

Original Article

The anti-depressant and anxiolytic properties of the lyophilized aqueous leaf extract of *Mimosa pudica* L. (Fabaceae)

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Abstract

Introduction: This study seeks to determine the antidepressant and anxiolytic properties of the lyophilized aqueous leaf extract of *Mimosa pudica* (LAL-MP) in mice.

Methods: LAL-MP was administered orally to mice at 50 – 500 mg/kg, daily for 14 days, after which mice were individually subjected to the forced swimming (FST) and tail suspension (TST) tests, the elevated plus maze (EPM) model and locomotor activity count.

Results: Generally, LAL-MP from 100 to 500 mg/kg exhibit antidepressant and anxiolytic properties in all 4 models of depression and dose levels at 400 and 500 mg/kg were found to be equipotent with the standard drug fluoxetine.

Conclusions: This is the first report on the combined antidepressant and anxiolytic properties of LAL-MP.

Keywords:

Mimosa pudica, Anxiolytic, Antidepressant

1. Introduction

The leaves of *Mimosa pudica* L. has been studied for its diuretic, anti-infective, wound healing, antifertility, aphrodisiac and antivenom properties [1]. A decoction of the leaves exhibit anticonvulsant effects at 1,000 – 4,000 mg/kg in various animal models of seizures although this study did not reveal any mood-stabilizing effects[2]. There is evidence on the antidepressant properties of the aqueous leaf extract at 6 and 8 mg/kg I.P. by the forced-swimming method [3]. However, no studies have been conducted to demonstrate the antidepressant and anxiolytic properties of the lyophilized aqueous leaf extract of *M. pudica* (LAL-MP). This study seeks to investigate the antidepressant and anxiolytic effects of LAL-MP in different models of depression and anxiety in mice.

2. Materials and Methods

2.1. Harvesting of Plant Material

The leaves of *M. pudica* were harvested in a farm located at San Carlos City, Pangasinan. The plant was authenticated at the Virgen Milagrosa University Foundation Herbarium. Leaves were washed with water to remove dirt and pressed dried in newspapers away from sunlight. Dried leaves were pulverized using a Wiley mill. One kilogram of powdered leaves were extracted by exhaustive cold maceration in a stainless steel percolator with distilled water. Combined aqueous extracts were freeze-dried in a Virtis-201 lyophilizer to yield 158.2 grams (15.8% w/w) of light brown powder which was designated in this study as the LAL-MP. Fluoxetine (i.e., positive control) and LAL-MP were prepared as a 5% (w/v) suspension in 5% (w/v) acacia mucilage.

2.2. Experimental Animals

Male Swiss mice weighing at least 20 grams were purchased from the Philippine Food and Drug Authority (Alabang, Muntinlupa City). They were acclimatized at 30°C with free access to food and water. Experiments conformed to protocols on the humane

handling of laboratory animals as approved by the Institutional Animal Care Use Committee.

2.3. Phytochemical Analysis

The LAL-MP was subjected to thin-layer chromatography (TLC) using 1 x 6 cm silica gel 60 F₂₅₄ TLC plates according to the methods of Guevara (2004)[4].

2.4. Sample Dosing

The LAL-MP was orally administered, daily for 14 days, at doses of 50, 100, 200, 300, 400 and 500 mg/kg with 5 mice assigned to each dose. Fluoxetine at 3.3 mg/kg (positive control) and 0.5 mL of 5% acacia mucilage (negative control) were given similarly. During the 14-day dosing, mice were given free access to food and water. The mice were subjected to the forced swimming, tail suspension, elevated plus maze and locomotor activity tests on the 14th day. Mice were fasted overnight with free access to water before commencing with these tests.

2.5. Forced Swimming Test

Mice were dropped individually in a 30 x 20 cms. glass aquarium containing water at a depth of 12 cms. and maintained at 30°C. Using a stopwatch, the total mobility time within a duration of 5 minutes (i.e., 300 seconds) was measured [5].

2.7. Tail Suspension Test

Mice were individually suspended at the edge of a table, 50 cms. above the floor, by adhesive tapes placed 1 cm. from the tip of the tail. The mobility time for a 5 minute (i.e., 300 seconds) suspension period was recorded [6].

2.8. Elevated Plus Maze (EPM) Test

The apparatus comprises of 2 open arms (35 x 5 cms.) and 2 closed arms (30 x 5 x 15 cms.) elevated at 12 inches. Mice were individually placed at the center of the maze facing one of the closed arms. The number of entries into the open arms and the % of time spent at the open arms were measured within a 5 minute (i.e., 300 seconds) observation period [7].

2.9. Locomotor Activity

Mice were individually placed in an activity cage (i.e., actophotometer) and the activity scores were measured within a 5 minute (i.e., 300 seconds) observation period [8].

2.10. Statistical Analysis

Means were compared by the 2-way analysis variance and 2-tailed t-test. Dose-response relationships were calculated by linear regression analysis.

3. Results

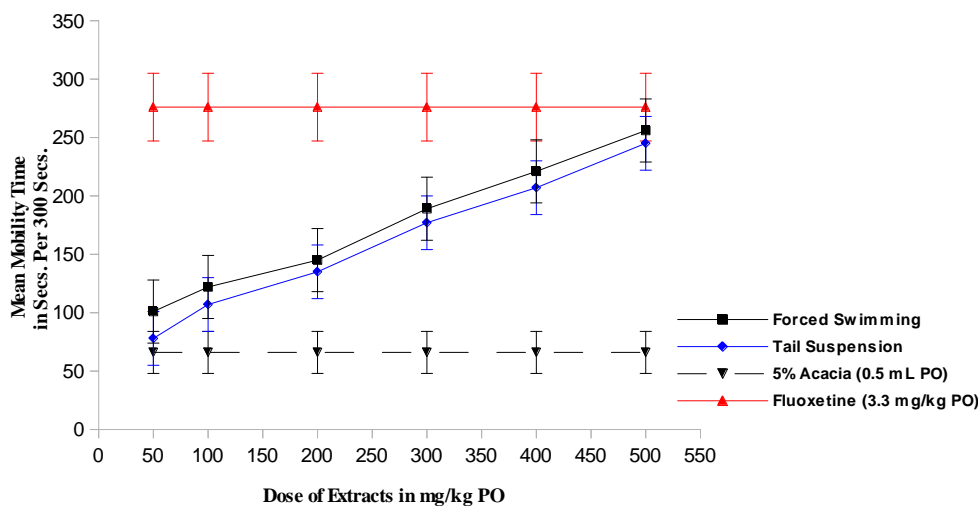
3.1. Phytochemical Screening

TLC analysis of LAL-MP revealed the presence of alkaloids, polyphenols (i.e., tannins and flavonoids), lignans, iridoids, triterpenes and unsaturated sterols and anthraquinones.

3.2. Forced-swimming Test

Figure I compares the mobility time obtained between the forced swimming (FST) and tail suspension tests (TST) at various dose levels of LAL-MP.

Figure I: Comparative Mobility Time Among Mice in Various Dose Levels of the Lyophilized Aqueous Leaf Extract of *Mimosa pudica* Between the Forced Swimming and Tail Suspension Tests



There is evidence of a dose-mobility relationship in both FST and TST ($r = 0.9823$ and 0.9855 , respectively). There is also a high correspondence between the 2 tests ($r = 0.9912$) in terms of mobility time. In both tests, significant difference in mobility time compared to acacia starts at 200 mg/kg. At 400 and 500 mg/kg, mobility times were comparable with fluoxetine ($p > 0.05$).

3.3. Elevated Plus Maze (EPM) Test

Figures II and III compares the number of entries and the % time spent in the open arms of the EPM, respectively, at various dose levels of LAL-MP in mice, showing a high agreement in both figures. Responses at all oral dose levels (50 - 500 mg/kg) were significantly higher than 5% acacia. At 400 and 500 mg/kg, the samples were equipotent with fluoxetine. Figures II and III showed high evidence of dose-response relationships ($r = 0.9910$ and 0.9923 , respectively).

Figure II: Open Arm Entries among Mice in the Elevated Plus Maze at Various Doses of the Lyophilized Aqueous Leaf Extract of *Mimosa pudica*

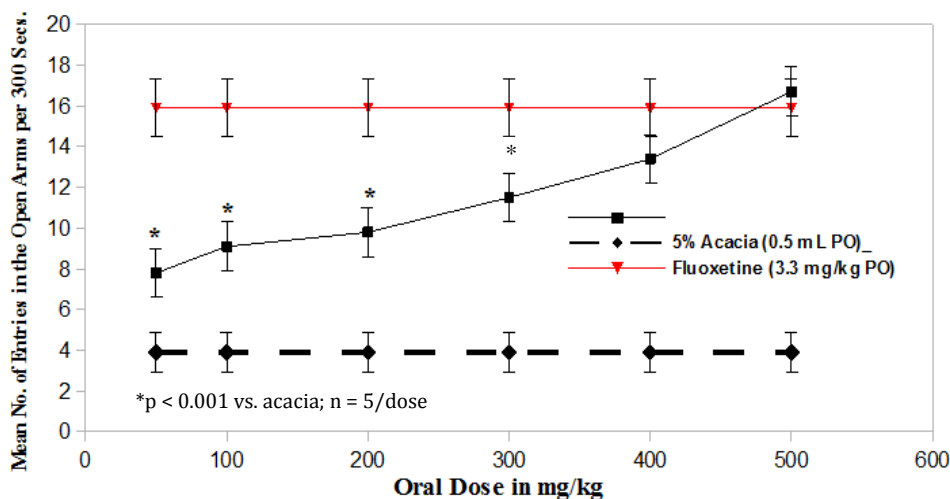
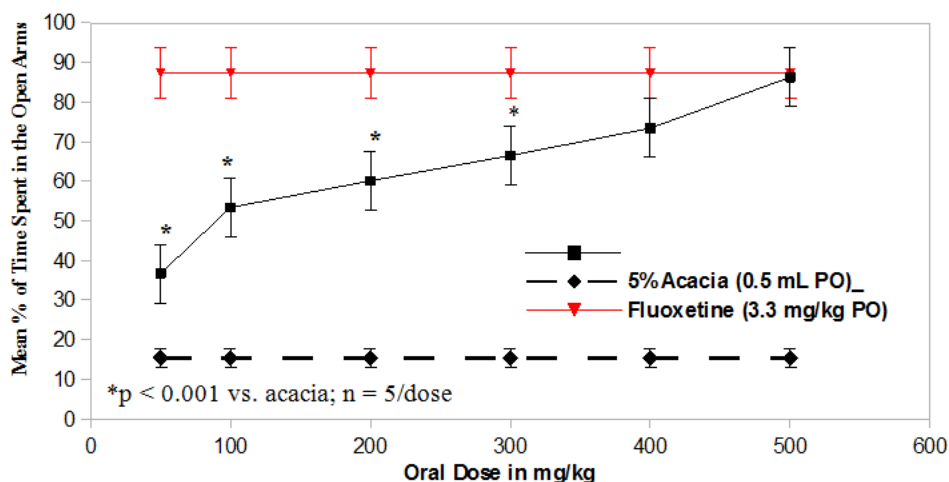
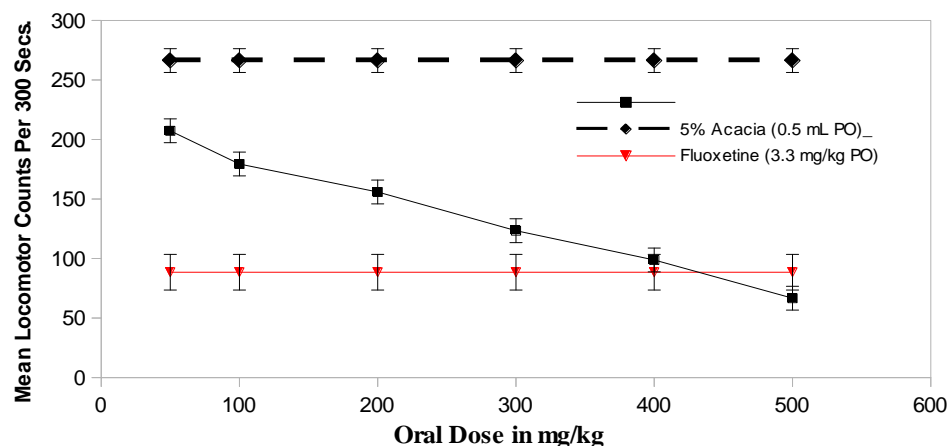


Figure III: Comparative Percentage of Time Spent Among Mice in the Open Arms of the Elevated Plus Maze at Various Doses of the Lyophilized Aqueous Leaf Extract of *Mimosa pudica*

3.4. Locomotor Activity

Figure IV compares the locomotor counts of mice treated with various dose levels of LAL-MP.

Figure IV: Comparative Locomotor Activities Among Mice with Various Doses of the Lyophilized Aqueous Leaf Extract of *Mimosa pudica*

There is a good negative correspondence between locomotor counts and dose levels ($r = -0.9877$) to indicate high dose-response relationship. The lyophilized sample at all dose levels (50 – 500 mg./kg) showed significantly lower locomotor counts than acacia. At 400 and 500 mg/kg, the responses are comparable to fluoxetine ($p > 0.01$).

4. Discussions

The fear due to height induces anxiety in mice when placed on an EPM. The ultimate manifestation of anxiety and fear is the decrease in locomotor activities and preference to remain at safer places (i.e., the 2 open arms). Anxiolytic drugs are expected to increase motor activity which corresponds to an increase in the time spent by an individual mouse in the open arms. The spontaneous decrease in basal locomotor activity counts recorded using an activity cage implies reduced anxiety.

The widespread use of FST is mainly due to its ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that mice following initial escape-oriented movements develop an immobile posture when placed inside an inescapable cylinder filled with water. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e., despair behavior) or the development of a passive behavior which is the loss of the ability to cope with stressful stimuli. Increase in

mobility characterized by swimming, aggression, escaping and the tendency to bite are, therefore, manifestations of anti-depressant effects. Similarly, in the TST, the increase in mobility due to these characteristics also corresponds to antidepressant effects.

Antidepressant therapy includes drugs mostly affecting reuptake or metabolism of monoamines such as epinephrine, norepinephrine and most importantly, serotonin. Recent data have suggest that glutamate N-methyl-D-aspartate (NMDA) receptors may be involved in the mechanism of antidepressant action. Several studies have demonstrated that the NMDA receptor antagonists exhibit antidepressant activities in to rodents using the FST [9]. Furthermore, NMDA receptor antagonists exhibit anxiolytic effects examined in the EPM. Thus, antidepressant and anxiolytic properties of NMDA receptor ligands suggest the involvement of glutamatergic system [10].

Treatment of depression requires chronic intake of antidepressants to cause manifestations of therapeutic effects since neuronal adaptive alterations seem to participate in mechanisms involving antidepressants and anxiolytic drugs. Therefore, it is important to examine if tolerance develops after prolonged treatment with an antidepressant or anxiolytic drug. The data in this study demonstrate that treatment with LAL-MP and fluoxetine, daily for 14 days, do not alter both measured behavioral responses. Moreover, challenge with acacia mucilage does not exhibit significant behavioral effects.

5. Conclusion

This study shows that the lyophilized aqueous leaf extract of *Mimosa pudica* (LAL-MP) exhibit oral antidepressant and anxiolytic properties from 100 to 500 mg/kg. At 400 and 500 mg/kg, LAL-MP is equipotent with 3.3 mg/kg of fluoxetine given orally.

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