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Anti-anxiety activity of *Mimusops elengi* barks extract in experimental animals.

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ABSTRACT

Mimusops elengi Linn (Sapotaceae) commonly known as Bakul, is a small to large evergreen tree. It is cultivated in gardens as an ornamental tree. Bark is used as a tonic, febrifuge, against odontopathy, inflammation. The part of plants was used in Thai traditional as rejuvenating and neuro tonic remedies, also *Mimusops elengi* is being screened for acetyl cholinesterase inhibitory activity. Despite the widespread uses of the plant, no scientific work is reported in literature regarding the effect of *Mimusops elengi* bark against anxiety like states therefore, the present study evaluates the anti-anxiety activity of methanolic, aqueous and n-butanol extract of bark of *Mimusops elengi* using elevated plus maze in swiss albino mice. Different doses of Test extract was evaluated in animals. Methanol extract at 50,100 and 200 mg/kg, aqueous extract at 100 and 200 while n-butanol extract at 200 mg/kg in mice were active but methanolic extract at 200 mg/kg was found to have more significant anxiolytic activity as compared to aqueous and n-butanol extract. Also activity of methanolic extract at 200 mg/kg was comparable with diazepam.

Keywords: *Mimusops elengi*, Elevated plus maze, Methanolic extract, Antianxiety.

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INTRODUCTION

Mimusops elengi L. (ME) (Sapotaceae) ("Syn.": Bakul, aulsari) is a small to large evergreen tree found all over the different parts of India. It has been used in the indigenous system of medicine for the treatment of various ailments. Bark of *Mimusops elengi* possesses cardio tonic, alexipharmic, stomachic, anthelmintic and astringent activity [1]. Phytochemical review reveals the presence of taraxerol, taraxerone, ursolic acid, betulinic acid, α -spinosterol, β -sitosterol glycoside, quercitol [2], lupeol [3], alkaloid isoretronecyl tiglate [4] and mixture of triterpenoid saponins [5] in the bark of ME. It is a rich source of tannin, saponin, alkaloids, glycoside, and ursolic acid. ME is appeared to be a good source of natural antioxidant. Various parts like flower are brain tonic and are useful as snuff to relieve cephalalgia. The existing line of treatment for anxiety and convulsions presents number of limitations with respect to side effects for example clinical uses of benzodiazepines are limited by their side effects such as psychomotor impairment, potentiating of other central depressant drugs and dependence liability Remedies from natural origin have drawn attention in the recent times because of multi factorial mechanisms with minimum of side effects. Also elevated plus-maze (EPM) model is well established animal model for testing anxiolytic drugs [6]. Hence we tested different doses of extract using elevated plus maze. For more sensitive measures of effects of new anxiolytic compounds, risk assessment behavior (behavior related to anxiety/fear) such as stretch attend postures (SAP) and head dips (HD) were also measured in addition to measure of time spent on and number of entries into arms in the EPM [7] which are supporting indicators of fear reducing substance effect [8].

MATERIALS AND METHOD

Collection and authentication of plant material:

Mimusops elengi bark was collected during May and June from Rajgurunagar, Pune district, Maharashtra State, India. The plant was identified and authenticated by Botanical Survey of India and a voucher specimen was deposited at Botanical Survey of India (voucher specimen sample no. GG 01).

Extraction of methanolic extract of ME (MEME):

The stem bark of ME was shade-dried and powdered in a grinder. The powdered material (100 g) was extracted with methanol using soxhlet extraction at 40°C. The extract was dried on a tray dryer at 40°C (10.2 % yield w/w).

Extraction of n-butanolic extract of ME (NBME):

The stem bark of ME was shade-dried and powdered in a grinder. The powdered material (100 g) was extracted with n-butanol using soxhlet extraction at 40°C. The extract was dried on a tray dryer at 40°C (6 % yield w/w).

Extraction of aqueous extract of ME (AQME):

The stem bark of ME was shade-dried and powdered in a grinder. The powdered material (100 g) was extracted with distilled water using soxhlet extraction at 40°C. The extract was dried on a tray dryer at 40°C (20 % yield w/w).

Table.1 Preliminary phytochemical analysis of bark of *M. elengi* in methanol , n-butanol and aqueous extract.

Constituents	MEME	NBME	AQME
Carbohydrates	+	+	+
Protein	+	+	+
Alkaloid	-	-	-
Steroids	+	+	+
Flavanoid	+	+	+
Terpenoids	+	+	+
Glycoside	+	+	+
Saponin	+	+	+
Tannin	+	+	+
Fixed oil	-	+	-

+ and - indicate presence and absence respectively.

EXPERIMENTAL ANIMALS

Swiss albino mice (25-30 g) of either sex were obtained from Serum Institute of India, Pune, India. The animals had free access to food pellets and water was available ad libitum. All the experiments were carried out between 10.00 h to 16.00 h at ambient temperature . The animals were drawn at random for test and control groups. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study.

ACUTE ORAL TOXICITY STUDIES

Adult albino mice of either sex were subjected to acute toxicity studies as per guideline (425) suggested by Organization for Economic Co-operation and Development (OECD). The mice were observed for 2 h for behavioral, neurological and autonomic profiles and for any lethality during next 48 h. The LD50 was found to be > 5000mg/kg, p.o

ELEVATED PLUS MAZE

Animals were divided into Eleven (I-XI) groups. Group I was a negative control and was given vehicle, consisting of simple syrup IP and carboxy methyl cellulose (2%), in a dose of 0.25ml. Group II was a positive control and was given standard drug, diazepam (2mg/kg, orally), suspended in the vehicle. Groups III to V received MEME at doses of 50 ,100 and 200 mg/kg p.o respectively, Group VI to VIII received n-butanolic extract of ME (NBME) at doses of 50, 100 and 200 mg/kg, p.o respectively , Group IX to XI received Aqueous Extract of

ME (AQME) at doses of 50 ,100 and 200 mg/kg, p.o respectively. All the test solutions, standard drug and control were administered orally 45 minutes prior to elevated plus maze test.

Elevated plus maze model

The elevated plus-maze apparatus which is either in the shape of plus or cross consist of two open arms (16 x 5 cm), two closed arms (16 x 5 x 12 cm for mice), and an open roof with the entire maze elevated (25 cm) from the floor. The animals were placed individually in the centre of the maze, head facing towards open arms .The amount of time spent on and number of entries in both open and closed arms whereas numbers of stretch attend postures and head dips in closed arms were measured manually during the 5 min test period. An anxiolytic response was defines as increased number of entries and time spent in the open arm of elevated plus maze. Following parameters were evaluated during the 5 min time period.

- a) First preference of mice to open and closed arm.
- b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm)
- c) Time each animal spends in each arm:
- d) Stretch attended postures
- e) Head dips

The apparatus was cleaned after each mouse was tested to remove any residue or odor

STATISTICAL ANALYSIS

Results are represented as Mean \pm SEM. The test extract, standard and control were analyzed with the help of one-way analysis of variance (ANOVA) followed by Dunnett's Test. P values < 0.05 were considered as statistically significant

RESULTS AND DISCUSSIONS

Pathological anxiety is one of the most common stress related mood disorders causing disability and premature death The elevated plus maze is a validated and reliable test for detecting both anxiolytic- and anxiogenic-like effects of agents [9,10]. Use of EPM for detecting anxiolytic effect of the drug was validated behaviorally, physiologically and pharmacologically, also drugs like yohimbine, pentylenetetrazole, caffeine and amphetamine have significantly reduced the percentage of entries and time spent on the open arms by rodents. Hence EPM is said to be a reliable method in testing anxiogenic behavior in rodents. In this animal model, an anxiolytic- or anxiogenic-like effect is evaluated by the relation of entries into the open arms to the total entries and the time spent on the open arms of the X-maze in comparison to the same parameters of the control group. An increase of the time and the

proportion of the entrances into the open arms without a changed locomotor activity is regarded as a powerful marker for an “anxiolytic” substance effect [6].

Table No 2: Anti-Anxiety activity of Methanolic Extract of *Mimusops elengi* (MEME) in the elevated plus maze.

Groups	Treatment	Time Spent (Seconds) in OA	Number of enteries		Number of SAP (CA)	Number of HD (OA)
			OA	CA		
I.	Control	9.833±0.9458	2.667 ± 0.2108	8.000 ± 0.3651	12.167±0.3073	5.667 ± 0.2108
II.	Diazepam 2mg/kg	31.333 ± 1.783**	6.333 ± 0.3333**	3.167 ± 0.4014**	5.167 ± 0.3073**	11.833 ± 0.7923**
III.	MEME 50	13.500 ± 1.057	3.500 ± 0.2236	5.000 ± 0.3651**	9.833 ± 0.5426**	7.500 ± 0.2236*
IV.	MEME 100	17.333 ± 0.7149**	4.333 ± 0.2108**	3.833 ± 0.3073**	7.667 ± 0.6667**	11.333 ± 0.5578**
V.	MEME 200	25.833 ± 1.621**	5.333 ± 0.3333**	4.000 ± 0.3651**	5.667 ± 0.4944**	13.500 ± 0.5627**
VI.	NBME 50	10.500 ± 1.057	3.333 ± 0.2108	7.167 ± 0.4773	11.667 ± 0.2108	6.333 ± 0.2108
VII.	NBME 100	11.333 ± 1.145	3.167 ± 0.3073	6.667 ± 0.4944	11.333 ± 0.3333	6.833 ± 0.1667
VIII.	NBME 200	18.500 ± 0.8062**	4.000 ± 0.2582**	3.833 ± 0.4773**	10.167 ± 0.3073*	8.333 ± 0.2108**
IX.	AQME 50	9.667 ± 0.9545	3.000 ± 0.2582	6.833 ± 0.3073	11.500 ± 0.2236*	6.000 ± 0.3651
X.	AQME 100	15.000 ± 1.366*	3.833 ± 0.3073*	6.333 ± 0.3333*	10.333 ± 0.6667**	7.500 ± 0.5000*
XI.	AQME 200	21.500 ± .8466**	5.167 ± 0.3073**	5.167 ± 0.4773**	7.000 ± 0.3651**	10.500± 0.2236**

Results are presented as mean ± SEM (n=6), data analysed by ANNOVA followed by Dunnett test. **p<0.01, * p<0.05 compared with normal control. OA –Open arm, CA-Closed arm, SAP-Stretch attend posture, HD-Head dipping. methanolic extract of *Mimusops elengi* (MEME) :50,100,200 mg/kg p.o., n-butanolic extract of *Mimusops elengi* (NBME): 50,100,200 mg/kg p.o., aqueous extract of *Mimusops elengi* (AQME):50,100,200 mg/kg p.o.

Mimusops elengi is said to contain ursolic acid and betulinic acid which are reported to have anxiolytic activity [11]. Ursolic acid is reported to possess inhibitory gamma-aminobutyric acid transaminase (GABA-T) activity which increase GABA levels in the brain [12, 13]. Anxiolytic activity of ME therefore can be due to inhibitory GABA activity which is further supported by results obtained by EPM. An arm entry was defined as all four feet in the arm. A decrease in close arm entry and an increase in open arm entry was observed following administration of the ME extract. In addition to the anxiolytic activity calculated by number of entry in open and closed arm fear or risk assessment behavior is calculated by an increase in head dips in open arm and a decrease in SAP in closed arm. Inclusion of SAP and HD as an additional parameter further helps in assessing the fear reducing effect of the drug. SAP is defined as mice stretching forward and then retracting to original position from closed (protected) or open (unprotected) arms. HD is defined as mice protruding the head over the edge of closed or open arms down towards floor [14]. Diazepam has not only increased the time

spent in open arm but has also shown as increase in HD and decrease in SAP and the activity of MEME was found to be parallel. The results obtained from EPM showed that MEME at 50, 100 and 200 mg/kg has shown significant anti-anxiety activity, while AQME at 100 and 200 mg/kg and NBME at 200 mg/kg were found to be active as evident from Table 2.

CONCLUSION

In conclusion it may be said that ME possesses significant anti-anxiety activity and hence may prove to be beneficial and an alternative in the treatment of anxiety like disorders. It can also be said as found out from these pre-clinical studies that the MEME at 200 mg/kg was highly effective and results obtained are equivalent to that of diazepam.

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