Research Article

Anxiolytic effects of Hydro-Alcoholic Extract of *Aegle Marmelos* Fruit (Bael fruit) in Swiss Albino Mice

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ABSTRACT

Introduction and Aim: Anxiety is the common psychiatric condition. This study was done to screen anti-anxiety activity of hydroalcoholic extract of *Aegle marmelos* (HAAM) fruit in Swiss albino mice.

Materials and Methods: 100 mg/kg and 200mg/kg extracts of *Aegle marmelos* fruit and a combination of 200 mg/kg extract and diazepam were compared with the Diazepam 1mg/kg and distilled water. Each group consisted of six Swiss albino mice. Anxiety was evaluated using the models Elevated Plus Maze (EPM) and Light Dark Arena (LDA). Time spent in open and closed arm in EPM and time spent in bright and dark area in LDA were calculated. Data was analysed by one -way ANOVA followed by Tukey Kramer's test.

Results: HAAM 200 mg/kg plus diazepam group has high value when compared with the control, standard, HAAM 100 mg and HAAM 200 mg. HAAM 200 had anxiolytic effect when compared to the standard. When HAAM 200 mg + diazepam was given, time spent in the light box and open arm was more and was found to be statistically significant when compared to control and standard. Two doses 100 mg and 200 mg has anxiolytic properties when compared to the control and standard. The combination of hydroalcoholic extract of *Aegle marmelos* (HAAM) 200 mg with the diazepam has also showed a significant anxiolytic effect when compared to the standard and control.

Conclusion: The result confirms anti-anxiety properties of *Aegle Marmelos* fruit. Hence *Aegle marmelos* fruit may be used as a promising agent for the treatment of anxiety.

Keywords: *Aegle marmelos*, Anti-anxiety, Elevated plus maze, Light Dark Arena, Swiss albino mice

1. INTRODUCTION

Anxiety disorder is a very common psychiatric condition. It is present in almost 15-20 % of the patients who come to hospital for some other medical condition. Anxiety which is a normal human emotion that serves as an adaptive response requires treatment when it is out of scale with the perceived threat. According to WHO, 1 in 13 people in the world suffers from anxiety. It is estimated that 970 million adults around the globe has mental disorder with anxiety and depression being the most common (WHO 2019) [1]. WHO also estimates that about 7.5 percent Indians suffer from mental disorders. Even though anxiety disorder is a common type of disorder, only less than 30% of the patients seek treatment [2]. Generally, anxiety is a way of expressing our emotion. It is a way of reacting to a stressful or novel situation which may be unexpected but when it comes to anxiety disorder, the individual will lack the quality of how to appropriately handle the situation. Anxiety disorders can be diagnosed in its early stage itself by taking a proper history, observing patient's behavior and gestures. Treatment is very effective when given early. It may bring drastic change in the behavior and life. Counseling the patient and also judicious use of psychotherapy and drugs like benzodiazepines can produce a drastic change. Conventional treatment options for anxiety have a very low margin of safety and they may cause unwanted side effects too. So researchers are trying to discover new compounds especially plant based

products which have lesser toxicity and less undesirable side effects. Commonly prescribed drugs for anxiety are benzodiazepines, azapirones, sedative antihistaminic, beta blockers, Selective Serotonin Reuptake Inhibitors (SSRIs). In the present study, anti-anxiety effect of *Aegle marmelos* fruit (Bael fruit) was evaluated in swiss albino mice.

Aegle marmelos is a moderate sized, slender and aromatic tree which belongs to family Rutaceae [3]. It is a common medicinal plant used in India. It is also called as Indian fruit, Holy fruit, golden apple, stone apple. The fruit and other parts of the plants like roots, flowers, ripe and seeds have been in used in traditional medicinal treatments for various diseases. It has been used to treat fever, intestinal ailments, dysentery, fertility control, ulcers, as antivirals, epilepsy [4]. The traditional physicians of Southern Chattisgarh use dry form of this fruit with mustard oil for treatment of burns [5]. The ripe fruit helps in digestion and also will reduce the inflammation of the rectum.

Previous studies have shown the anti-anxiety effect of *Aegle marmelos* leaves, roots and other parts. Bael fruit contains bioactive compounds like coumarin, xanthotoxol, aegeline, marmeline etc which have anti-oxidant effect [6]. Therefore, it would be beneficial to evaluate the anti-anxiety effect of *Aegle marmelos* fruit.

2. Materials & Methods

Materials

After the approval from the Institutional Animal Ethics Committee (IAEC), the study was performed in the department ethnopharmacology laboratory.

Experimental animal

Healthy adult male and female Swiss albino mice weighing 25-30g aged 3-4 months were used for the study. The mice selected were experimentally naive. They were housed in clean polypropylene cages, six mice in each cage, under standard housing conditions maintained at a room temperature of 24±2^{o}C with a 12-hour light and dark cycle in the departmental animal house (347/PO/RES/RC-L/01/CPCSEA). They had free access to standard pellet diet and water *ad libitum.* The mice were marked using hematoxylin stain for identification and were allowed to acclimatize to the laboratory conditions for one week prior to the experiments. They were handled with care as per CCSEA guidelines. All the experiments were carried out during light cycle.

Plant material

Fruit in the form of dry powder was purchased from an authorized vendor.

Preparation of the extract

In a soxhlet apparatus, 300g dried powder was extracted with 1.5L of petroleum ether, chloroform and methanol at 60-70°C for 10-12hours. The solvent which was used were of analytical quality. Rotavapour was used to remove methanol from the extract resulting in a semisolid mass. The yield of the methanol extract was 10.2% (w/w). Before being used for the experiment, the extract was kept in the refrigerator in sterile amber coloured storage vials [7].

Experimental animal

Animals were randomly divided into five groups containing 6 mice of either sex in each group. Total number of mice used = 30

Group I: Control Normal Saline, Per oral (PO)

Group II: Standard: Diazepam 1mg/kg

Group III: Test drug: Hydro-Alcoholic extract of *Aegle marmelos* (HAAM) 100mg/kg, Per oral (PO)

Group IV: Test drug: Hydro-Alcoholic extract of *Aegle marmelos* (HAAM) 200mg/kg, PO

Group V: HAAM 200 mg/kg + Diazepam1mg/kg, PO

Dose

Based on the acute toxicity studies done previously, the dose chosen was 100mg/kg and 200mg/kg of Aegle marmelos [8]. The required amount of dose to be given was calculated based on the animal body weight. The drugs were administered once daily orally for 14 days. On the 14th day after one hour of administration of drug, the animals were screened for anti-anxiety activity. The experiments were done after giving a washout period of 6 weeks between the two experiments.

Methodology

The study was carried out using two animal models which are widely validated for measuring

anxiety in rodents- Elevated Plus Maze and Light Dark Arena.

Elevated Plus Maze (EPM)

The elevated plus maze combines three potential anxiogenic factors – novelty, height and open space. The cross shaped maze consists of four arms (two open arms and two closed arms) that are interconnected by a central platform The model was first presented by Montgomery in 1958 and Handley and Mithani subsequently altered it to cross shape maze. When the animal was placed in the centre of EPM, they experience fear because of height induced anxiety. The mice will prefer to stay in safer and darker places [9].

The maze is suspended 50cm above the ground level. The mouse was placed on the central platform facing one of the enclosed arms and observed for 5 minutes. The test was recorded using a video camera attached to a computer. During the 5-min test, the number of open and closed arm entries, plus the time spent in open and closed arms were recorded. The arena was cleaned with spirit after each test. The results were tabulated.

Light Dark Arena (LDA)

The maze is divided into two parts, 1/3 with opaque walls and a cover (dark compartment). Whereas the remaining 2/3 is open and illuminated (light compartment). The opening between the two compartments permits the mouse to move from one chamber to the other [9].

The mouse was placed in the light compartment and observed for 5 minutes. The test was recorded using a video camera attached to a computer. During the test period, the time spent in light and dark compartment and number of crossings were recorded. The arena was cleaned with spirit after each test. The results were tabulated

Statistical analysis

Data was compiled and analysed using the statistical software SPSS v24.0. Results were represented as Mean±SEM (Standard Error of Mean). Statistical significance between means was analysed using one way analysis of variance (ANOVA) followed by Tukey Kramer test. p value <0.05 was considered significant and p value <0.01 was considered highly significant.

3. Results

Table 1 Effect of HAAM on anxiety using LDA

SI No.	Group	Time spent in light box (seconds)	Time spent in dark box (seconds)
I	Control normal saline	74±4.3	226±4.7
II	Standard (Diazepam 1mg/kg)	151±6.4*	149±6.7*
III	HAAM 100mg	138±6.81*	152±6.5
IV	HAAM 200mg	156.33±7.5*	139.75±8.1
V	HAAM 200mg plus Diazepam 1mg/kg	178±6.5*	141±10.1*

HAAM: Hydro Alcoholic extract of Aegle Marmelos
One way ANOVA followed by Tukey Krammer test
* p value <0.05 – significant compared to Group I

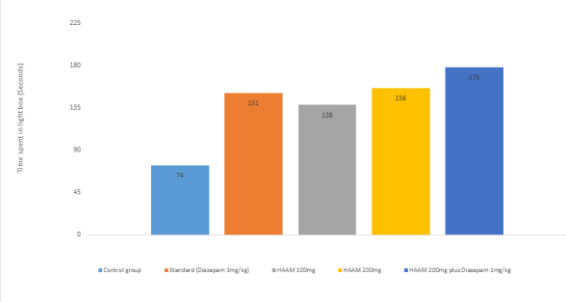

Figure 1 : Time spent in light box (seconds) (N=6)

Table 2-Effect of HAAM on anxiety using EPM

SI No.	Group	Time spent in open arm (seconds)	Time spent in closed arm (seconds)
I	Control normal saline	63.75±7.77	237±7.8
II	Standard (Diazepam 1mg/kg)	156±6.25*	133±6.7
III	HAAM 100mg	144±6.2	156±6.5
IV	HAAM 200mg	155±4.7*	139.25±3.9*
V	HAAM 200mg plus diazepam 2mg/kg	160±5.3*	129±2.7*

One way ANOVA followed by Tukey Krammer test
*P value <0.05 – significant compared to Group I

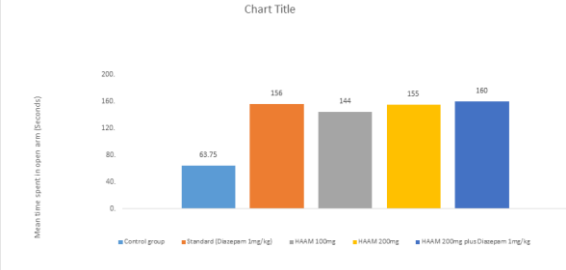

Figure 2: Time spent in open arm (seconds) (N=6)

4. Discussion

Anxiety is a common psychiatric problems faced in the current era. Anxiety is an adaptive response which helps the individual to face the difficult situations and challenges in life. But

when this anxiety becomes persistent, it will interfere in the daily routine of the person. It is a chronic disabling condition and there may be any preceding history of childhood abuse or punishment, family history of mental disorders and low socio-economic status.

The current anxiolytic therapies have a very narrow margin of safety but are still frequently used for the treatment of anxiety disorders. They may have undesirable effect like sedation, memory disturbance, physical dependence and also drug interaction

The current trend is to use many herbal medicines as an alternative for anxiety disorders. Preclinical trials are essential for understanding the bioactive component of the medicinal plants. The effect of the herbal extract depends on the method of extraction so it is necessary to conduct number of trials to screen the herbs for their therapeutic effects.

Aegle marmelos is considered one of the promising herbal alternatives, as evidenced by provisional uses and pharmacological studies. In this study, preclinical evaluation of hydroalcoholic extract of *Aegle marmelos* at two doses 100mg/kg and 200mg/kg was used and also combination of 200mg/kg dose with the standard diazepam was assessed. This study employs widely used models for anxiety such as Elevated Plus Maze (EPM) and Light And Dark Arena (LDA). A comparative study of the hydro-alcoholic extract of *Aegle marmelos* fruit pulp was done to screen the anti-anxiety effects. Elevated plus maze and Light and dark arena are well known paradigm and are very successful in assessing the anxiety like behaviour of rodents.

EPM Model

The results of the EPM model suggest that the mean time spent in the open arm was 156±6.25 seconds when the mice were given the standard drug diazepam. The mean time spent was 144 seconds when HAAM 100mg was given. The mean time spent in open arm when HAAM 200mg was given is 155 seconds and when diazepam 1mg/kg and HAAM 200mg/kg was combined and given, the mean time spend in open arm was160±5.3 seconds. These result shows that HAAM 100mg has significant value when compared with the control and is having lesser effect when compared with the standard diazepam. HAAM 200 mg is significant when compared with the control group and is having similar effect when compared with the standard group. Among the test group, highest value was for HAAM 200mg/kg plus diazepam group when compared with the control, standard, HAAM 100mg and HAAM 200mg.

LDA Model

The mean time spent in the light box was 151±6.4 seconds when standard (diazepam) was given. The mean time decreased to 138 seconds when HAAM 100mg was given. The time spent in the lightbox was 156.33±7.5seconds when HAAM 200mg was given. The results showed that HAAM 200mg has significant anxiolytic properties when compared to control. And HAAM 200 has equivalent anxiolytic effect when compared to the standard diazepam. When HAAM 200mg+diazepam was given, the mean time spent in the light box was 178±6.5 seconds and this was also found to be statistically significant when compared to control group and standard. Kothari et al [10] reported that methanolic extract of *Aegle marmelos* leaf showed significant anxiolytic and anti-depressant activities by increasing monoamine levels at post synaptic sites which is confirmed by experimental setups like open field test, actometer and forced swimming test. The research carried out by Arul, Miyazaki, Dhananjayan *et al.,* [11] demonstrated that *Aegle marmelos* leaves have anti-inflammatory properties. Rajan, Gokila, Jency, Brindha, Sujatha *et al.,* [12] screened the DPPH induced antioxidant activity of methanol and aqueous extract of *A.marmelos* fruit pulp for radical scavenging method. Both the extracts have shown promising antioxidant properties.

5. Conclusion

The study's findings confirm the anti-anxiety properties of the fruit of *Aegle marmelos.* The hydroalcoholic extract of *Aegle marmelos fruit* shows that the two doses 100mg and 200mg has anxiolytic properties when compared to the control and standard. The combination of hydroalcoholic extract of *Aegle marmelos* 200 mg with the standard Diazepam showed a

significant anxiolytic effect in comparison to the standard and control groups. As a result, *Aegle marmelos* fruit may be useful in the treatment of anxiety. A systematic research can be conducted to determine the additional efficacy of *Aegle marmelos* fruit.

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Conflict of Interest
None declared

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Ethical Information
This study was approved by Institutional Animal Ethics Committee with clearance number YU/IAEC/18/2019

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