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Neuropharmacological Profile of Ethanolic Extract of *Baliospermum Montanum Roots in Mice*

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ABSTRACT

Baliospermum montanum, Euphorbiace family commonly known as Danti in Ayurveda is of therapeutic significance and is utilized traditionally for the treatment of various ailments. The plant's roots are considered as laxatives, anthelmintic, diuretic, diaphoretic, rube facient, febrifuge and tonic. On preliminary basis neuropharmacological profile of ethanolic extract of roots of *Baliospermum montanum* was assessed for Locomotor activity using Actophotometer, muscle relaxant activity using Rota-rod Apparatus, pentobarbital-induced hypnosis and Anticonvulsant activity by Maximal electroshock test was performed. Ethanolic extract of *Baliospermum montanum* roots decreased the motor activity and showed significant muscle relaxation along with potentiation of pentobarbital-induced sleeping time and decreased the duration of tonic hind leg extension of seizures activity. The results conclude that the extract has significant central nervous system depressant activity. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Keywords: *Baliospermum montanum,* CNS Depressant, neuropharmacological profile.

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INTRODUCTION

Baliospermum montanum (Willd.) Muell-Arg Euphorbiaceae, commonly known as Danti is a leafy under shrub, distributed in outer range of Himalayas from Kashmir to Assam and in moist deciduous forests elsewhere in India[1].The plant is reported to have valuable effects as digestive, anthelmintic, diuretic, diaphoretic, rube facient, febrifuge and tonic. It documented to be useful in anemia, asthma, bronchitis, constipation, fever, leprosy, leucoderma, jaundice and wounds [2]. The phytochemical studies on the roots of *Baliospermum montanum* showed the presence of five phorbolesters, which are montanin, balios per min, 12-deoxyphorbol-13-palmitate, 12-deoxy-16-hydroxyphorbol-13-palmitate and 12-deoxy-5β-hydroxyphorbol-13-myristate. A preliminary phytochemical study on the roots revealed presence of flavonoids, glycosides, sterols and absence of alkaloids, saponins and terpenoids[3].The crude organic and aqueous extracts of roots of *Baliospermum montanum* have been screened for pharmacological activities and reported to possess antioxidant, anticancer[4], antidiabetic[5], antibacterial[6], anthelmintic[7], free radical scavenging[8], hepatoprotective[9] andimmunomodulatory[10] activities. The present study has been carried out to explore the activity of *Baliospermum montanum* roots on central nervous system.

MATERIALS AND METHOD

Collection of Plant Material

The roots of *Baliospermum montanum* were collected from the local medicinal plant supplier and were authenticated by Dr. P. Veera Reddy, Professor, Government Ayurvedic College, Warangal, Telangana, India.

Drugs and Chemicals

Diazepam (Ranbaxy Research Laboratories, India), Phenobarbitone sodium (Rhone-Poulenc India Limited, India, Phenytoin (Cadilla Healthcare Ltd, India) and Tween 80(S.D Fine Chemicals, India).All other reagents used for the experiments were of high analytical grade.

Preparation of Plant Extract

The roots of *Baliospermum montanum* were shade dried. The dried roots were pulverized in an electrical processor and then the powder was separated.50 gram of dried powder material was extracted in a soxhlet apparatus with 200 ml. of absolute alcohol. The ethanolic extract was then distilled, evaporated and dried in vacuum. All the extracts were kept in desiccator and stored in a refrigerator for pharmacological experiment.

Animals Used

Albino mice of either sex, weighing about 25-30 grams were used in experiments. Mice were

accommodated in polypropylene cages with not more than three animals per cage and kept under standard condition (12 hours light / dark cycle; relative humidity 48%; temperature 25± 3^{o}C) and had free access to standard rat/mice pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for7 days before commencement of experiments. Mice were divided into three groups containing 6 mice in each group and treatments were given according to the assessment that was carried out. The experimental procedures were reviewed and approved by Institutional animal ethical committee and experiments conducted according to CPCSEA.

Acute Toxicity Studies

A preliminary pharmacological study was carried out to evaluate the gross behavioral effects and safety effects of the *Baliospermum montanum* extract. The acute toxicity study was carried on mice weighing about 20-25gm as per ICH Topic S7Aguidelines[11].Overnight fasted mice received the test extract at a dose of 100mg/kg intraperitoneally and mortality was observed for 14days. If no mortality was observed for any mice, then the procedure was repeated again with doses of300, 1000 and2000 mg/kg intraperitoneally. The animals were observed continuously for 2 h for behavioral, neurological and autonomic profiles along with the percentage of mortality observations were tabularized according to Irwin's table [12]. For this the following checklist was utilized:

Stimulation: Hyperactivity, Piloerection, Twitching, Rigidity, Irritability, Jumping, Clonic convulsions, Tonic convulsions

Depression: Ptosis, Sedation, Loss of righting reflex (sleep), Loss of traction, Loss of Pinnal reflex, Catatonia, Ataxia, Loss of muscle rigidity, Analgesia.

Autonomic reflexes: Straub's tail, Laboured respiration, Cyanosis, Reddening, Abnormal secretions, balancing.

Methods Employed in Screening of CNS Activity

Assessment of Locomotor activity using Actophotometer[13]

This activity was measured using Actophotometer which works on photoelectric cells, which are joined in circuit with a counter. When the beam of light falling on the photoelectric cell is interrupted by the mice, a count is recorded. This test can demonstrate a CNS depressant or stimulant activity profile. Animals were divided into three groups of six mice each. Standard pellet diet and water was provided *ad libitum* to the animals. The equipment was turned on and mice were placed individually inside the activity cage of Actophotometer for 10 min and basal activity score was noted. Group 1was treated as control and administered with 1ml of 1% Tween

80; Group 2-was treated with ethanolic extract of *Baliospermum montanum* (200 mg/kg, i.p) and Group 3-was treated with Standard (Diazepam 4 mg/kg, i.p). After 30 min of mice are placed again in Actophotometer for 10 min and the activity was monitored. The difference in activity before and after treatment was noted and the percentage change in the activity was calculated.

Assessment of Muscle Relaxant Activity using Rota-Rod Apparatus[14]

The loss of muscle grip is an indication of skeletal muscle relaxation. The difference of fall off time from the rotating rod between the control and treated animal is taken as an index of muscle relaxation. Before performing this experiment, the animals were trained to remain on rotarod apparatus (with the rod rotating at a speed of 25 rpm) for 3 min. Animals remaining on Rota-Rod for 2 min or more in low successive trials were selected for testing; After training, the mice were divided into three groups of six mice each. Group 1 was treated as control and administered with 1ml of 1% Tween 80; Group 2-was treated with ethanolic extract of *Baliospermum montanum* (200 mg/kg, i.p) and Group 3-was treated with Standard (diazepam, 4 mg/kg i.p). 30 Minutes after treatment the same test was repeated. The fall off time from the rotating rod was noted. The difference in the fall off time from the rotating rod between the control and the treated mice (standard-Diazepam/extract) was taken as an index of muscle relaxation.

Assessment of Pentobarbital-Induced Hypnosis[15]

In this method, mice of either sex were randomly taken and divided into three groups containing six mice in each. Group 1 was treated as control and administered with 1ml of 1% Tween 80; Group 2-was treated with ethanolic extract of *Baliospermum montanum* (200 mg/kg, i.p) and Group 3-was treated with Standard (diazepam, 4 mg/kg i.p). After 30 min mice received an intraperitoneal injection of pentobarbital sodium (40 mg/kg). The time between the loss and recovery of the righting reflex was taken as the sleeping time. The percentage effect of on pentobarbital-induced hypnosis was calculated considering the right reflex in control as 100%.

Assessment of Anticonvulsant Activity by Maximal Electroshock Test[16]

Maximal electroshock test is the most widely used animal model in the antiepileptic drug discovery. MES produces convulsions mainly by opening the voltage dependent sodium ion channels thereby causing the repetitive firing of action potential. In the electrically-induced seizure experiment, the maximal electroshock method will be employed. In brief, tonic convulsions of the hind extremities of the mice were induced by passing 50 hertz alternating electrical current of 150 milliamps for 0.2 seconds through corneal electrodes. Animals were divided into three groups containing six mice in each. Group 1 was treated as control and administered with 1ml of 1% Tween 80; Group 2-was treated with ethanolic extract of

Baliospermum montanum(200 mg/kg, i.p) and Group 3-was treated with Standard (Phenytoin, 25 mg/kg i.p.) for 15 days prior to the induction of seizures. The percentage protection from hind limb tonic extension seizure and the duration of seizure were recorded.

Statistical Analysis

Values were expressed as Mean ± standard error of the mean. The Significance of differences among the group was assessed using one way analysis of variance (ANOVA). The test followed by Dunnett's multiple comparisons test of significance. p values less than 0.05 were considered as statistically significant.[17]

RESULTS AND DISCUSSION

This study has been carried out to establish the central nervous system properties of ethanolic extract of *Baliospermum montanum* roots. For the screening of neuropharmacological profile of extract assessment of Locomotor activity using Actophotometer, muscle relaxant activity using Rota-rod Apparatus, pentobarbital-induced hypnosis and Anticonvulsant activity by Maximal electroshock test was performed. One of the important pharmacological actions of antianxiety agents of benzodiazepine class of drugs is CNS depressant effect along with muscle relaxing property. The skeletal muscle relaxant together with taming or calming effect these agents reduces anxiety and tension. Locomotor activity indicates an index of wakefulness or alertness whereas loss of muscle grip on Rota rod indicates muscle relaxation[18]. Ethanolic extract of *Baliospermum montanum* roots reduced the spontaneous locomotor activity from 53.02% when compared with control 3.03% (Table 1) and showed significant muscle relaxation (Table 2) which is an indication of CNS depressant property as like diazepam which acts by potentiating GABAergic inhibition triggering chloride channel opening with resulting membrane hyperpolarization.

Table 1: Effect of *Baliospermum montanum* extract on Locomotor activity in mice

Treatment	Dose, i.p	Locomotor activity observed for 10 min		Percentage change in activity
		Before treatment	30 minutes after treatment	
Control	1ml of 1% Tween	398 ± 4.14	384 ± 5.16	3.03
Extract	200 mg/kg	413 ± 5.38	294 ± 8.84*	53.02*
Diazepam	4 mg/kg	408 ± 6.91	109 ± 7.23**	73.28**

Table 2: Effect of *Baliospermum montanum* Extract on Muscle Relaxant Activity in Mice

Treatment	Dose, i.p	Fall of time (sec)		Percentage change in activity
		Before treatment	After treatment	
Control	1ml of 1% Tween	74±2.87	71±3.61	4.05
Extract	200 mg/kg	68±5.54	29±6.72*	57.35*
Diazepam	4 mg/kg	72±5.79	16±4.63**	77.78**

Values are mean ± S.E.M. (n=6) One way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control

Further, the present results suggest that ethanolic extract of *Baliospermum montanum* roots possesses CNS-depressant action. Extract showed marked potentiation of pentobarbital-induced sleeping time with significant reduction in the onset and prolongation of sleep duration induced by pentobarbitone which is indicated by the loss of righting reflex (Table 3) [19].

Table 3: Effect of *Baliospermum montanum* extract on pentobarbital-induced hypnosis in mice

Treatment	Dose, i.p	Onset of action (min)	Duration of action (min)	% Effect
Control	1ml of 1% Tween	8.81±0.91	36.24±3.15	100
Extract	200 mg/kg	5.85±0.44*	45.98±5.21*	126.88
Diazepam	4 mg/kg	3.10±0.65**	56.61±0.62**	156.21

Values are mean ± S.E.M. (n=6) One way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control

The MES test determines the electroconvulsive threshold and seizure magnitude in rodents and mainly used for screening of drugs such as valproate and phenytoin which acts by voltage dependent blockade of sodium ion channels responsible for generation of action potential[20]. The Ethanolic extract of *Baliospermum montanum* at the dose of 250 mg/kg protected 83.33 % of mice and decreased the duration of tonic hind leg extension and altered the frequency of seizures elicited by Maximal Electroshock to a significant extent when compared with the control 66.67% (Table 4). The present result showed that *Baliospermum montanum shows the anticonvulsant effect by* blocks the frequency of voltage dependent sodium channel conductance there by preventing the repetitive firing of action potential.

Table 4: Effect of *Baliospermum montanum* Extract on Maximal Electroshock Induced Seizures in Mice

S.no	Treatments	Dose, i.p	Duration of	Quantal	%
1	Control	1ml of 1%	19.53±0.29	4/6	66.67
2	Extract	200 mg/kg	13.36 ± 0.38*	5/6	88.33
5	Phenytoin	25 mg/kg	7.71±0.31**	6/6	100

Values are mean ± S.E.M. (n=6) One way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control

CONCLUSION

From the present series of experiments, it can be concluded that the ethanolic extract of *Baliospermum montanum* roots possessed positive locomotor depressant, skeletal muscle relaxant, sedative potentiating and anticonvulsant effects in the experimental rodent models

indicating its prominent depressant action on the central nervous system, as shown by these important neuropharmacological properties in Swiss albino mice. Purification of the plant extract and further definitive studies may reveal the exact mechanisms and constituents behind the observed neuropharmacological activities of *Baliospermum montanum* root.

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