

Evaluation of antidepressant properties of hydroalcoholic extract of Aegle marmelos fruit (Bael fruit) in Swiss albino mice: A pre-clinical study

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ABSTRACT

Introduction: Depression is a prevalent psychiatric disorder affecting 3.8% of the global population. The clinically available antidepressants can cause serious adverse effects. Aegle marmelos fruit has shown a wide range of pharmacological effects. The present study was carried out to elucidate the possible antidepressant activity of Aegle marmelos (Bael) fruit in Swiss albino mice. **Objective:** To evaluate the possible antidepressant activity of Aegle marmelos fruit extract in Swiss albino mice. **Methods:** A total of 30 (n=30) male Swiss albino mice weighing 25-35 g, divided into five groups of six animals each were used for the study. Aegle marmelos fruit powder was subjected to solvent extraction in a Soxhlet apparatus. The antidepressant activity of the hydroalcoholic extract of Aegle marmelos fruit was evaluated by using two models – Forced Swimming Test (FST) and Tail Suspension Test (TST). **Results:** One-way ANOVA was used for multiple comparisons followed by a post hoc (Bonferroni test) for comparison between groups. Aegle marmelos fruit at the dose of 100mg/kg and 200 mg/kg significantly reduced the immobility time in both the tests (TST and FST) compared to the control. The reduction in duration of immobility at the dose of 200 mg/kg was comparable to the standard drug Imipramine. **Conclusion :** The Hydroalcoholic extract of Aegle marmelos has significant antidepressant activity in both the animal models of depression (FST and TST).

KEYWORDS: Aegle marmelos fruit, anti-depressants, Forced Swimming Test, Tail Suspension Test

INTRODUCTION

Mental disorders have caused a major disease burden to the world and have increased in the past few years.

In the present scenario, about 12% of the disease burden is constituted by psychological disorders, with approximately 450 million people suffering from it as estimated by the WHO. [1] Depression is a psychiatric disorder characterized by tiredness, feelings of guilt, low self-esteem, and sadness, loss of interest, disturbed sleep, disturbed appetite, and poor concentration. Approximately 3.8% of the global population is affected with depression in 2020, including 5.0% among adults and 5.7% among adults older than 60 years. [2] Depression is also the major contributor to suicide deaths, which number close to 800,000 per year. Oxidative stress is one of the key factors which play an important role in the etiopathogenesis of depression. Monoamines are the excellent scavengers of hydroxyl and oxy radicals, deficient in depressive patients due to increased monoamine metabolism and degradation. Increased production of hydroxyl and superoxide anions and the inability to destroy them due to deficient antioxidant mechanisms in the brain may produce increased oxidative stress in patients suffering from depression. [3]

Aegle marmelos (commonly known as bael) is a plant of Indian origin that belongs to the Rutacea family. All parts of the plant like stem, root, leaves, and fruit have medicinal values and have been used as medicine for a long time. Previous studies have shown that the fruit have a wide range of pharmacological effects like free radical scavenging, antioxidant, antibacterial, antiviral, anti-diarrheal, gastro protective, and anti-cancer activity, etc. Previous study conducted by using methanol extract of Aegle marmelos leaves has shown significant anti-anxiety and antidepressant activity in mice. [4] Hence, it would be worthwhile to screen the anti-depressant activity of hydroalcoholic extract of Aegle marmelos fruit in Swiss Albino mice. The objectives of the study were to study the antidepressant activity of the hydroalcoholic extract of Aegle marmelos fruit in animal

models of depression and to evaluate the antidepressant activity of the hydroalcoholic extract of *Aegle marmelos* fruit and compare it with the standard drug Imipramine.

MATERIALS AND METHODS:

Animals:

Institutional Animal Ethical Committee (IAEC) clearance (YU/IAEC/3/2020) was obtained before conducting the study. Swiss albino mice of either sex weighing 25-35 g, inbred in the animal house of the Department of Pharmacology, Yenepoya Medical College (347/PO/ReBi-S/Rc-L/01/CPCSEA) were used for the study. The mice were housed under standard conditions in the departmental animal house with a temperature maintained around 24±2°C with a 12:12 hour light: dark cycle. The animals were given a standard pellet diet and water ad libitum. Animals were acclimatized to the laboratory conditions for seven days before conducting the study. The animals were handled according to the CCSEA (Committee for Control and Supervision of Experiments on Animals) guidelines.

Collection of plant material:

Preparation of the extract:

Dry powder of the bael fruit was purchased from an authorized dealer (Kamdhenu Baelgiri Powder sold by KamDhenu Laboratories). Dried powder was successively extracted with 70% alcohol in a soxhlet apparatus at 60-70°C for 10-12 h.^[5] Solvents used were of analytical grade. Alcohol was removed from the extract using rotavapor and a semisolid mass was obtained. It was stored in at used for experiment 0°C until used.

Drugs:

The standard antidepressant drug Imipramine tablets of 5 mg was purchased from an institutional pharmacy store for experimental purpose after indenting through the Head of the department.

Inclusion and Exclusion criteria:

Inclusion criteria: Swiss albino mice weighing between 25-35g aged 3-4 months and healthy with normal behavior and activity.

Mice less than 25g and more than 35, age below 3 months and above 4 months and animals previously used in other experiments were excluded.

Experimental Design

Animals were divided into 5 different groups for each test. Each group consists of 6 mice of either sex.

Group I: Control Normal Saline 10 ml/kg, Per oral (PO)

Group 2: Standard: Imipramine 5 mg/kg, PO

Group 3: Test drug: HAAM 100 mg/kg, PO

Group 4: Test drug: HAAM 200 mg/kg, PO

Group 5: HAAM 200 mg/kg + Imipramine 5 mg/kg, PO

Dose

The dose selected was 100 mg/kg and 200 mg/kg of *Aegle marmelos* based on the acute toxicity studies previously done. The selected dose was extrapolated.^[6] The required amount of extract to be given was calculated according to the bodyweight of animals. The extract was administered as a once a day dose per orally for 14 days. On the 14th day, after an hour of administration of extract, animals were screened for anti-depressant activity.

Antidepressant activity of hydroalcoholic extract of *Aegle marmelos* fruits was evaluated by using two models –Forced Swimming Test (FST) and Tail Suspension Test (TST). The experiment was conducted in the Ethnopharmacology laboratory of the Department of Pharmacology, Yenepoya Medical College between 8:00 A.M. to 2:00 P.M. The food and water was discontinued during the study period. Animals were weighed and an appropriate dose of extract was given per orally (p.o) to the different groups. The experiment was conducted sixty minutes after the administration of the drug.

Forced Swim Test:

Mice were housed in individual cages one day prior to experiment. Each mouse was placed inside a vertical Plexiglass cylinder (40 cm x 18cm and containing 15 cm of water at 25°C) and were observed for 6 minutes. The evaluation was initiated after 2 minutes. Duration of immobility was noted for each mouse. The antidepressant activity was represented by increase in the duration of mobility or decrease in the duration of immobility. After the test, animals were allowed to dry and returned to their home cages.^[7]

Tail Suspension Test:

Each mouse was suspended upside down 50 cm above the ground with the help of a stand using an adhesive tape placed approximately 1 cm from the tip of the tail. They were observed for 5 minutes. Mice were considered immobile when it was hanging freely without any movements. Duration of immobility was noted for each mouse. The antidepressant activity was represented by increase in the duration of mobility or decrease in the duration of immobility. After the test, animals were allowed to dry and returned to their home cages.^[7]

Statistical analysis

Results were represented as Mean. One-way analysis of variance (ANOVA) was used for simultaneous comparison of

groups. For those variables that were significant in ANOVA, multiple comparisons between the groups were made with the Tukey Kramer Test at $P = 0.05$. Result showing the level of significance $P \leq 0.05$ is considered as statistically significant. The statistical analysis was carried out using the software SPSS version 24. P -value < 0.05 was considered as significant.

RESULTS

Effect of HAAM on depression was done using Tail suspension test (TST) and Forced swim test (FST).

Effect of Aegle marmelos fruit extract on depression — Tail suspension test (TST) and Forced swim test (FST)

Table 1 and Figure 1 shows that administration of HAAM 100 mg/kg and 200 mg/kg treated groups showed a significant decrease in immobility period ($p < 0.05$) as compared to the control group. The combination of HAAM 200 mg + Imipramine 5 mg also showed similar efficacy when compared with HAAM 100 mg and 200 mg.

Group	Treatment group	Dose (mg/Kg)	FST Duration of Immobility (Sec)	TST Duration of Immobility (Sec)
1	Control (Normal Saline)	10ml	133.83	171.5
2	Imipramine	5	59.5	102.5
3	HAAM*	100	74.5	126.83
4	HAAM*	200	73.6	121.17
5	HAAM* + Imipramine	200+5	70.83	119.17

*HAAM -Hydroalcoholic extract of Aegle marmelos

Table 1: Effect of Aegle marmelos fruit extract on Forced Swim Test (FST) and Tail Suspension Test (TST)

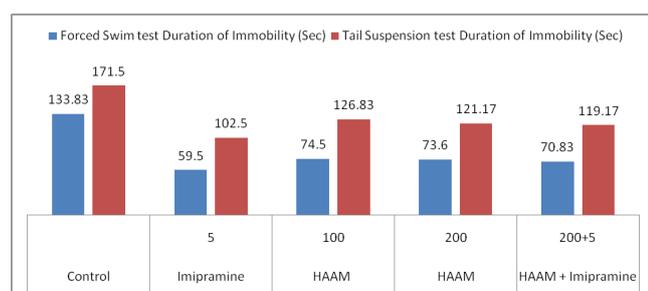


Figure 1: Effect of Aegle marmelos fruit extract on immobility time (in seconds)

DISCUSSION

The present study was performed to evaluate the antidepressant activity of hydroalcoholic extract of Aegle marmelos fruits in experimental animal models. Our study showed a significant decrease in immobility in behavioral models of mice after administering the extract for a period of 14 days. Various animal models are available for demonstrating the antidepressant activity of potential drugs. Forced swim test (FST) and tail suspension test (TST) are the two commonly used animal models to evaluate the antidepressant activity. In both the models, period of immobility is used to assess the antidepressant activity. Decrease in the period of immobility was considered as an indicator of antidepressant activity.

Depression is a chronic psychiatric condition. There are various hypotheses explaining the pathophysiology of depression, and the monoamine hypothesis is the most common and widely accepted among them.^[8] It is well said that depletion of monoamines leads to depression. It is undoubtedly known that serotonin depletion plays an important role in the pathophysiology of depression. This is again substantiated by the fact that Selective Serotonin Reuptake Inhibitors (SSRIs), which increase serotonin levels play a vital role in the pharmacotherapy of depression.^[9] Literature also suggests that oxidative stress plays an important role in the pathophysiology of depression and associated neuropsychiatric diseases.^[10] Similar study done by Pojala et al showed that fruit of Aegle marmelos exhibited anti depressant activity when compared with the standard group.^[11] Currently available antidepressants not only affect the levels of neurotransmitters but also modify the imbalance between oxidant and antioxidant substances. It also causes numerous adverse effects and also the clinical response is not obtained immediately.^[12] Therefore, there is a need for an antidepressant drug with better clinical profile to explore more therapeutic options with safer herbal preparations.

The phytoconstituents present in Aegle marmelos are flavonoids, alkaloids, coumarins, phenols, terpenoids and tannins.^[13, 14] These constituents of Aegle marmelos may be responsible for the antidepressant activity. Further studies need to be carried out to find out the exact mechanism of action responsible for this antidepressant activity

CONCLUSION

Traditional herbal medicines have an important role in the primary healthcare of our country. Plant materials can be a potential source to isolate and manufacture novel compounds, still, the beneficial effect has to be appropriately tested for its efficacy and safety before concluding their superiority over non-herbal drugs. Without appropriate preclinical and clinical studies, one cannot recommend the use of traditional medicines. From this study, it can be concluded that hydroalcoholic extract of Aegle marmelos fruits shows significant antidepressant activity in the doses of 100 mg/kg and 200 mg/kg, although

there was no dose-dependent action seen between the two doses.

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