University, Gulbarga.

PREPARATION OF PLANT EXTRACT : The aerial parts of the plant were dried in the shade upto 10 weeks. The dried plant material was powdered in a mixer grinder. The powder was exhaustively extracted with ethanol in a soxhlet apparatus for atleast 10 h. The solvent was removed by evaporation under reduced pressure. The dried semisolid EEPQ was kept in a refrigerator till further analysis.

ANIMALS : Male Swiss Albino mice weighing 20-25 gms were used for the experiments. The animals were

purchased from Mahaveera Enterprises, Hyderabad, Andhra Pradesh, India, and were housed in polyethylene-walled cages in groups of six, in the animal house of Luqman College of Pharmacy, Gulbarga. The animals were used after an acclimatization period of atleast one week to the laboratory environment and provided with standard food pellets and water *ad libitum*. However, the animals were deprived of food and water before 18 h of the experiment and were kept on a 12 h light : 12 h dark regime at 23 ± 2^0 C and relative humidity of 45 ± 2% prior to and throughout the experiment. The protocol for the study was approved by Institutional Animal Ethical Committee.

DRUGS :. Chlorpromazin was purchased from Sun Pharmaceuticals Ltd., Silvarsa, India. Pentazocin(Fortwin) was purchased from Ranbaxy Laboratories Ltd., Ahmedabad, India. Phenytoin Sodium was purchased form Embiotic Pvt. Ltd. Bengaluru, India. Diazepam was purchased from Ranbaxy Laboratories Ltd., Nihalgarh, Himachal Pradesh, India. Other chemicals used were of analytical grade.

EXPERIMENTAL:
The animals were divided into four groups. The Group-1 animals were administered with normal saline (control), Group-2 animals were given the standard, Group-3 and Group-4 animals received EEPQ at 400 mg/kg i.p. and 800 mg/kg i.p. respectively.

SPONTANEOUS MOTOR ACTIVITY : This was determined using Actophotometer (Inco- Ambala India).The actophotometer had photoelectric cells which were connected in circuit with a counter when the beam of light falling on the photocell was cutoff by the animal, a count was recorded. The instrument had square area in which the animal moved[3]. Chlorpromazin (13mg/kg i.p.) was used as the standard drug. Each animal was kept for 10 minutes in the cage. Readings were taken at 0 h, 1 h, 2 h, 4 h, and 6 h of administration of the dose.

ANTINOCICEPTIVE ACTIVITY : Antinociceptive activity was evaluated using Eddy's Hot Plate instrument (Inco-Medicraft Analgesiometer, S.No.A-10-042). The animals were individually placed on Eddy's Hot Plate maintained at constant temperature (55^0C) and the reaction of animals such as paw licking or jump response was taken as the end poin[4]. Pentazocin (10 mg/kg i.p.) was used as the standard drug. Reaction times were determined at 0 h, 1 h, 2 h, 4 h, and 6 h of the administration in all the groups. 15 seconds was taken as cutoff time for the reaction.

MUSCLE RELAXANT ACTIVITY :
GRIP STRENGTH TEST : A Rota-rod (Inco-Ambala, Instruments and chemicals Pvt. Ltd. model town, Ambala City -03) was used to measure the grip strength in mice. The instrument (a horizontal rotation device) was set at a rate of 16 revolutions per minute[5,6]. The animals were placed on the rotating rod and fall off time i.e, when the animal falls from the rotating rod, was recorded, which was taken as grip strength. Diazepam (26 mg/kg i.p.) was used as the standard drug.Grip strength in all the groups was measured before and at 30 minutes, 1 h, 2 h, 4 h, and 6 h of the administration of the dose.

ANTICONVULSANT ACTIVITY : Convulsions were induced in mice through an electric shock (150 mA) using Electro-convulsometer (Inco-Ambala, Model No.100-3). Phenytoin sodium (30 mg/kg i.p.) was used as the standard drug. The time of recovery of animals from convulsions in all the groups was recorded[7].

PHYTOCHEMICAL SCREENING : The quail tative phytochemical analysis of the crude EEPQ was carried out by employing standard conventional protocols[8,9].

STATISTICAL ANALYSIS : Results were calculated as mean ± S.E.M. where appropriate, one-way ANOVA followed by Post hoc Dunnet's t-test was employed. Values $P<0.05$ were considered significant, using Instat @ Graph Pad Software.

RESULTS

EEPQ at the doses of 400 and 800 mg/kg i.p. reduced the spontaneous motor activity in mice.Results were found to be significant when compared with the vehicle treated control group (Table-1). This effect was dose dependent and was observed within 30 min of drug administration and persisted upto 4 h.

EEPQ, at both concentrations, showed significant antinociceptive activity in mice. The effect was found to be significant from 30 min of administration of the dose to 4 h (Table-2).

The EEPQ showed no significant effect on grip streangth in mice.The effect was persisted only upto 1 h of the drug administration(Table-3).

PQ extract showed significant anti convulsant activity in mice compared to the controlgroup.(Control=138.33±10.4265;400mg/kg=78.167±8.894*s;800mg/kg=56.500±8.922**s; Standard=55.667 8.894**s; Dunnet's t-test ; * Significantly different at $P<0.05$; ** Significantly different at $P<0.01$).

Table 1 : Effect of EEPQ On Spontaneous motor activity in mice

Group	Treatment	Dose (mg/kg)	Total activity during				
			0 h	1 h	2 h	4 h	6 h
1	Control	----	584.33 ± 43.353	448.33 ± 47.042	289.17 ± 24.034	253.33 ± 24.370	300.67 ± 21.290
2	Chlorpromazin	13	399.333 ± 21.751	** 113.17 ± 1.014	** 63.333 ± 2.246	** 71.000 ± 4.290	** 95.000 ± 4.487
3	EEPQ	400	414 ± 39.087	** 97.500 ± 22.558	** 68.833 ± 20.550	** 85.833 ± 24.479	ns 227.67 ± 46.035
4	EEPQ	800	549.1666 ± 69.865	** 171.50 ± 46.355	* 118.17 ± 44.863	* 132.17 ± 29.283	ns 275.00 ± 60.848

Values are mean ± SME ;n=6 in each group; *Significantly different at p<0.05;** significantly different at p<0.01; ns-Non-significant

Table 2 : Antinociceptive effect of EEPQ in mice

Group	Treatment	Dose (mg/kg)	Reaction time in seconds at					
			0 h	30 min	1 h	2 h	4 h	6 h
1	Control	----	2.958 ± 0.1535	2.988 ± 0.1462	3.118 ± 0.1721	2.373 ± 0.05608	2.642 ± 0.1588	2.407 ± 0.06200
2	Pentazocin	10	3.422 ± 0.04331	** 5.298 ± 0.03859	** 6.143 ± 0.04673	** 6.172 ± 0.06284	** 6.450 ± 0.02875	ns 2.328 ± 0.06300
3	EEPQ	400	2.657 ± 0.1603	* 3.587 ± 0.1619	** 4.500 ± 0.1428	** 5.572 ± 0.1340	** 3.888 ± 0.2354	2.973 ± 0.2270
4	EEPQ	800	2.682 ± 0.1620	ns 3.062 ± 0.1628	** 5.082 ± 0.2686	** 6.117 ± 0.1694	ns 3.045 ± 0.3246	3.092 ± 0.1878

Values are mean ± SME ;n=6 in each group; *Significantly different at p<0.05;** significantly different at p<0.01; ns-Non- significant

Table 3 : Effect of EEPQ on muscle relaxant activity in mice

Group	Treatment	Dose (mg/kg)	Grip strength in seconds					
			0 h	30 min	1 h	2 h	4 h	6 h
1	Control	----	358.17 ± 11.620	370.33 ± 19.416	379.23 ± 12.456	381.50 ± 12.132	376.33 ± 11.071	363.17 ± 19.511
2	Diazepam	26	370.50 ± 18.936	* 202.17 ± 13.893	* 153.17 + 13.946	** 125.23 ± 11.923	** 103 ± 33 ± 4.310	347.17 ± 14.831
3	EEPQ	400	332.50 ± 12.233	* 309.17 ± 10.778	* 326.23 ± 9.127	ns 362.01 ± 11.923	359.50 ± 10.987	337.23 ± 6.940
4	EEPQ	800	351.23 ± 18.609	** 176 ± 18.195	** 157.07 ± 17.046	ns 363.23 ± 7.472	373.07 ± 6.805	392.33 ± 6.820

Values are mean ± SME ;n=6 in each group; *Significantly different at p<0.05;** significantly different at p<0.01; ns-Non.significant

DISCUSSION

EEPQ showed significant reduction in spontaneous motor activity. The activity is a measure of the level of excitability of the CNS[10]. This decrease in activity may be closely related to sedation resulting from depression of the central nervous system[11]. The reduction in spontaneous motor activity could be due to inhibitory effects of the extract on the CNS.

EEPQ showed significant antinociceptive activity. The inhibitory action of EEPQ on the CNS which was demonstrated in the present study may contribute to the antinociceptive properties of the extract. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception . The ability of the extract to prolong the reaction latency to the pain thermally-induced in mice by the hot plate further suggests central analgesic activity[12].

Further the study clearly demonstrates that the EEPQ has no significant effect on *in vivo* muscle relaxant activity as indicated by negative results obtained in the grip strength test. The effect was significant only upto 1h of the administration. The reduction in time to recover from electrically induced convulsions by EEPQ indicates anticonvulsant effect of the plant. This activity again could be due to the inhibition of excitatory mechanisms in the CNS[13]. Treatment with maximal electro shock (MES) causes significant increase in the level of norepinephrine,dopamine and acetylcholine activity in the brain[14]. It has been also reported that there may be an increase in the turnover of norepinephrine by MES induced seizures. An excitatory effect on the cerebellum, to activate inhibitory pathways that extend to the cerebral cortex,has been suggested to contribute to the anticonvulsant effects of phenytoin. The anticonvulsant effect of EEPQ is almost similar to the phenytoin. Hence it can be said that EEPQ may follow the similar mechanism of anticonvulsant activity as that of phenytoin.

Phytochemical analysis of the extract showed the presence of alkaloids, flavonoids, saponins, steroids/ terpenoids, tanning, glyeosides. The saponins and flavonoids have been reported to be responsible for sedative effect and likewise for inhibition of spontaneous motor activity in mice[15]. These results are supported by several researchers[16,17,18,19]. Thus the reported pharmacological activities of EEPQ could be attributed to the presence of saponins and flavonoids. Further studies concerned with isolation and characterization of these secondary metabolites are in progress.

CONCLUSION

Our experimental data clearly demonstrates the depressant effect of EEPQ on CNS. However, to know the exact mechanism of action of EEPQ further study with purified/bioactive compounds is warranted.

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