Evaluation of Antidepressant and Antianxiety activity of Ethanolic leaf extract of Aegle marmelos
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ABSTRACT

Background: The aim of this study is to evaluate antidepressant and antianxiety activity of Ethanolic leaf extract of Aegle marmelos.

Methods: Plant materials are collected from Punjab. Ethanolic extract was screened for different phytochemical constituents. Acute toxicity studies are done according to OECD guidelines. Ethanolic extracts were screened for antidepressant activity in Wistar Albino rats (forced swim test) and antianxiety activity (elevated plus maze). Ethanolic extract Aegle marmelos shows significant antidepressant and antianxiety activity compared to control. Presence of alkaloid, saponins and steroids were identified in the extract.

Results: The result suggests that the ethanolic extract of Aegle marmelos contains some active principles which may be responsible for these activities.

INTRODUCTION

Aegle marmelos (Bael), one of the oldest and most popular species in the world belonging to the family Rutaceae, is found abundantly throughout India. Aegle marmelos, is one of the most useful Indian medicinal plants; it has numerous of use in day to day life. Physicochemical studies prove that bael fruit is rich in nutritional value, and this is being used from several years ago. Other nutritive elements of bael are protein, fat, minerals, fibers, carbohydrates, calcium, phosphate, potassium, iron, vitamins A, vitamin B1, nicotinic acid, riboflavin, vitamin C.

In many countries, herbal therapies are among the most popular all "alternative treatments". Aegle marmelos has been used for centuries as an herbal medicine. Its stem, bark, root, leaves and fruits have medicinal value. The ancient systems of medicine, including Roman, Ayurveda, Greek, Siddha and Unani have mentioned its therapeutic applications in cardiovascular disorders, diabetes, diarrhea and dysentery. Other actions like antifungal, antibacterial, antiviral, hypoglycemic, antioxidant, antiviral and cardio protective effects have been studied using various parts of the plant [1-6]. Besides its antioxidant properties, Aegle marmelos unripe fruit aqueous extract interacts by various other mechanisms in a complex way to elicit its therapeutic effects.

Aegle marmelos is reported to have number of coumarins, alkaloids, steroids, and essential oils [7-8]. Root and fruits contain coumarins such as scoparone, scopolin, umbelliferone, marmesin and skimming.

Fruits in addition contain xanthotoxol, imperatorin and alloimperatorin and alkaloids like aegeline and marmelline. It also contains polysaccharides like galactose, arabinose, uronic acid and L-rahaminose, which may obtain after hydrolysis. Different types of carotenoids have been reported in the Aegle Marmelos, these are responsible for the imparting yellow pale color to fruit. Marmelosin, skimmingine and umbelliferone are the therapeutically active principle of bael plant. Minor constituents are like ascobic acid, sitosterol, crude fibers, tannins, α amyrin, carotenoids, and crude proteins are also resent. Apart from these chemical constituents more than 100 compounds have been isolated these are skimmingine, aegelin, lupeol, cineole, citral, citronellal, cuminaldehyde, eugenol, marmesin, marmelosine, luvangetin, aurapten, psoralen, marmelide, figarine, marmin, and tannins have been proved to be biologically active against various major and minor disease.

One-eighth of the total population of the world and has been affected by anxiety. It has very important area of interest in psychopharmacology since last decade. Anxiety is characterized by excessive fear, motor tension, sympathetic hyperactivity, apprehension, and vigilance syndromes. Benzodiazepines are the major class of compounds that are used in anxiety and they are the most common prescribed treatment for anxiety, despite the important unwanted side effects that they produce such as sedation, muscle relaxation, ataxia, amnesia, ethanol and barbiturate potentiation and tolerance. In quest of finding new therapeutic agents for the treatment of neurological ailments, medicinal plant research worldwide, has progressed constantly demonstrating the pharmacological effectiveness of different plant species in a variety of animal models.

MATERIALS AND METHODS

Leaf collection and identification

The leaf specimens were collected in the month of April from Punjab, India and authenticated by Professor Dr. Girish, Herbal Science Laboratory, Centre for Advanced Studies in Botany, University of horticulture and forestry, Nauni, Solan (H.P) India.
After a thorough investigation leaves were checked for any pathological disorders and contamination of other plants and were washed with distilled water subjected for drying for 15-20 days.  

**Preparation of extracts**

Dried leaves were powdered in a mechanical grinder. The powdered plant sample (1 kg) was packed into a Soxhlet apparatus and extracted exhaustively with ethanol (95%) for 24 h. The ethanolic extract was concentrated using a rotary evaporator at 40°C. Then extract was kept in refrigerator at 50°C for experimenta-

**Preliminary phytochemical screening**

The different qualitative chemical tests were performed for establishing the profile of the leaf extracts for its chemical composition. Detection of alkaloids-Mayer’s test, Wagner’s test, Hager’s test Detection of saponins by foam test

Detection of phytosterols -Libermann-Burchard’s test Detection of phenolic compound-Ferric chloride test Detection of flavonoids-Alkaline reagent test

**Acute toxicity study**

Swiss Albino mice of either sex (20 - 25 gm weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 423 and animals were observed for mortality and behavioral changes. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Evaluation of antidepressant and antianxiety activity [10]**

Forced swim test (FST) – Wister albino rats (200-250g) were divided in to 3 groups of 6 animal. Forced swim test was proposed as a model to test for antidepressant activity. Rats were forced to swim individually in a glass jar (25 × 12 × 25 cm3) containing fresh water of 15 cm height and maintained at 25°C (+ 3°C). After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. A rat was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility periods were studied after administering drugs in separate groups of animals. Each animal was used only once. Imipramine 50mg/Kg used as standard.

**Elevated plus maze (EPM)**

This test has been widely validated to measure anxiety in rodents. The plus-maze combines three potential anxiogenic factors –novelty, height and open space. Briefly, the cross-shaped maze consists of four arms that are interconnected by a central platform. Two opposing arms are surrounded by side-end-walls (closed arms), whereas the remaining two arms are unprotected (open arms). The set-up consists of a maze of two open arms (25 cm ×5 cm), crossed with walls (35 cm high) and central platform (5 cm ×5 cm). The maze is suspended 50 cm above the room floor. The animal was placed on the central platform, facing one of the enclosed arms and observed for 5 minutes. During the 5-min test period, the time spent in open and enclosed arms were recorded. Diazepam 4mg/Kg used as standard.

**Statistical Analysis**

Results were expressed as mean ± Standard deviation (SD). Statistical analysis was performed using One-way analysis of variance (ANOVA). P <0.05 was considered statistically significant.

**RESULTS**

Preliminary phytochemical screening of ethanolic extract of *Aegle marmelos* leaf revealed the presence of alkaloids, saponins, steroids. The observation of acute toxicity study indicated that there was no death in 2000mg/kg dose after 72hr. In forced swim test the ethanolic extract of *Aegle marmelos* (400mg/kg) and imipramine(50mg/kg) shows significant antidepressant activity(P < 0.001).EAM(400mg/Kg) treated animals (Group II) showed a significant (p<0.001) increase in the time spent in open arms by EPM test on comparing with the normal (Group I).

![Fig 1. Effect of EAM on elevated pluz maze](image)

**Table 1: Effect of EAM on pluz maze**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Time spent in each arm in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1ml distilled water</td>
<td>open: 8.06±1.4; closed: 260±6.21</td>
</tr>
<tr>
<td>test</td>
<td>EAM - 400mg/Kg</td>
<td>55.13±8.9*; 152.46±11*</td>
</tr>
<tr>
<td>standard</td>
<td>diazepam 4mg/Kg</td>
<td>52.58±1.6*; 160.27±72*</td>
</tr>
</tbody>
</table>

Values were expressed as means ±SD of five animals in each group.  

*** = P<0.0001 (significant), when all groups compared with control group.
Fig 2. Effect of EAM on forced swim test

DISCUSSION

Now a days the herbal researchers are concentrating to identify the drugs from the natural resources, in order to stay away from the synthetic drugs and its adverse effect. Hence A. marcellos leaf proved as potential resources for treatment of anxiety and depression. A. marcellos contains a number of phytoconstituents, which are the key factors in the medicinal value of this plant. Almost all parts of this plant such as leaf, fruit, seed, bark and root are used to cure a variety of diseases. Based on the earlier report the fruit of A. marcellos has not been reported for the psychiatric disorders. As the pharmacologists are looking forward to develop new drugs from natural sources, development of modern drugs from A. marcellos can be emphasized for the control of various diseases.

CONCLUSION

This study demonstrated that ethanolic extract of Aegle marcellos shows significant anti-depressant and anti-inflammatory activity. Further studies were needed to understand actual mechanism of action.

REFERENCES