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***J. Nat. Prod. Plant Resour.*, 2014, 4 (1):19-22**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Evaluation of anxiolytic activity of hydro-alcoholic extract of *Madhuca Longifolia* leaves

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ABSTRACT

In the present study, Anxiolytic activity of hydro-alcoholic extract of Madhuca longifolia leaves (HAEML) was evaluated. The closed field test showed that after administration of hydro- alcoholic extract of Madhuca longifolia leaves (100 mg/kg) or diazepam (1 mg/kg), there was significant decrease in the number of rearing, assisted rearing and number of squares traveled compared to the control group which might be attributed to the anxiolytic activity of the plant extract. In the elevated plus maze test, hydro - alcoholic extract of Madhuca longifolia leaves (100 mg/kg, p.o.) showed significant ($p<0.05$) increase in the time spent in the open arm and diazepam treated group showed more significant groups compare to control.

Keywords: Anxiolytic activity, *Madhuca longifolia*, Diazepam

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INTRODUCTION

The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants[1].

India has one the world´s richest medicinal plant heritages and it is largest producer of medicinal herbs and is slightly called the “Botanical Garden” of the world. It has biogeography zone and 25 biotic provinces. About 8,000 species of plant are used in local health tradition.

India is a leading producer and exporter of medicinal plants. Medicinal plants, their extracts and pure natural products are produced in the Indian herbal drug industries. There are about sixty well recognised manufacturing of traditional drugs and around 1200 small manufacturing facilities. There are many as 700 species of medicinal plants used in number of herbal formulations available in India[1].

Madhuca longifolia belongs to the family Sapotaceae also called as mahua in India. It grows in the sub – tropical region of Indo – Pak subcontinent and it is economically very important . Parts of *Madhuca longifolia* are used as stimulants, demulcents, emollients, astringents. For itching, swelling, fractures and snake bites, as well as for diabetes mellitus[3] bark of the plant is used. The Mahua oil is used for the treatment of the skin diseases, rheumatism, headache and as a laxative. Fruits of the plant are used as astringents[2] and are employed as a lotion for curing chronic ulcers, acute and chronic tonsillitis, pharyngitis. Anxiety-related disorders such as generalized anxiety, panic, obsessive-compulsive disorder, phobias or post-traumatic stress are the most common mental illness and major cause of disability in the world. In most of the countries mental disorders usually occurs at same point of

the time observed in most of the people. Benzodiazepines are used as anxiolytic drugs with well-known benefits, their side effects includes sedation, muscle relaxation, anterograde amnesia and physical dependence[11].

Medicinal plants constitute an important part of Indian pharmacopoeia, British pharmacopoeia etc. These plants are used as traditional medicines. However, many of these traditional medicines can be developed as potential drugs after scientific validation[8].

MATERIALS AND METHODS

Plant material

Fresh leaves of *Madhuca indica* Syn. *Madhuca longifolia* (Sapotaceae), were collected from Kanpur (U.P), and remove adhering material. The powder was prepared from dried leaves. The hydro ethanolic extract of *Madhuca longifolia* leaves (MLE) was prepared by extraction using Soxhlet apparatus with mixture of ethanol and water (1:1)[7].

Selection of dose

The dose was selected 100 mg/kg from the acute toxicity study test[7].

Animals

Experiments were performed on either sex of rats (100–150 g). From the animal house of Institute of Foreign Trade and Management (I.F.T.M) Moradabad animals were procured and kept at room temperature of about 24-26 °C, with access to standard food pellets and water *ad libitum*, maintained on a natural day–night cycle (12 hrs dark: 12 hrs light). For at least ten days before exposure to behavioural experiments, animals were acclimatized. Experiments were carried out between 10:00-17:00 hrs. By the Institutional Animal Ethics Committee, I.F.T.M, Moradabad, (U.P.) experimental protocol was approved.

Experimental protocols

Thirty minutes and one hour time interval between drug administration and behavioural tests were maintained in case of oral administrations respectively. Three groups of animal were taken and each group contain six animals.

Control group

Received vehicle, 2ml p.o. for 7 successive days and test was performed 7^{th} day.

Test group

Received 100 mg/kg, p.o. for 7 successive days and test was performed on 7^{th} day.

Standard group-

Received diazepam 1mg/kg, p.o. for 7 successive days and test was performed on 7^{th} day.

Behavioural tests

Elevated plus maze test

The plus-maze apparatus consisted of two open arms ($35\times5cm^2$) crossed with two closed arms $35\times5\times20$ cm^2.The arms were connected together with a central square of 5×5 cm^2

The apparatus was elevated to the height of 25cm in a dimly illuminated room. Rats were treated with HAEML (100 mg/kg, p.o.), diazepam (1mg/kg p.o.) or vehicle 30 min before being placed individually in the centre of the apparatus, facing the closed arm. The time spent and the numbers of entries in both the open and the closed arms were recorded for 10 min. An entry was defined as having all four paws within the arm[6].

In the plus-maze apparatus, there are two open arms (16×5 cm for rat and 50×10 cm for rats) and two closed arms (16×5 × 12 cm for rat and 50×10×40 cm for rats), and an open roof with the entire maze elevated (25 cm for rat and 50 cm for rats) from the floor. At the centre of the elevated plus-maze with their head facing open arm, animals were individually placed. In the five minute period of test the following were recorded –

The animal preference for the “first entry”, the “number of entries” of animals into the open or closed arms and the “time spent” by animals in each arm of the maze. Each animal was used only once and the test was carried out during a fixed time of the day. The rationale is that the open arms are more fear-provoking and that the ratio of either time spent on open: closed arms or entries into open-closed arms reflect the relative “safety” are closed arms compared with relative “fearfulness” of open arms. Anxiolytic would be expected to increase the proportion of entries into and spent on open arms[5].

1. Weigh and number the animals. Divide them into two groups each consisting of 4-6 rat. One group is used as control and other for drug (diazepam) treatment.
2. Place the animals individually in the centre of the maze, head facing towards open arm and start the stop watch and note following parameters for 5 min:
3. First preference of rat to open or enclosed arm.
(a) Number of entries in open and enclosed arms (An arm entry defined as the entry of four paws into the arm).
(b) Average time each animal spends in each arm (Average time = total duration in the arm/number of entries).
4. Inject diazepam to the test group. After 30 min place the animals individually in the centre of the maze and note all parameters as described under step 2.
5. Compare the preference of the animal to open / enclosed arm, average time spent in open arm and number of entries in open arm in each group[5].

RESULTS AND DISCUSSION

In the present study, HAEML consisting the leaves of *Madhuca longifolia* produced significant anxiolytic activity in rat using elevated plus maze test due to presence of flavonoids, triterpenoids, steroids, sapogenins, saponins. The closed field test also showed that after administration of HAEML (100 mg/kg) or diazepam (1 mg/kg), there was significant decrease in the number of rearing, assisted rearing and number of squares traveled compared to the control group which might be attributed to the anxiolytic activity of the plant extract[8].

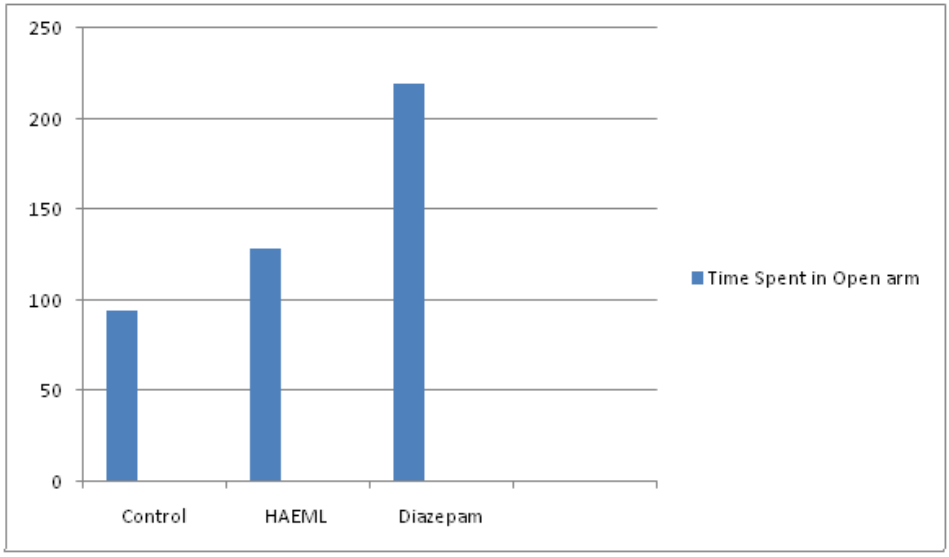
In the elevated plus maze test, HAEML (100 mg/kg, p.o.) showed significant ($p<0.05$) increase in the time spent in the open arm and diazepam treated group showed more significant groups compare to control[8].

Table 1 - Effect of Haeml and diazepam in elevated maze plus test

S No.	Group	Dose	Time spent in open arm (seconds)
1.	Control(vehicle)	2 ml	94.16±2.13
2.	HAEML	100mg/kg	128.33±2.19*
3.	Diazepam	1mg/kg	219.67±1.89**

Values are in mean (s) ±SEM
*Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple compression test**p<0.05.*

Figure 1: Effect of HAEML graph



CONCLUSION

The anxiolytic effects of HAEML could be due to the interaction of chemical constituents of the plant with the GABA/benzodiazepine receptor complex in brain[9]. To evaluate the "psychomotor performance" and "emotional aspects of rodents"[10] elevated plus-maze test is used. The results showed that HAEML significantly increased the time spent on the open arms and decreased the number of entries closed arms. This type of effect is observed with the drugs that act on GABA/benzodiazepine receptor complex and inhibit reuptake of serotonin[9].

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