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Evaluation of Sedative and Hypnotic activities of Ethanolic extract of *Biophytum sensitivum Linn.* DC

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ABSTRACT: Background: The traditional peoples are extensively used the leaflets of *Biophytum sensitivum* Linn plant for inducing sleep. **Aim:** The present research work was aimed to evaluate the sedative and hypnotic activities of ethanolic extract (EE) of *B. sensitivum Linn.* **Methods:** The phyto-constituents of *B. sensitivum* from leaflets was extracted by Soxhlation method by using ethanol as solvent. The EE of *B. sensitivum* was tested for presence of Alkaloids, Glycosides, Carbohydrates, Flavonoids, Saponins and Tannins. The non-toxicity of ethanol plant extract was confirmed by performing acute toxicity study by using Albino mice as animal model. The sedative and hypnotic activities of EE of *B. sensitivum* at doses of 50, 100 and 200 mg/kg of body weight (b.w.) of mice were investigated using Hole board test, Hole cross test, Open field method and Rotarod method. The Diazepam at the dose of 1 mg/kg b.w., was used as a standard drug for all the experimental methods. **Results:** The EEBS was found to nontoxic to the animal (Mice). The EE of *B. sensitivum* showed marked reduction for crossing number of holes, decreasing the number of movements and head dip in central hole. The EE of *B. sensitivum* shows sedative and hypnotic activities in a dose dependent manner. The sedative and hypnotic activities exhibited by EEBS are well comparable with the standard drug, Diazepam. **Conclusion:** Thus it could be concluded that the plant *B. sensitivum* possesses significant sedative and hypnotic activities. Thus this plant could be successfully use for safe and effective management of insomnia.

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INTRODUCTION:

Sedative are drugs that reduces excitement and calm down the patient without inducing sleeping. It also acts as an anxiolytic agent. In the case of a large dose, it may produce hypnosis. For sedative, the site of action in the limbic system which regulates throughout and also the mental function. Hypnotics are drugs that produce sleep [1]. These types of drugs are used for the initiation of sleep and for maintain sound sleep duration. Hypnotics at higher dose may produce an effect like a general anaesthetic. The main site of function is the midbrain.

Currently marketed sedative and hypnotics drugs tends to produce serious side effects starting from physical dependence, tolerance, digestive and immune system dysfunction to deterioration of cognitive functions. The newer approaching drug with fewer side effects has been suggested to combat different psychiatric disorders [2].

The plant, *Biophytum sensitivum* Linn. DC, belonging to family *Oxiladaceae*, is commonly known as little tree plant. This plant is an annual herb in whose leaves are sensitively crowded into a rosette on the top of the stem. The length of the leaves is 3.8 to 7.5 cm long, petioles are short, rachis is slender and leaflets are opposite (Fig 1). It is widely distributed in throughout hotter parts of India to tropical Africa [3], where this plant has been used as traditional and folk medicine to treat a wide range of diseases that are anti-bacterial, anti-inflammatory, anti-oxidant, anti-tumor, radio protective, antigenic, wound healing, anti-diabetic, cardio-protective, anti-fertility, anti-metastatic, immune-modulatory, anti-hypersensivity in nature [4]. However so far there is no any scientific report demonstrated about the sedative and hypnotic activities of *B.* sensitivum. This gives us a new idea to design the present study to evaluate the sedative and hypnotic activities of this plant.

Fig 1. The leaflets of *B. sensitivum* Plant.

MATERIAL AND METHODS:

The Diazepam of company Cipla Ltd., was procured from Jeypore Sub Divsional Hospital, Jeypore, Koraput. The ethanol of analytical grade was purchased from Himedia, New Delhi. The Soxhlet apparatus used are of Borosil grade, India. All other chemicals, reagents and accessories were procured from an authorised Dealer.

Collection of plant materials and Identification:

The whole plant is collected from the Kandhamala District of Odisha in August 2019. The plant samples were identified by a Scientist of M.S. Swaminathan Research Foundation, Biju Patnaik Herbal Research, Umuri, Jeypore, Koraput, Odisha.

Plant Extraction:

The collected plant was cleaned and shade dried for a month with care of that no such fungal growth should occur or any insects shall not be able to infect the plant. The dried leaflets were powdered in coarse form. The powdered dried leaflets (About 250 g) were extracted by using Soxhlet apparatus with 500 ml ethanol for continuous 72 h. About 6 to 7 solvent cycle was run to complete the extraction process. The extract was then filtered by a Whatmann Filter paper no 4. The extract was concentrated on a water bath at a temp not exceeding 60 °C. The extract was packed in air tight container and stored in dark place for further study [5,6].

Phytochemical screening:

The EE of *B. sensitivum* was evaluated for detection of plant constituents that are Alkaloids, Glycosides, Carbohydrates, Flavonoids, Saponins and Tannins, as per the standard procedures mentioned in specified standard literature books [5,6].

Animals:

Young adult Albino mice of either sex (25 to 30 g) were used in this study after obtaining animal ethical committee clearance. The mice were maintained under standard condition in the animal house (CPCSEA approved regd. No.1960/PO/Re/S/16/CPCSEA) in department of the Pharmacology of Jeypore College of Pharmacy, Jeypore. The mice were kept in polypropylene cages (Polylab Ltd., India) under standard condition of pellet diet, water and *ad labitum*. Animals were acclimatized under a standard laboratory conditions and were kept in a 12 h day and night cycle for seven days before experiment.

Acute toxicity study:

The mice were divided in to control and three test groups each containing 6 no of animals. The EE of *B. sensitivum* was administered to the animals by orally at a doses of 1000, 2000 and 3000 mg /kg of b.w. The mice were given access to food and water *ad libitum* and all animals were observed for any significant behavioral change, allergic symptoms and mortality for 72 h [7,8].

Sedative and Hypnotic activity study:

***Drugs and Treated Animal groups*:**

Diazepam was used as standard sedative and hypnotic drug. It was administered at a dose of 1mg/kg of body weight of mice in intra-peritoneal route. The normal

saline water was used as control. The mice were divided into 5 groups. Each group containing 6 mice. The group I was treated with normal saline water as control in dose of 2 ml/kg b.w. The group II was treated with Diazepam as standard drug at a dose of 1mg/kg b.w. The groups III to V were treated with EE of *B. sensitivum* at doses of 50, 100 and 200 mg/kg b.w.

***Hole Board test*:**

In this test, a platform of 60 × 30 cm diameter with 16 evenly spaced holes are done. After 15 and 30 min of administration of test and standard drugs in all treated animal groups, each animal was given free move on the platform and number of head dips into holes are counted for 5 min [8,9].

***Hole cross test*:**

For this experimental test a wooden cage is prepared having size of 30×20×14 cm with a fixed partition in middle having a hole of 3 cm diameter [12]. Before treatment of drugs, all animals were allowed to pass through the middle hole and the number of passage was counted. After 15 and 30 min of administration of test and standard drugs, the experimental animals allowed to pass through the middle hole and the number of passage by the mice through the middle hole was counted at 30, 60 and 90 min respectively [8-10].

***Exploratory Behavior test by the open- field method*:**

These tests are specifically designed for mice for observing exploratory behavior. The original maze is having a series of squares, which are painted in black and white, a wall mounted up to 50 cm and placed in a dimly light room. Before treatment of drugs, all animals of each group were allowed to pass through the middle hole and the number of passage was counted. After 15 and 30 min of administration of test and standard drugs, the experimental animals were released to the room around the box and no of squares visited by animals was counted at 30, 60 and 90 min respectively [8,10].

***Test for Motor Co-ordination (Rotarod test)*:**

The test was performed by using Rotarod apparatus (RR 01, Orchid Sci, India). After 15 and 30 min of administration of test and standard drugs, each mice was placed on the Rotarod for 3 min and the effect was calculated [8-10].

Statistical Analysis:

To analyses the data for statistical significance, all results were expressed as mean with standard deviation. One way analysis of variance (ANOVA) was carried out and the statically comparison among the groups were performed with Tukey Krammer test using a static package programme. P< 0.05 was considered as significant [11].

Table 1. Photochemical screening of ethanolic extract of *B. sensitivum*.

Phytochemical	Name of test	Result
Alkaloids	Mayer's test	+
	Hager's test	-
	Wagner's test	-
	Tannic acid test	-
Glycosides	General test for glycoside	+
Flavonoids	HCl reduction test	+
Carbohydrates	Fehling's test	+
	Bendicts Test	+
Saponins	Forthing test	-
Tannins	Ferric chloride test	-
	Alkaline reagent test	+

+ and – sign represents present and absent.

RESULTS AND DISCUSSION:

The Soxhlation method was found to be efficient method for successful extraction of phyto-constituents from the plant *B. sensitivum*. The percentage of the yield of the extract was 10 %. The phtochemical screening study revealed that the EE of *B. sensitivum* contains Glycosides, Carbohydrates, Flavonoids and Tannins (Table 1). From the acute toxicity study it was observed that no such significant behavioural change or mortality was found. The oral administration of EE of *B. sensitivum* in a quantifying manner (1000, 2000 and 3000 mg/kg b.w.) also did not produce any allergic and hypersensitive reaction. Thus it confirmed that the EE of *B. sensitivum* is non-toxic to the living body. So it is found that the safety dose of the EE of *B. sensitivum* is considered as up to 3000 mg/kg b.w. The experimental procedure followed for the evaluations are Hole board test, Hole cross test, Open field test and, Motor co-ordination test. During these test oral administration of EE of *B. sensitivum* in all doses (50, 100 and 200 mg/kg b.w.) caused a marked reduction for the crossing of the number of holes. For further observations, the experiment was continued for next 90 min. But similar types of reactions are shown by the animals. The effect is shown in Table 2. The Open-field test shows same

type of responses which are observed and inhibits the locomotors activity and exploratory behaviour which indicated that the EE of *B. sensitivum* has CNS depressant activity (Table 3).

Table 2. The sedative and hypnotic response data of EE of *B. sensitivum* by Hole Board Test.

Groups/ Drugs	Dose (mg/kg)	Response	
		No. of head dips	Inhibition (%)
I/ Control	0.1 ml/kg	56.2±2.27	0
II (DZM)	1	14.0±2.04	76.43
III/ EEBS	50	39.8±2.41	24.23
IV/ EEBS	100	28.6±2.71	43.66
V/ EEBS	200	15.4±1.48	69.46

DZM – Diazepam, EE – Ethanolic extract. The no of head dips values are presented as the Mean ± Standard deviation (n=6). *P<0.05 compared with the control group (one way ANOVA).

For the assay of sedative and anxiolytic activities, the Hole cross test was done. By observing their exploratory behaviour, we found that expression of an anxiolytic state in animals might be reflected in their movements by decreasing the number of movement and head dip within the central hole. Our experimental design gives us the result that EE of *B. sensitivum* in doses of 50, 100 and 200 mg/kg b.w., having sedative activity. This methodological tabulation of responses are given in Table 4. Previously it is observed that the standard drug possess a good muscle relaxant activity along with CNS depressant activity. By considering the marked drug, Diazepam as standard, EE of *B. sensitivum* shows an inducing sedative activity by affecting the general activity and motor co-ordination of the experimental animals. The actions on motor co-ordination are given in Table 5. The EE of *B. sensitivum* shows sedative and hypnotic activities in a dose dependent manner. The sedative and hypnotic activities exhibited by EE of *B. sensitivum* are well comparable with the standard drug, Diazepam.

Table 3. The sedative and hypnotic (Number of passage) response data of EE of *B. sensitivum* by Head Cross Test.

Groups/ Drugs	Dose (mg/kg)	Number of passage through the holes			
		Pre-treatment	30 min	60min	90 min
I/ Control	0.1 ml/kg	18.0 ±1.58	16.60±0.67	13.2±0.93	12.90±0.40
II (DZM)	1	23.2±1.91	5.52±1.69	4.01±0.37	2.3±1.2
III/ EEBS	50	20.6±1.49	14.6±1.54	10.8±1.20	6.93±1.16
IV/ EEBS	100	21.0±1.006	8.80±1.49	6.79±1.20	3.6±0.76
V/ EEBS	200	21.4±0.84	5.79±0.64	1.69±0.71	1.3±0.39

DZM – Diazepam, EE – Ethanolic extract. The values are presented as the Mean ± Standard deviation (n=6). *P<0.05 compared with the control group (one way ANOVA).

Table 4. The sedative and hypnotic response (Number of squares visited by animals) data of EE *B. sensitivum* by Open Field Test.

Groups/ Drugs	Dose (mg/kg)	The Number of squares visited by animals around the box			
		Pre-treatment	30 min	60 min	90 min
I/ Control	0.1 ml/kg	102.3±2.78	93.2±3.31	82.01±3.76	65.8±1.88
II (DZM)	1	89.4±6.08	47.4±1.66	29.4±2.33	13.6±1.09
III/ EEBS	50	85.2±5.83	49.4±3.32	43±3.50	22.3±5.58
IV/ EEBS	100	91.2±6.04	40.2±8.36	32.2±4.87	14.7±2.93
V/ EEBS	200	92.3±5.34	41.5±1.50	21.1±2.26	6.3±0.48

DZM – Diazepam, EE – Ethanolic extract, EEBS – Ethanolic extract of *B. sensitivum*. The values are presented as the Mean ± Standard deviation (n=6). *P<0.05 compared with the control group (one way ANOVA).

CONCLUSION:

It could be concluded that the plant *B. sensitivum* Linn. DC possesses strong sedative and hypnotic activities. This plant primarily assessed that *B. sensitivum* might be used to calm down the CNS, which could be helpful for management of Insomnia disease. Still further extensive research study has to be done to isolate the active chemical moiety from the EE of *B. sensitivum*, which might be responsible for exhibiting sedative and hypnotic activities.

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