ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.2010

International Journal of Pharma and Bio Sciences

EVALUATION OF ANXIOLYTIC ACTIVITY OF METHANOLIC EXTRACT OF *SAPINDUS MUKOROSSI* GAERTN. IN MICE

AVIJIT CHAKRABORTY*, P. AMUDHA , M. GEETHA AND N. SURJIT SINGH

Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Chennai- 97,India

*Corresponding author email avijitchakraborty84@gmail.com

ABSTRACT

The present study was undertaken to evaluate the anxiolytic activity of methanolic extract of *Sapindus mukorossi* Gaertn.(Sapindaceae). The anxiolytic activity was evaluated by Elevated plus maze, Y-maze, Hole-board, Actophotometer, and Marble-burying behavior models. The efficacy of the extract (200 and 400 mg/kg) was compared with the standard anxiolytic drugs Diazepam (2 mg/kg) and Fluoxetine (10 mg/kg). The result showed that the extract significantly increased the number of entries and time spent in the open arm in the elevated plus maze. The results also showed that the extract significantly increased the number of head dipping and line crossing, decreased the numbers of visits to the three arms, locomotor score and number of marble-buried in Hole-board, Y-maze, Actophotometer and Marble-burying behavior models respectively. Present study confirms that the extract showed significant anxiolytic activity at both dose levels which is comparable with standard anxiolytics Diazepam and Fluoxetine.

KEYWORDS

Elevated Plus Maze, Y-maze, Hole-board, Actophotometer, Marble-burying behavior.

INTRODUCTION

Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat [1]. Anxiety effects one-eighth population worldwide and has become an important research area in the field of psychopharmacology [2]. Benzodiazepines (BZDs), barbiturates, tricyclic antidepressants (TCA's) have been used for long time to treat anxiety disorders. The serious side effects associated with these drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (BZD's, barbiturates and alcohol), sexual dysfunction, anticholinergic, antihistaminic effects (TCA's) have limited their use in patients [3]. Due to this many pharmaceutical companies are conducting studies to find an alternative medicine

or plant-derived medications with more specific anxiolytic effects [4].

The plant *Sapindus mukorossi* Gaertn., of family Sapindaceae is a well known plant in the Indian medicinal system and has historically been used in folk remedies as an expectorant [5], emetic [6], contraceptive [7], for treatment of excessive salivation [5], epilepsy [5, 8] and chlorosis [5]. They are a popular ingredient in Ayurvedic shampoos and cleansers.

A survey of literature on *Sapindus mukorossi* Gaertn. has revealed only a few pharmacological reports of the plant. No major investigated reports were found for its CNS activity; therefore, we undertook the present study to determine the anxiolytic activity of fruits and seeds of *Sapindus mukorossi* Gaertn .by using different animal models for anxiety.

MATERIALS AND METHODS

Collection and Authentication of Plant Material:

The plant materials were collected from a local distributer from Tripura and the plant material was identified and authenticated by resident botanist, Prof. Dr. P. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai.

Preparation of Methanolic Extract of *Sapindus mukorossi* Gaertn.

The fruits and seeds of the plant were dried in the shade and made powder. The powder (210g) was extracted using methanol as a solvent in Soxhlet apparatus (60-70ºC). The filtrate was evaporated to dryness at 40^0C. The methanolic extract of *Sapindus mukorossi* Gaertn. (MESM) yielded a thick brown semi-solid residue.

Phytochemical Screening [9, 10]:

The extract was subjected to preliminary phytochemical screening by the methods previously described by Kokate and Jayaraman J.

Drugs and Chemicals:

Diazepam (Ranbaxy Laboratories Ltd., Mumbai) and Fluoxetine (Pfizer Ltd., Mumbai) were used as the standard anxiolytic drugs. Methanol was purchased locally and was of analytical grade. Distilled water was used as vehicle.

Preparation of Test Doses:

The extracts were suspended in the vehicle in such concentrations as to administer 200 and 400 mg/kg doses to mice through the per oral route.

Animals:

Inbred adult albino mice (20-25 gms) of either sex were obtained from the animal house of C.L.Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited, Bangalore) and drinking water was provided ad libitum. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The animals were divided into four groups, each consisting of six mice and were used in all sets of experiments. Institutional Animal Ethical Committee approved the protocol of the study.

Acute Toxicity Study:

The procedure was followed as per OECD 423 guidelines. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioral, Neurological toxicity and mortality 14 days.

Elevated Plus Maze Model [2, 11]:

The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof. The MESM (200 and 400 mg/kg) and vehicle were administered for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment. The standard drug was given at a dose of 2 mg/kg p.o.

60 min before starting the experiment. After proper treatment each mouse was placed at the center of the maze with its head facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as: the number of entries into the open or closed arms and time spent by the mouse in each of the arms. An arm entry was defined as the entry of all four paws into the arm.

Y – Maze Model [12]:

Y- Maze is made of black painted wood or grey plastic. Mice were treated with the MESM (200 and 400 mg / kg p.o.) or vehicle for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min prior to experiment and kept individually in one arm of the apparatus. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. For a period of 10 min. the total numbers of visits to different arm were measured.

Hole –Board Model [2]:

The Hole-board apparatus was used as described earlier. The apparatus consists of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. The MESM (200 and 400 mg/kg) and vehicle were administered for 5 days p.o. once daily and the last dose was given on the 5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment. For a period of 10 min. the number of line crossing and number of head dipping were calculated.

Locomotor Activity [2]:

The locomotor activity was measured by using an Actophotometer. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. The MESM (200 and 400 mg/kg) and vehicle were administered for 5 days once daily p.o. and the last dose was given on the 5th day, 60 min before starting the experiment. The standard drug was given at a dose of 2 mg/kg p.o. 60 min before starting the experiment and the animals were kept in the Actophotometer individually. The locomotor activity was measured for a period of 10 min.

Marble-Burying Behavior Model [13]:

In this method animals were individually placed in transparent; poly carbonate cages (22 x 32 x 13.5 cm) containing a 5 cm layer of saw dust and 24 glass marbles (1.5 cm in diameter) were evenly distributed on the saw dust in the cages. The MESM (200 and 400 mg/kg) and the vehicle were administered once daily p.o. for 5 days and the last dose was given on the 5th day, 60 min prior to experiment. The standard drug was Fluoxetine was given at a dose of 10 mg/kg p.o. 60 min prior to the experiment and kept in the cages for a period of 30 min. and the number of marbles at least two-third buried in the saw dust was recorded.

Statistical Analysis:

The data were expressed as mean ± standard error mean (SEM). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test was followed by Dunnett's 't'-test, p values less than 0.05 were considered as significance.

RESULTS

Phytochemical Screening:

The preliminary phytochemical analysis of MESM showed that the plant contains carbohydrates, flavanoid, flavanones, glycoside, saponin but alkaloid, sterol, protein, tannin, phenols, steroids are absent.

Acute toxicity Study:

Acute oral toxicity studies revealed the non-toxic nature of MESM. There was no morbidity observed or any profound toxic reactions found at a dose of 2000 mg/Kg p.o. which indirectly pronouns the safety profile of the plant extract.

Elevated Plus Maze Model:

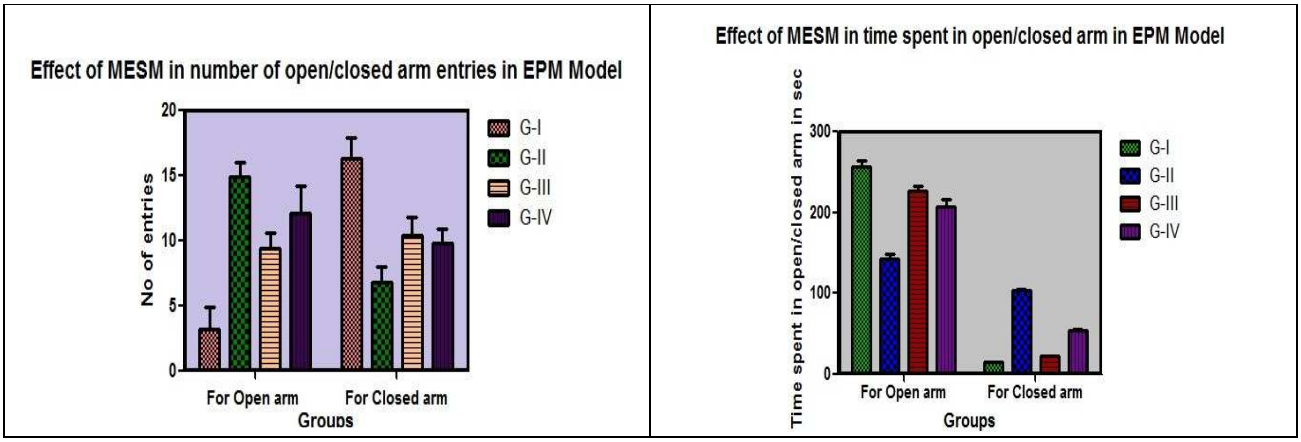
The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard Diazepam.

Table-1

Effect of MESM on animals in EPM model:

Group	Treatment	Time spent in the open arm (s)	Time spent in the enclosed arm (s)	No. of entries in open arm	No. of entries in enclosed arm
I	Vehicle	14.5 ± 1.41	256 ± 7.90	3.2 ± 1.70	16.3 ± 1.60
II	Diazepam 2mg/Kg	103 ± 1.54***	142 ±7.10***	14.9 ± 1.10***	6.80 ± 1.20***
III	MESM 200 mg/kg	22.2 ± 1.10**	226 ± 6.80*	9.4 ± 1.20*	10.4 ± 1.40*
IV	MESM 400 mg/kg	54.3 ± 1.93***	207± 9.40***	12.1 ± 2.10**	9.80 ± 1.10**

*n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*



Y-Maze Model:

A significant decrease in the number of visits in the three arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. Both the doses of MESM showed a

significant decrease in the number of visits in the three arms of the Y-maze which was comparable with the standard Diazepam.

Table-2

Effect of MESM on animals in Y-maze model:

Group	Treatment	Number of visits
I	Vehicle	59.7 ± 5.7
II	Diazepam 2mg/Kg	29.8 ± 4.3***
III	MESM 200 mg/kg	39.6 ± 4.7*
IV	MESM 400 mg/kg	36.3 ± 3.9**

*n = 6, *p < 0.05, **p < 0.01, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*

Hole-Board Model:

The number of line crossing and head dipping was increased significantly in case of Diazepam treated animals as compared to the control animals. The MESM at both dose levels showed an increase in the number of line crossing and head dipping significantly as compared to the control animals.

Table-3
Effect of MESM on animals in Hole-board model

Group	Treatment	No. of head dipping	No. of line crossing
I	Vehicle	20.7 ± 1.33	61.5 ± 1.41
II	Diazepam 2mg/Kg	42.2 ±1.58***	162 ± 5.66***
III	MESM 200 mg/kg	26.2 ± 1.42*	73.5 ± 1.38*
IV	MESM 400 mg/kg	29.8 ± 1.17***	114 ± 2.55***

*n = 6, *p < 0.05, ***p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*

Locomotor Activity:

A significant decrease in the locomotor score was observed for Diazepam when compared to the control animals. Both the doses of MESM showed significant decrease in the locomotor score when compared to the control animals.

Table-4

Effect of MESM on animals in locomotor activity:

Group	Treatment	Locomotor activity for 10 min.
I	Vehicle	540 ± 12.7
II	Diazepam 2mg/Kg	303 ± 9.8***
III	MESM 200 mg/kg	480 ± 16.4*
IV	MESM 400 mg/kg	465 ± 14.7**

*n = 6, * p < 0.05, ** p < 0.01, *** p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*

Marble-Burying Behavior Model:

A significant decrease in the number of marble buried was observed for the standard Fluoxetine when compared to the control animals. The MESM at both dose levels showed significant decrease in the number of marble buried which was comparable with the standard Fluoxetine.

Table-5

Effect of MESM on animals in Marble-burying Behavior model:

Group	Treatment	No of marble buried
I	Vehicle	19.7 ± 1.20
II	Fluoxetine 10mg/Kg	7.4 ± 1.70***
III	MESM 200 mg/kg	14.4 ± 1.00*
IV	MESM 400 mg/kg	12.1 ± 1.40**

*n = 6, * p < 0.05, ** p < 0.01, *** p < 0.001 (one way ANOVA followed by Dunnett's 't' test)*

DISCUSSIONS

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic, serotonergic neurotransmission in etiology, expression and treatment of anxiety [14, 15]. The adrenergic and dopaminergic systems have also been shown to play a role in anxiety [16].

Despite the widespread traditional use of *Sapindus mukorossi* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrated that the methanolic extract of *Sapindus mukorossi* had anxiolytic activity in mice in several animal models of anxiety like by EPM, Y-maze, Hole-board, Actophotometer and Marble-burying Behavior models.

The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the $GABA_A$-benzodiazepine complex [17]. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances

such as the benzodiazepines [18]. In EPM, naïve mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics [19].

In this study, we observed that MESM (200 and 400mg//kg) induced significant increases in the both number of entries and time spent in the open arms and the number of entries and time spent in the closed arms were reduced in the EPM model.

The results obtained in the Y-maze model showed that the number of visits in the three arms decreased significantly for all groups when compared to the control animals, which supports the anxiolytic activity of MESM.

In the Hole-board model a significant increase in the exploratory head-dipping and line crossing behavior were observed after treatment with 200 and 400 mg/kg of MESM, thus reinforcing the hypothesis that it has anxiolytic activity.

Locomotors activity is considered as an index of alertness and a decrease in that indicates a sedative effect [20]. Both the doses 200 and 400 mg/kg of the extract showed a decrease in the locomotors score, thus indicating the sedative effect of the extract.

The marble-burying behavior model has been suggested as a useful model for evaluating anti-obsessive-compulsive disorder drugs because no change in the intensity of marble-burying behavior occurred during repeated testing (this is considered as compulsive behavior) [21]. Both the doses 200 and 400 mg/kg of the extract decreased significantly the number of marble-buried.

Earlier reports on the chemical constituents of the plants and their pharmacology suggest that plants containing flavanoids, saponins and tannins possess activity against many CNS disorders [22]. Phytochemical tests of MESM revealed the presence of saponin and flavanoid. It may possible that the mechanism of anxiolytic action of MESM could be due to the binding of any of these phytochemicals to the $GABA_A$-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the $GABA_A$ receptor [23]. The plant *Sapindus mukorossi* also contains flavones which may responsible for its anxiolytic activity.

So the anxiolytic activity of MESM might involve an action on GABAergic transmission or effects on serotonergic transmission or due to its mixed aminergic potentiating effect.

CONCLUSION

From the above observations we can conclude that methanolic extract of *Sapindus mukorossi* possesses anxiolytic activity at both the dose level which is comparable with the standards. However further studies are required to know the exact mechanism of action of MESM as anxiolytics.

REFERENCES

1. Kulkarni SK, Reddy DS, Animal behavioral models for testing anti-anxiety agents. Meth Find Exp Clin Pharmacol, 18 (3): 219-230, (1996).
2. Yadav AV, Kawale LA, Nade VS, Effect of *Morus alba* L. (mulberry) leaves on anxiety in mice. Indian Journal of Pharmacol, 40: 32-36, (2008).
3. Kulkarni SK, Singh K, Bishnoi M, Comparative behavioral profile of newer antianxiety drugs on different mazes. Indian Journal of Expt. Biol, 46: 633-638, (2008).
4. Rabbani M, Sajjadi SE and Mohammadi A, Evaluation of the anxiolytic effect of *Nepeta persica* Boiss. in mice. eCAM, 5 (2): 181–186, (2008).

5. Maiti PC, Roy S, and Roy A, Chemical investigation of Indian soapnut, *Sapindus laurifolius* Vahl. Cellular and Molecular Life Sciences, 24 (11): 1091, (1968).
6. Garg S, Doncel G, Chabra S, Upadhyay SN and Talwar GP, Synergistic spermicidal activity of neem seed extract, reetha saponins and quinine hydrochloride. Contraception 50: 185–190, (1994).
7. Setty BS, Kamboj VP and Khanna NM, Screening of Indian Plants for biological activity Part. VII. Spermicidal activity of Indian plants. Indian Journal of Expt. Biol, 15: 231–232, (1977).
8. Ojha P, Maikhuri JP, Gupta G, Effect of spermicides on *Lactobacillus acidophilus* in vitro - nonoxynol-9 vs. *Sapindus* saponins. Contraception, 68 (2) : 135-138, (2003).
9. Kokate CK, Practical Pharmacognosy, 5th Edn, Vallabh Prakasham: 107-121, (1991).
10. Jayaraman J, Laboratory Manual in Biochemistry, 1st Edn, New age international (p) Ltd: 51, (1981).
11. Kumar S and Sharma A, Anti-anxiety Activity Studies on Homoeopathic Formulations of *Turnera aphrodisiaca* Ward. eCAM, 2 (1): 117–119, (2005).
12. Monique V, Willy M, Francoise D, Michel LM, Herve S, and Stefania M, Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress induced corticosterone secretion. The Journal of Neuroscience, 2626-2636, (1997).
13. Toshiharu S, Michihiko I, Shigeyuki C, Anxiolytic- like activity of MGS0039, a potent group II metabotropic glutamate receptor antagonist, in a marble-burying behavior test. European Journal of Pharmacology, 501: 121-125, (2004).
14. Graeff FG, Guimares FS, de Andrade TG and Deakin JF, Role of 5-HT in stress, anxiety and depression. Pharmacol. Biochem. Behav, 54: 129–141, (1996).
15. Griebel, G, 5-hydroxytryptamine pathways in anxiety and its treatment. Pharmacol. Ther, 66: 103–148, (1995).
16. Clement Y and Chapouthier G, Biological bases of anxiety. Neuroscience and Biobehavioral Reviews, 22 (5): 623-633, (1998).
17. Dhonnchadha BAN, Bourin M, Hascoet M, Anxiolytic-like effects of 5-HT_2 ligands on three mouse models of Anxiety. Behavioural Brain Research, 140: 203-214, (2003).
18. Maribel HR, Yolanda GB, Sergio M, Gabriela DV, Glauce SBV, Jaime T, Guillermo R, Antidepressant and anxiolytic effects of hydroalcoholic extract from *Salvia elegans.* Journal of Ethnopharmacology, 107: 53–58, (2006).
19. Hellion-Ibarrola MC, Ibarrola DA, Montalbetti Y, Kennedy ML, Heinichen O, Campuzanoa M, Tortoriello J, Fernandez S, Wasowski C, Marder M, De Limad TCM, Mora S, The anxiolytic-like effects of *Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae) in mice. Journal of Ethnopharmacology, 105: 400–408, (2006).
20. Thakur VD, Mengi SA, Neuropharmacological profile of *Eclipta alba* (Linn.) Hassk. Journal of Ethnopharmacology, 102: 23–31, (2005).
21. Njung'e K and Handley SL, Evaluation of marble-burying behavior as a model of anxiety. Pharmacology Biochemistry and Behavior, 38 (1): 63-67, (1991).
22. Bhattacharya SK, Satyan KS, Experimental methods for evaluation of psychotropic agents in rodents: I-Anti-anxiety agents. Indian Journal of Experimental Biology, 35: 565-575, (1997).
23. Adeyemi OO, Yemitan OK, Taiwo AE, Neurosedative and muscle-relaxant activities of ethyl acetate extract of *Baphia nitida* AFZEL. Journal of Ethnopharmacology, 106: 312–316, (2006).