IJPSR (2014), Vol. 5, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 September, 2013; received in revised form, 20 November, 2013; accepted, 16 January, 2014; published 01 February, 2014

SCREENING OF BEHAVIOURAL, MUSCLE CO-ORDINATION & ANXIOLYTIC ACTIVITIES OF METHANOLIC EXTRACT OF *SOPHORA INTERRUPTA* (BEDD)

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Keywords:

Sophora interrupta, Behavioural, muscle coordination and Anxiolytic activities

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ABSTRACT: The aim of this study was to evaluate the behavioural, muscle coordination and anxiolytic activities of methanolic extracts of *Sophora interrupta* (papilionaceae) in mice. The behavioural, muscle coordination and anxiolytic activities were evaluated by using various models of CNS using mice. Diazepam was used as standard drug for muscle coordination and anxiolytic studies. The extracts were administered orally at 400 mg/kg. The results of the present study indicates that the methanolic extract of *Sophora interrupta* leaves are effective in inducing a significant protection against behavioural, muscle coordination and anxiolytic activities, as evidenced by various CNS models with respect to control. This study confirmed the behavioural, muscle coordination and anxiolytic activities of this plant as it is used in traditional medicine.

INTRODUCTION: Anxiety and related disorders will become the second leading cause of disability in both developed and developing countries by the year 2020 [1]. Benzodiazepines have been extensively used for the last 40 years to treat several forms of anxiety [2]. Anxiety and musculoskeletal disorders are extremely dramatic and debilitating disorders and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and musculoskeletal disorders, it is impossible to offer effective treatment strategies for the patients. Various herbal remedies are present that possess lesser side effects than the conventional drugs and thus are safer to use.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(2).526-31

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(2).526-31

MATERIALS AND METHODS: *Sophora interrupta* Bedd belongs to family *Papilionaceae* found in Tirumala and it is commonly called as Adavibillu. Since past it is used as Antibacterial, Antifungal and Anticancer, antioxidant, antiasthamatic, antipyretic, cardiotonic, anti-inflammatory, diuretic activities [3]. From the preliminary phytochemical studies it was identified that it has constituents like alkaloids, flavonoids, glycosides, phenols, carbohydrates and proteins [4].

Collection and authentication of plant materials: The plant material was collected in the month of June 2011 from Srichalam hills and a specimen was dropped in the herbarium and the leaves were authenticated by Dr. Madhavachetty. The collected powdered material was shade dried and pulverized.

Solvent for extraction: Petroleum ether and methanol

Preparation of the extract: The dried powders of leaves of *Sophora interrupta* were defatted with

petroleum ether (60-80ºC) in a Soxhlet Apparatus by continuous hot- percolation. The defatted powder material (marc) thus obtained was further extracted with methanol with same method. The solvent was removed by distillation under low pressure and evaporation.

The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Phytochemical Screening: The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

1. **Test for reducing sugars (Fehling's test):** The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

2. **Test for anthraquinones:** The individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

3. **Test for terpenoids (Salkowski test):** To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

4. **Test for flavonoids:** A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

5. **Test for saponins:** To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

6. **Test for tannins:** About 0.5 g of the individual extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (FeCl3) was added and observed for brownish green or a blue-black coloration

7. **Test for alkaloids:** 0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

8. **Test for cardiac glycosides (Keller-Killiani test):** To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

EXPERIMENTAL ANIMALS: Healthy albino rats of either sex between the age of 2-3 months and weighing 150-200 gms and Albino mice weighing 18-25 gm were used for the present study. The animals were kept in well aerated laboratory cages in the animal house and were allowed to acclimatize to the laboratory environment for a period of 2 weeks before the commencement of experiment.

They were maintained on standard animal feed and drinking water ad libitum during the stabilization period.

All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg.no (1447/po/a/11/ CPCSEA)

Experimental Protocol:

Treatment: The extract of *Sophora interrupta* was freshly dissolved in a suitable amount of distilled water to be acutely administered per os (p.o.) by an intra-gastric cannula in mice, or intraperitoneally (i.p.) in rats. One hour after p.o. or 30 min after i.p. administration, the animals were submitted to the various CNS tests. Doses of the extract and the time intervals were determined in preliminary tests. Diazepam (1 mg/kg) was dissolved in 40% propylene glycol and distilled water, respectively, immediately prior to use and given intraperitoneally. All administrations were performed in a dose volume of 1 ml/kg body weight. Control groups received only distilled water in the same volume by the same route. The Animals were fasted 18hrs prior to the experiment; treatment group was done as follows,

Group-1: Control administered with 1 ml of distilled water orally.

Group-2: Standard drug diazepam 1mg/kg.

Group-3: 400 mg/kg b.w of *Sophora interrupta* orally.

Behavioural activity: Behavioural activity was evaluated by using Hole-board model

Hole board test: Hole board test is a generally used method for screening the potential anxiolytic character of the drugs. The Hole board consists of wooden chamber (40×40×25cm) with 16 holes (diameter of 3 cm) on the floor, elevated from the ground so that the mice could peep through the holes. Each mouse will be placed individually in the apparatus for recording the parameters like latency to the first head dips, number of head dips in the holes, total time spent with the head dips, no. of rearings, number of defecation units.

Anti-anxiety activity: Anxiolytic activity was evaluated by using

1. Staircase model
2. Elevated plus maze model
3. Dark and light model
4. Swim test

1. **Staircase model:** Staircase consists of five identical steps of 2.5cm high, 10cm wide and 7.5cm deep. The internal height of the walls is constant along whole length of the staircase. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears are counted over a 3 min period. A step is considered to be climbed only if the mouse had placed all four paws on the step.

2. **Elevated plus maze model:** The plus maze apparatus consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with the plus maze elevated (25cm) from the floor, was used to observe anxiolytic behaviour in animals. All the animals in the different groups were administered the normal water, extract and standard drug orally using a tuberculin syringe fitted with oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus maze apparatus 45min after the administration of the dose. Each mouse was placed at the centre of the elevated plus maze with its head facing the open arm. During this 5min experiment the behaviour of the mouse was recorded as

   i. Preference of the mouse for its first entry into the open arms

   ii. The number of entries into the open arms or closed arms

   iii. Average time spent by the mouse in the open arms (Average time= total duration in the arms/number of entries)

During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals. Similar observations were recorded for the standard group (Diazepam 2mg/kg) as well as the control group (vehicle 1ml) [5].

3. **Dark and light model:** The light/dark box consist of a light, open topped, opaque, plexiglass box connected to a dark, closed topped, plexiglass box. Each compartment measuring (30×40×40cm). The boxes were

connected by a small opening that allows the rat to cross between chambers. Each rat was placed individually in the center of the light compartment and observed for the next 5 minutes for the number of crossing between two compartments and time spent in the light and dark compartments. All the animals were placed in this and the observation was noted.

4. **Swim test:** Mice weighing 18-25g are used. They are bought to the laboratory at least one day before the experiment and are housed separately in cages with free access to food and water. Mice are individually forced to swim inside a vertical Plexiglas cylinder (height 40cm; diameter 18cm; containing 15cm of water maintained at 25ºc). Mice placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom.

Skeletal muscle relaxant activity or Muscle co-ordination Test: The muscle co-ordination activity was evaluated by Rotarod apparatus.

Rotarod apparatus: The Rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The animals were trained to remain for 3min on the rod rotating at a speed of 25rpm. On the next day either vehicle or methanolic extract of *Kigelia africana* (200mg/kg) was administered orally and their ability to remain on the rotating rod was assessed before and 30min after the oral administration. The fall-off time from the rod was noted for each animal [6].

STATISTICAL ANALYSIS: The results of study were subjected to one way Analysis of Variance (ANOVA) followed by Dunnett's test. Values with $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION:

Phytochemical screening: Phytochemical screening of the extracts of *Sophora interrupta* showed the presence of various chemical constituents, mainly alkaloids, flavonoids, tannins, saponins, phenols, terpinoids, glycosides and sugars.

Hole board test: The statistical summary of the latency to 1st head dips, number of head dips are presented **in Table 1**. The doses when administered orally have shown significant increase in latency to 1st head dips, and number of head dips for every 30min.

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *SOPHORA INTERRUPTA* LEAVES ON HOLE BOARD MODEL IN MICE

Treatment group	Dose	No. of head dips				% Activity
		30 min	60 min	90 min	120 min	
Control	1ml/kg/p.o	24±1.84	27±0.28	25±0.92	29±0.37	-
Diazepam	1mg/kg/i.p	15±2.85***	19±0.49***	13±0.24***	16±0.92***	42.39
MESI	400 mg/kg/p.o	20±0.71**	28±0.42**	21±0.56**	18±0.39**	37.38

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.5, **p<0.1,***p<0.05,****P<0.001 vs. control.

Staircase test: The statistical summary of the rearing and number of steps climbed is presented in **Table 2**. After 60 and 90 min of treatment, a reduction in anxiety linked behaviour was indicated by a reduction in number of rearing and sedation that was evaluated by number of steps climbed. The dose of methanolic extract of *Sophora interrupta* leaves (400 mg/kg/p.o) and standard drug (Diazepam, 1mg/kg/i.p) significantly reduced the number of rearings as well as Number of steps climbed.

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *SOPHORA INTERRUPTA* LEAVES ON STAIR CASE MODEL IN RATS

Treatment group	Dose	No. of rearings	No. of steps climbed	% Activity
Control	1ml/kg/p.o	30±0.91	26±1.34	-
Diazepam	1mg/kg/i.p	37±0.56****	10±0.26****	62.47
MESI	400 mg/kg/p.o	19±0.78**	17±0.47**	36.1

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.5, **p<0.1,***p<0.05,****P<0.001 vs. control.

Elevated plus maze test: The dose of methanolic extract of *Sophora interrupta* (400 mg/kg/p.o) significantly increased the time spent and no of entries into open arms when compared with control showed in **Table 3**.

TABLE 3: EFFECT OF METHANOLIC EXTRACT OF *SOPHORA INTERRUPTA* LEAVES ON ELEVATED PLUS MAZE APPARATUS IN RATS

Treatment group	Dose	No of entries (counts/5min)		Time spent (5min)		% Activity
		Closed arm	Open arm	closed arm	Open arm	
Control	1ml/kg/p.o	9±0.21	9±0.81	19±0.74	91±0.85	-
Diazepam	1mg/kg/i.p	3±0.12****	3±0.28****	39±0.26****	31±0.23****	66.6
MESI	400 mg/kg/p.o	5±0.43**	5±0.39**	75±0.49**	53±0.43**	44.4

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.5, **p<0.1,***p<0.05,****P<0.001 vs. control.

Dark and light model: Table 4 indicates the significant increase in the time spent in light compartment with administration of *Sophora interrupta* at a dose of 400mg/kg when compared to control.

TABLE 4: EFFECT OF METHANOLIC EXTRACT OF *SOPHORA INTERRUPTA* LEAVES ON DARK/LIGHT BOX TEST IN RATS

Treatment group	Dose	No. of entries in light chamber		Time spent in light chamber		% Activity
		Before treatment	After treatment	Before treatment	After treatment	
Control	1ml/kg/p.o	7±0.58	7±0.69	179±10.99	245±27.57	-
Diazepam	1mg/kg/i.p	4±0.91****	4±0.42****	71±6.87****	71±7.58****	71.7
MESI	400mg/kg/p.o	9±0.46**	9±0.24**	126±19.5**	128±17.5**	59.4

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.5, **p<0.1,***p<0.05,****P<0.001 vs. control.

Swim test: The values are represented in **Table 5** in this test when doses administered orally have shown significant decrease in duration of swimming. **Rotarod apparatus:** Represented in Table 6 In this test, methanolic extract of *Sophora interrupta* (400 mg/kg/p.o) significantly reduced the time spent by the animals on revolving rod when compared to control.

TABLE 5: EFFECT OF METHANOLIC EXTRACT OF *SOPHORA INTERRUPTA* LEAVES ON SWIM TEST IN RATS

Treatment group	Dose	Duration of immobility in 5 min period (sec)
Control	1ml/kg/p.o	20±0.14
Diazepam	1mg/kg/i.p	122±5.46****
MESI	400 mg/kg/p.o	79±0.47**

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.5, **p<0.1,***p<0.05,****P<0.001 vs. control.

TABLE 6: EFFECT OF METHANOLIC EXTRACT OF *SOPHORA INTERRUPTA* LEAVES ON ROTA ROD APPARATUS TEST IN MICE

Treatment group	Dose	Fall of time(sec)				%Activity
		30 min	60 min	90 min	120 min	
Control	1ml/kg/p.o	191±16.7	118±18.3	151±14.4	150±13.9	-
Diazepam	1mg/kg/i.p	35±0.3***	37±0.7***	28±0.9***	38±0.7***	76.38
MESI	400 mg/kg/p.o	68±4.74**	51±3.89**	46±3.76**	69±4.46**	55.17

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test. *p<0.5, **p<0.1,***p<0.05,****P<0.001 vs. control.

DISCUSSION: The present study investigated the putative central effects of the methanolic extract of the leaves of *Sophora interrupta* a plant generally used in folk medicine as a sedative and antihypertensive remedy. Thus, given acutely at single doses of 100mg/kg the extract of *Sophora interrupta* produced significant dose-related decreases rearing and grooming behavior. The evaluation of the putative anxiolytic activity of *Sophora interrupta* was performed with the EPM, hole-board, and Stair case. In the EPM, rats with i.p. treatment showed a significant increase in both

the number of entries and the percentage of time spent in the open arms of the maze, similar to the effects observed after administration of the reference anxiolytic drug diazepam. The oral treatment of mice with the extract promoted a slight enhancement of the percentage of entries in open arms at 400 mg/kg. Moreover, this treatment produced a decrease on head-dipping and stretch-attend postures along with an increase on rearing. Altogether, these results could indicate a mild anxiolytic-like activity of the extract of *Sophora interrupta* leaves.

However, the same treatment was unable to induce any significant effect in mice evaluated in the hole-board tests, which could be attributed to different kinds of anxiety. The results obtained after i.p. administration of the extract in rats demonstrate the high potency of the CNS effects of this plant.

Chemical studies have reported the presence of several compounds on different parts of the plant. It is well known that the Methanolic extract of leaves of *Sophora interrupta* which is proposed as its hypotensive principle. In conclusion, our results provide evidence that the Methanolic extract of the leaves of *Sophora interrupta* possesses CNS properties. Besides, anxiolytic-like effects are suggested by the EPM experiments, stair case and hole board and the motor coordination is through rota rod and traction test. However, further studies are necessary to confirm and extend these results.

The findings presented here are relevant because they validate, for the first time, the folk uses of *Sophora interrupta*, an important medicinal plant.

REFERENCES:

1. Kim, J.H, S.Y Lee, Jang C.G, Antidepressant like effects of Albizzia julibrassin in mice: involvement of 5-HT_{1A} receptors system. Pharmacol. Biochem. And Behav. 2007; 87: 41-47.
2. Jordan, A.D, Kordik, C.P., Reitz, A.B and Sanfillopo, P.J. Novel anxiolytic agents 1994 to present. Expert Opin.Ther.Pat. 1996; 6: 1047-1060.
3. Flowering plant of Chittoor district Andhra Pradesh, India. Students offset printers, Tirupathi 2008; 101.
4. Cai YZ, Luo Q, Sun M, Cork H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci, 2004; 74: 2157-2184.
5. P. K.Warrier. Indian medicinal plants: A compendium of 500 species**.** 1994; 4:3811.
6. Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM. African journal of pure and applied chemistry, 2010; 4(9): 206-212.

How to cite this article:
Veeresh K, Vijusha M, Rajani and Hemamalini K: Screening of behavioural, muscle co-ordination & anxiolytic activities of methanolic extract of *Sophora interrupta* (Bedd). *Int J Pharm Sci Res* 2014; 5(2): 526-31.doi: 10.13040/IJPSR.0975-8232.5(2).526-31