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Research Article

ASSESSMENT OF ANXIOLYTIC ACTIVITY OF AQUEOUS EXTRACT OF *MANGIFERA INDICA* L. LEAVES IN RODENTS EXPOSED TO CHRONIC UNPREDICTABLE MILD STRESS

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ABSTRACT
The purpose of this study was to characterize the anxiolytic activity of an Aqueous extract prepared from the leaves of *Mangifera indica* L (AEMI) using an elevated plus maze (EPM) model in rat and staircase model in mice. In elevated plus maze (EPM) model, At dose of *Mangifera indica* (250mg/kg and 500mg/kg) treated group showed significant increase in the % time spent in open arm and no. of entries in open arm when compared to CUMS. Significant decrease in SOD, CAT levels and increase in lipid peroxidation level was observed in stressed rats. *Mangifera indica* (250mg/kg and 500mg/kg) treated group improved SOD, CAT, and controlled the lipid peroxidation in liver tissue. In stair case model, at dose of *Mangifera indica* (250mg/kg, 500mg/kg) treated group showed significant increase in the no. of step climbed and decrease the no. of rearing when compared to CUMS. These results indicate that AEMI is an effective anxiolytic agent and could be useful in alternative treatment. So Present study confirms that the extract showed significant anxiolytic activity at both dose levels which is comparable with standard anxiolytics Diazepam (2mg/kg in rat and 4mg/kg in Mice p.o).
KEYWORDS: lipid peroxidation, Anxiolytic, Elevated plus-maze, staircase, Chronic unpredictable mild stress.

INTRODUCTION

According to the World Health report, approximately 450 million people suffer from a mental or behavioural disorder[1]. This data is 12.3% of the global burden of disease, and predicted to rise up to 15% by 2020[2].Stress is a state of threatened homeostasis provoked by psychological, physiological or environmental stressors[3]. Stressor is a stimulus either internal or external, which activates the hypothalamic pituitary adrenal axis and the sympathetic nervous system resulting in a physiological change[4]. Stressful condition can precipitate anxiety and depression, which can lead to excessive production of free radicals which in turn results in oxidative stress, an imbalance in the oxidant/antioxidant system.

Anxiety is a normal response to stress, a feeling of apprehension or fear, combined with the symptoms of increased sympathetic activity. A clinical problem may arise if anxiety becomes persistent that interferes with everyday performance. Clinical symptoms of anxiety include panic disorder, agoraphobia, other phobias and generalized anxiety[5].The prevalence of such syndromes in the general population is about 10-20% and there is high rate of morbidity with depressive disorders[6].The ratio of anxiety in female and male is 2:1. Although the maximum prevalence of generalized anxiety and agoraphobia- panic is in 50-64 age groups; the age of onset of most of anxiety disorders is in the young adulthood (twenties and thirties)[7].

Mangifera indica L. (family-Anacardiaceae), popularly known as mango is one of the most important tropical plants marketed in the world. It grows in tropical and subtropical regions. Various parts of plant are used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhoea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles[8].The Ayurvedic texts recommend the use of mango leaves as anti-anxiety but there is a lack of scientific evidence for the anxiolytic activity[9, 10].Hence the study aimed to evaluate the anxiolytic activity of aqueous extract of *Mangifera indica* L. leaves in using elevated plus maze model in rats and stair case model in mice after subjecting to chronic unpredictable mild stress (CUMS).

MATERIAL AND METHODS

Drug and Chemicals:

Aqueous extract of *Mangifera indica* leaves was obtained from Ansar Industries Private Limited, Surat having Batch No-MI-313. Diazepam: Commercially available (Ranbaxy 10mg/2mL) were used as the standard anxiolytic drugs. Distilled water was used as vehicle.

Preparation Of Test Doses:

The extracts were suspended in the vehicle in such concentrations as to administer 250 and 500 mg/kg doses to rat and mice through the per oral route.

Animals:

Experimental study was carried out using Wistar Albino male rats weighing between 160-180g and Albino mice weighing between 25- 35g. The animals were procured from TRC, Laboratory animal facilities, Ahmedabad. All the animals were maintained under standard laboratory conditions i.e. temperature of 20 ± 20C; relative humidity 45 – 55 % and a 12 hour light/ dark cycle. The animals were fed standard mice pellet *ad libitum* under hygienic conditions. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The approval from the Institutional Animal Ethical Committee (Ref No.GHB/IAEC/08/2011-12).

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 rat; observed for signs of behavioral, Neurological toxicity and mortality 14 days.

Elevated plus maze model:

It is considered one of the most widely validated tests for assaying sedative and anxiolytic substances[13,14]. The elevated plus maze apparatus consists of four arms, two open arms (11 × 50 cm) with 0.5 cm ledges and two enclosed arms of the

same size with 50 cm high walls. The arms were attached to a central square (10 cm2) and shaped as a plus sign. The entire apparatus was elevated 48 cm above the floor. For testing, each rat will be placing individually at the centre of the maze, facing one of the closed arms. During 5 min test period the following parameters were recorded.

- The number of entries into open arms
- The number of entries into closed arms
- Time spent in the open arms
- Time spent in the closed arms

Dose Selection:

Diazepam: 2 mg/kg oral dose of diazepam in rats[11].

Mangifera indica extract: 250 mg/kg and 500 mg/kg in rats[12].

Treatment protocol:

Animals were divided into 5 groups of 10 animals in each group:

Group 1 was administered vehicle and not subjected for chronic unpredictable mild stress (CUMS). (Negative control)

Group 2 was administered vehicle and subjected for CUMS for 21 days. (Positive control)

Group 3 was administered Diazepam and subjected for subjected for CUMS for 21 day. (Standard)

Group 4 was administered *Mangifera indica* (250mg/kg) and subjected for CUMS for 21 day.

Group 5 was administered *Mangifera indica* (500mg/kg) and subjected for CUMS for 21 day.

Stair case model:

It is rapid reliable and simple screened method for testing of anxiolytics in mice[15,16]. Staircase consists of five identical steps, 2.5 cm X 10 cm X 7.5 cm. The inner height of the walls is constant (12.5 cm) along the 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 were 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. In order to simplify the observation, the numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behaviour of the next animal. The following parameters were recorded

- Number of steps climbed
- Number of rearing

Dose Selection:

Diazepam: 4 mg/kg oral dose of diazepam in mice.

Mangifera indica extract: 250 mg/kg and 500 mg/kg in mice[11].

Treatment Protocol:

Animals were divided into 5 groups of 10 animals in each group.

Group 1 was administerd vehicle and not subjected for chronic unpredictable mild stress (CUMS). (Negative control)

Group 2 was administered vehicle and subjected for CUMS for 14 days. (Positive control)

Group 3 was administered Diazepam and subjected for subjected for CUMS for 14 day. (Standard)

Group 4 was administered *Mangifera indica* (250mg/kg) and subjected for CUMS for 14 day.

Group 5 was administered *Mangifera indica* (500mg/kg) and subjected for CUMS for 14 day.

Statistical analysis:

All results are expressed as MEAN ± SEM. Statistical analysis was performed using the Graph pad prism 5, Graph pad software, USA. One way ANOVA followed by Dennett's *t* test. Differences with P<0.05 were considered statistically significant.

Table 1: Evaluation of aqueous extract of *Mangifera indica*, on elevated plus maze test in rats.

GROUPS	%TSOA MEAN ±SEM	%TSCA MEAN ±SEM	NO.OF OAE MEAN ±SEM	NO. OF CAE MEAN ±SEM
Group I **Vehicle control**	25.12 ± 4.27	62.91 ± 3.62	8.25 ± 1.06	7.12 ±1.06
Group II **Vehicle + CUMS**	8.95 ± 2.71^{***a}	86.95 ± 3.04^{***a}	4.00± 1.29^{**a}	5.25 ±1.41$^{ns\ a}$
Group III **Diazepam (2mg/kg) + CUMS**	40.12 ± 1.19^{***b}	52.16 ± 1.86^{***b}	8.25±0.59^{**b}	5.75 ±0.64$^{ns\ b}$
Group IV ***Mangifera indica* (250mg/kg) + CUMS**	31.62 ± 1.31^{***b}	52.28 ± 1.40^{***b}	8.62 ±0.68^{**b}	7.50 ±0.73 $^{ns\ b}$
Group V ***Mangifera indica* (500 mg/kg) + CUMS**	31.62 ± 1.31^{***b}	53.57 ± 1.38^{***b}	8.12 ±0.83^{*b}	7.37±1.10 $^{ns\ b}$

Data was analyzed using one way ANOVA followed by Dunnett's *t* test, ns- not significant, *P<0.05, **P<0.01, ***P<0.001, n=10, [a] when compare with vehicle control group, [b]when compare with CUMS group.

TSOA: Time spent in open arm, **TSCA:** Time spent in closed arm, **OAE:** Open arm entries, **CAE:** Closed arm entries, **CUMS:** Chronic unpredictable mild stress

Table 2: Evaluation of aqueous extract of *Mangifera indica*, on Stair case model in mice

GROUPS	NO. OF STEP CLIMBING IN 3 MIN MEAN ± SEM	NO. OF REARING IN 3 MIN MEAN ± SEM
Group I **Vehicle control**	10.80 ± 0.89	20.00 ± 2.04
Group II **Vehicle + CUMS**	3.90 ± 0.58^{***a}	30.60 ± 1.30^{***a}
Group III **Diazepam (4 mg/kg) + CUMS**	20.20 ± 0.78^{***b}	16.00 ± 1.24^{***b}
Group IV ***Mangifera indica* (250mg/kg) + CUMS**	16.00 ± 1.30^{***b}	19.00 ± 1.04^{***b}
Group V ***Mangifera indica* (500 mg/kg) + CUMS**	18.00 ± 1.23^{***b}	17.00 ± 0.67^{***b}

Data was analyzed using one way ANOVA followed by Dunnett's *t* test, ***P<0.001.n=10, [a] when compare with vehicle control group, [b]when compare with CUMS group.

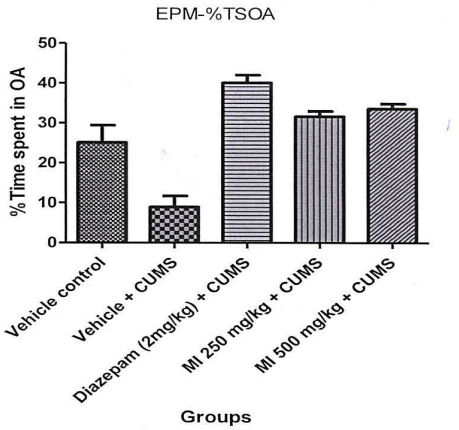

Figure 1: Elevated Plus Maze (EPM) model: % Time spent in open arm

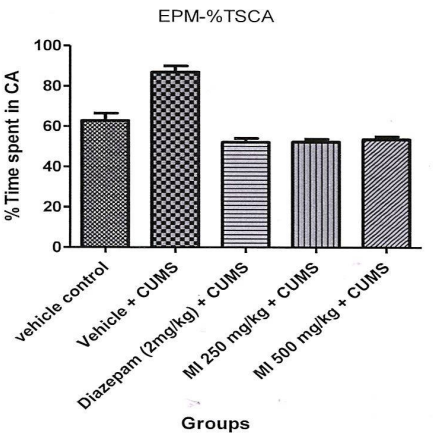

Figure 2: Elevated Plus Maze (EPM) model: % Time spent in closed arm

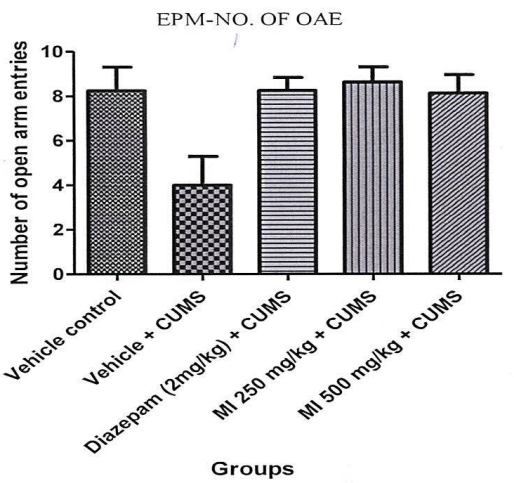

Figure 3: EPM- Number of open arm entries

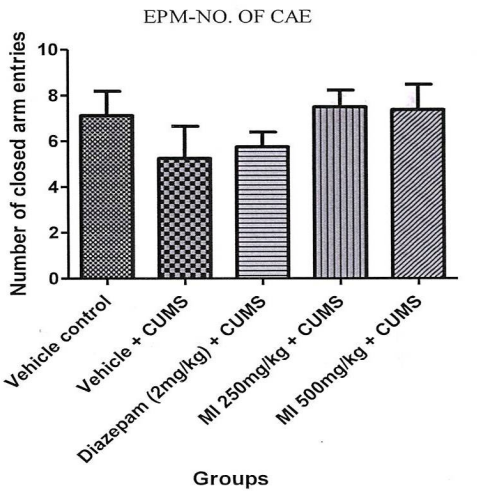

Figure 4: EPM-Number of closed arm entries

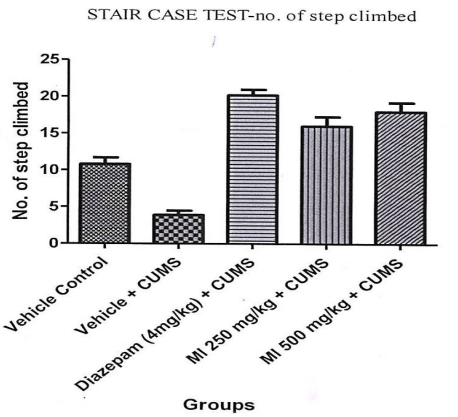

Figure 5: Stair case test: No. of step climbed

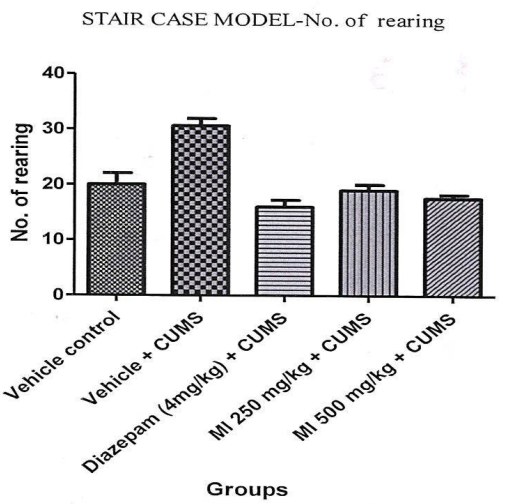

Figure 6: Stair case test: No. of rearing

RESULT

Elevated plus maze model:

The rats were pre-treated with *Mangifera indica* and Diazepam for 21 days and simultaneously subjected to CUMS, and on 22nd day animals were observed and the result are tabulated in Table 1 and Figure 1-4:

- CUMS (group 2) showed significant decrease (P<0.001) in % time spent in open arm when compared to vehicle control (group 1). Diazepam (2mg/kg) (group 3), *Mangifera indica* (250mg/kg) and *Mangifera indica* (500mg/kg) showed significant increase (P<0.001) in % time spent in open arm when compared to CUMS (group 2).
- CUMS (group 2) showed significant increase (P<0.001) in % time spent in closed arm when compared to vehicle control (group 1). Diazepam (group 3), *Mangifera indica* (250mg/kg) (group 4) and *Mangifera indica* (500mg/kg) (group 5) showed significant decrease (P<0.001) in % time spent in closed arm when compared to CUMS (group 2).
- CUMS (group 2) showed significant decrease (P<0.01) in no. of open arm entries when compared to vehicle control (group 1). There was an increase in the no. of entries in open arm in the group treated with 250mg/kg of aqueous extract of *Mangifera indica* (P<0.01), 500mg/kg aqueous extract of *Mangifera indica* (P<0.05) and 2 mg/kg Diazepam (P<0.01), when compared to CUMS.
- CUMS (group 2) showed decrease in no. of closed arm entries when compared to vehicle control (group 1) which is not significant. Diazepam (group 3), *Mangifera indica* (250mg/kg) (group 4) and *Mangifera indica* (500mg/kg) (group 5) showed increase in no. of closed arm entries when compared to CUMS (group 2) which is not significant.

Stair case model:

The mice were treated with *Mangifera indica* and Diazepam for 14th days and simultaneously subjected to CUMS, and on 15th day animals were observed and result are tabulated in Table No. 2 and figure 5-6:

- CUMS (group 2) showed significant decrease (P<0.001) in the no. of step climbed when compared to vehicle control (group 1). Diazepam (4mg/kg) (group 3), *Mangifera indica* (250mg/kg) (group 4) and *Mangifera indica* (500mg/kg) (group 5) showed significant increase (P<0.001) in the no. of steps climbed when compared to CUMS (group 2).
- CUMS (group 2) showed significant increase (P<0.001) in the no. of rearing when compared to vehicle control (group 1). Diazepam (4mg/kg) (group 3), *Mangifera indica* (250mg/kg) (group 4) and *Mangifera indica* (500mg/kg) (group 5) showed significant decrease (P<0.001) in the no. of rearing when compared to CUMS (group 2).

DISCUSSION

The present work was performed to evaluate the anxiolytic activity of aqueous extract of *Mangifera indica* leaves in using elevated plus maze model in rats and stair case model in mice after subjecting to chronic unpredictable mild stress (CUMS).

In elevated plus maze model, CUMS rats exhibited anxious behaviour as evidenced by the % time spent in open arm was decreased significantly (P<0.001) and also the no. of entries in open arm was decreased significantly (P<0.01) when compared to control group. Aqueous extract of *Mangifera indica* (250mg/kg and 500mg/kg) and Diazepam (2mg/kg) treated group, % time spent in open arm was increased significantly (P<0.001) as compared to CUMS group. There was an increase in the no. of entries in open arm in the group treated with 250 mg/kg of aqueous extract of *Mangifera indica* (P<0.01), 500 mg/kg aqueous extract of *Mangifera indica* (P<0.05) and 2 mg/kg Diazepam (P<0.01), when compared to CUMS. This showed that *Mangifera indica* has anxiolytic property.

In stair case model, CUMS mice exhibited anxious behaviour as evidenced by significantly decreased in the no. of step climbed (P<0.001) and significantly increased in the no. of rearing (P<0.001) when compared to control group. Aqueous extract of *Mangifera indica* (250mg/kg and 500mg/kg) and Diazepam (4 mg/kg) treated group, number of step climbed was increased significantly (P<0.001) when compared to CUMS which shows anxiolytic effect. Aqueous extract of *Mangifera indica* (250mg/kg and 500mg/kg) and Diazepam (4 mg/kg) treated group, number of rearing was decreased significantly (P<0.001) when compared to CUMS which shows anxiolytic effect.

CONCLUSION

Mangifera indica at doses 250mg/kg and 500mg/kg showed anxiolytic activity in the models tested. Antianxiety and antistress activities may be due to the large no of chemical constituents present in *Mangifera indica.* More investigations are necessary to prove the anxiolytic activity of *Mangifera indica* by other models. However further studies are necessary to identify the exact mechanism of action of *Mangifera indica* as anxiolytic activity.

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