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Evaluation of CNS activity of *Bramhi Ghrita*

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Abstract

OBJECTIVE: To evaluate the CNS activity of *Bramhi Ghrita*, a polyherbal formulation containing *Bacopa monneri*, *Evolvulus alsinoids*, *Acorus calamus*, *Saussurea lappa* and cow's ghee. **MATERIALS AND METHODS:** The effect of *Bramhi Ghrita* on motor coordination, behavior, sleep, convulsions, locomotion and analgesia was evaluated in mice using standard procedures. **RESULTS:** The formulation exhibited reduced alertness, spontaneous locomotor activity and reactivity. It also antagonized the behavioral effects of d-amphetamine, potentiated the pentobarbitone-induced sleep and increased the pain threshold. *Bramhi Ghrita* protected mice from maximum electroshock and pentylene tetrazole-induced convulsions.

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Full Text

Introduction

Panchagavya is a term used in Ayurveda to describe five important substances obtained from the cow, namely milk, ghee (clarified butter fat), curd, urine and dung. A good number of formulations described in Ayurveda contain panchagavya components either alone or in combination with other substances of herbal, mineral or animal origin. In spite of vast reports in Ayurvedic texts on the applications of panchagavya substances, very meager work has been carried out on their chemical, biochemical, pharmaceutical and pharmacological aspects. Cow urine concoction (CUC) is a popular Nigerian herbal preparation containing cow urine. Over 50 components have been identified in CUC. Its major pharmacological actions include anticonvulsant and hypoglycemic effects.[1] Fulzele et al (2002) reported immunostimulant activity of the polyherbal formulation of Ashtamangal ghrita.[2]

Bramhi Ghrita (BG) is also one of the panchagavya formulations mentioned in Ayurveda containing *Bacopa monneri* (8 g), *Acorus calamus* (4 g), *Evolvulus alsinoids* (4 g), *Saussurea lappa* (4 g) and cow's ghee (80 g).[3] This formulation is used traditionally as a memory enhancer and as an anticonvulsant. *Bacopa monneri* is a well-

known nootropic plant having sedative,[4] tranquilising,[5] memory-enhancing,[6],[7],[8] antioxidant,[9] and hepatoprotective effects.[10] *Acorus calamus* is reported for its carminative,[11] sedative and tranquilizing actions.[12] *Saussurea lappa* has been reported to possess antiulcer,[13] anti-inflammatory,[14] immunostimulant,[15] antispasmodic, hypotensive and respiratory depressant actions.[16] *Evolvulus alsinoids* has been used traditionally as a brain tonic and sedative,[17] anthelmintic, antiepileptic and against leucoderma.[18] Cow's ghee (clarified butter fat) is described in Ayurveda as a memory enhancer, anticonvulsant and anti-inflammatory agent.[19] In spite of considerable literature available on some herbal components of *Bramhi Ghrita*, there is no known data regarding the pharmacological evaluation of this formulation on the central nervous system. The present study is focused on the evaluation of the CNS activity of *Bramhi Ghrita* in mice.

Materials and Methods

Animals: Male Swiss albino mice weighing 25-30 g were used. They were housed in groups of five under standard laboratory conditions (temperature 23±1°C, relative humidity 55±5% and lighting 08:00-20:00 h) with food (Lipton India Ltd. pellets) and water ad libitum. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during day (08:00-16:00 h). The institutional animal ethical committee approved the study protocol.

Bramhi Ghrita (BG): The formulation 'Bramhi Ghrita' was obtained as a gift sample from Go-Vigyan Anusandhan Kendra, Deolapar Dist., Nagpur (M.S.), India and was used as received. The formulation was prepared by an expert Ayurvedic practitioner. BG contains dried finely powdered herbs: aerial parts of *Bacopa monneri* (4% w/w), aerial parts of *Evolvulus alsinoids* (4% w/w), roots of *Acorus calamus* (4% w/w) and roots of *Saussurea lappa* (4% w/w), uniformly mixed and blended with cow's ghee (84% w/w). The formulation was fed in the form of suspension prepared using 1% gum acacia solution. The vehicle (gum acacia solution) was used as control in all experiments.

Behavioral effect: Behavioral effect of BG (100 mg/kg, 300 mg/kg p.o.) was assessed by the method described by Irwin et al, (1968).[20] The mice were divided into groups of 6 animals each. After treatment with BG the animals were observed after 30 min of administration up to 2 h for behavioral changes. Prior to BG, the behavioral pattern upon administration of the vehicle was studied. The observation parameters consisted of body position, locomotion, rearing, respiration, righting reflex and lacrimation.

Pentobarbital-induced sleeping time: The animals were divided into 4 groups (n=5). Group I animals were treated orally with vehicle and pentobarbitone sodium 45 mg/kg, i.p. Groups II, III and IV received BG orally at a dose of 100, 300 and 500 mg/kg, respectively. Pentobarbitone sodium (45 mg/kg, i.p.) was injected 30 min before oral administration for all the groups. The time elapsed between loss and recovery of the righting reflex was noted and taken as sleeping time.

Motility test: Four groups of animals (n=5) were fed orally with BG at a dose of 100, 300 and 500 mg/kg or vehicle, respectively. Thirty min after drug administration the spontaneous locomotor activity was recorded using an activity cage (Actophotomotor, Centronics Mumbai) with automatic counting of animal movements on the cage floor. The locomotor count for each animal was recorded for 5 minutes at 30-minute intervals for 2 h.

Amphetamine antagonism: Four groups of mice (n=5) were treated with BG (100, 300 and 500 mg/kg respectively) or vehicle orally 30 min before d-amphetamine administration (2 mg/kg, i.p.). The motor activity was measured for 5 min at 30 min intervals for 2 h after the injection of amphetamine.

Analgesic activity (Tail flick method): Four groups of mice (n=5) were treated orally with BG (100, 300 and 500 mg/kg) or vehicle. Thirty minutes after oral administration, basal reaction time to radiant heat was noted, by placing the tip of the tail on a radiant heat source (Tail flick test apparatus, Techno India) for 30, 60 and 120 min. The tail withdrawn from the heat source was taken as end point. A cut-off period of 10 sec was taken to prevent the damage to the tail.

Motor coordination: Four groups of mice (n=5) were fed orally with BG (100, 300 and 500 mg/kg) or vehicle and the effect on motor coordination was assessed using rotarod apparatus.[21] The animals were trained to remain for 3 min on the rod rotating at a speed of 25 rpm. On the next day either vehicle or BG (100, 300 and 500 mg/kg) was administered orally and their ability to remain on the rotating rod was assessed before and 30 min after the oral administration. The fall-off time from the rod was noted for each animal.

Convulsions: The BG was screened for anticonvulsant activity (100, 300, 500 and 750 mg/kg, orally) by administering it 60 min before pentylenetetrazole or maximum electroshock.[22],[23]

Pentylentetrazole-induced seizures: Six groups of mice (n=8) were treated with pentylentetrazole (80 mg/kg s.c.) 60 min after oral administration of either BG or vehicle or diazepam (4 mg/kg, i.p.). The animals were observed for 30 min for onset, presence or absence of clonic convulsions and mortality.

Maximum electroshock-induced seizures: Mice were divided into 6 groups (n=8). Group I served as control and received only vehicle. Groups II, III, IV and V were treated orally with BG at a dose of 100, 300, 500 and 750 mg/kg, respectively. Group VI received diazepam (4 mg/kg, i.p.). The animals received a current of 45 mA for 0.2 sec duration through electroconvulsimeter (Techno India) using corneal electrodes, after 60 min of oral administration of BG or vehicle or diazepam. The incidence and duration of extensor tonus was noted. A complete abolition of hind limb tonic extension was considered as 100% protection.

Statistical analysis: The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's 't' test or Student's 't' test and Kruskal-Wallis test followed by Dunn's test. Behavioral assessment

The animals were observed for 2 h after oral administration of BG (100 and 300 mg/kg, p.o.) and the data are presented in [Table:1]. The animals treated with vehicle showed normal body position, locomotion, respiration and righting reflex. Lacrimation was not observed in the vehicle-treated group.

Pentobarbital-induced sleeping time

The pretreatment with the formulation BG significantly potentiated pentobarbital-induced sleep. The result is shown in [Figure:1].

Motility test

BG exhibited significant decrease in spontaneous locomotor activity in mice [Figure:2].

Amphetamine Antagonism

The formulation BG antagonized the hyperactivity induced by amphetamine (2 mg/kg, i.p.) in the dose of 300 mg/kg and 500 mg/kg [Figure:2].

Analgesic Activity

BG significantly increased the pain threshold in mice (tail flick method). The results are shown in [Figure:3].

Motor Coordination

The animals treated with BG remained on the rotating rod for 3 minutes in all the doses tested. The formulation BG did not induce any motor incoordination.

Pentylentetrazol- induced seizures:

BG significantly delayed or abolished clonic seizures induced by PTZ. The results are summarized in [Table:2].

Maximum Electroshock-induced seizures

BG produced dose-dependent decrease in the duration of hind limb extensor phase. The results are shown in [Table:2].

Discussion

The formulation BG showed significant CNS depressant actions, such as reduced alertness and locomotion, and diminished response to touch and noise in a dose-dependent manner. BG did not have hypnotic action of its own but potentiated the pentobarbitone-induced sleeping time. It antagonised the amphetamine effects. Such effects are observed with antipsychotic agents.[24] BG also showed antinociceptive action by tail flick method. BG inhibited MES and PTZ-induced convulsions in a dose-dependent manner. This may also suggest that the anticonvulsant action of the formulation is mediated by the chloride channel of the GABA / benzodiazepine receptor complex. In lower doses i.e. up to 300 mg/kg p.o., BG showed reduction in the tonic extensor phase in MES-induced seizures and slightly prolonged the onset of action in PTZ-induced convulsions but the data were not

statistically significant. Similar observations were also noted with Unmadnashak Ghrita.[25] The present findings are indicative of an association of a CNS depressant and anticonvulsant effect with BG. Further studies are needed to find out the exact mechanism of action of the formulation.

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