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Research Article

Anti-depressant Activity of the Seeds of *Zanthoxylum armatum* on Swiss Albino Mice

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Abstract

The present study was designed to study the anti-depressant activity of the seeds extract of *Zanthoxylum armatum* using forced swim test and tail suspension test on swiss albino mice. The anti-depressant activity of the seeds of *Zanthoxylum armatum* was assessed using Chronic Unpredictable Mild-Stress (CUMS) induced depression in mice. The animals were treated with the Methanolic extract of seeds of *Zanthoxylum armatum* orally at two doses of 100, 200 mg/kg body weight for eight days after (CUMS) induced depression in mice. These results demonstrate that Methanolic extract of *Zanthoxylum armatum* has got anti-depressant potential.

Keywords: *Zanthoxylum armatum*; Methanolic Extracts; Anti-depressant Activity; Imipramine

Introduction

Depression is a global mental disorder that has high incidence, high recurrence, and high self-mutilation and suicide rates and can be mainly characterized by persistent depressed mood, loss of interest and enjoyment, anxiety, a significant reduction in volitional activity, cognitive impairment, mental retardation and other symptoms [18]. The neurotransmitter levels in the brain such as norepinephrine, serotonin and dopamine changes in case of the depression [2,3]. It has been most commonly found to affect the people between the ages of 20 and 40 with 8 - 12% prevalence rate [6]. Although the antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs) are available for the treatment, depression still continues to be a major medical problem [14]. In search of the agents having fast onset of action, less side effects and wide safety margin for the management of mood disorder, various plants are being used as complementary and alternative medicines.

The monoaminergic hypothesis of depression is still valid for showing the depressive symptoms. According to this hypothesis, the depletion of serotonin, noradrenaline and dopamine in the central nervous system is associated with depression [5].

The monoaminergic hypothesis is unable to fully explain the depression complexity and delay in therapeutic effect of antidepressant drugs, other complementary theory such as neurotrophic hypothesis is developed, which postulates that low levels of neurotrophic factors mainly the brain derived neurotrophic factor (BDNF) is associated with depression [5].

Among various plants that are being used for their medicinal properties, the plant *Zanthoxylum armatum* is also known for variety of its medicinal properties.

Antidepressants are the drugs which act by elevating the mood in the depressive illness. They affect the monoaminergic transmission in the brain.

Classification of antidepressants [17]

- **Reversible inhibitors of MAO-A (RIMAs)**
  - Moclobemide, Clorgyline.
- **Tricyclic antidepressants (TCAs)**
  - **NA + 5-HT reuptake inhibitor:** Imipramine, amitriptyline, doxepin.
  - **Predominantly NA reuptake inhibitor:** Desipramine, reboxetine.
- **Selective serotonin reuptake inhibitors (SSRIs)**
  - Fluoxetine, sertraline, citalopram, fluvoxamine.
- **Serotonin and noradrenaline reuptake inhibitors (SNRIs)**
  - Venlafaxine, Duloxetine.
- **Atypical antidepressant**
  - Trazodone, bupropion, mianserin, mirtazapine.

Mechanism of action of imipramine

It is a tricyclic antidepressant which inhibit norepinephrine transporter (NET) and serotonin transporter (SERT) which mediate active reuptake of biogenic amines Noradrenaline (NA) and Serotonin (5-HT) into their respective neurons and thus potentiate them. TCAs inhibit monoamine reuptake and interact with variety of receptors viz. muscarinic, alpha adrenergic, histamine, 5-HT_1, 5-HT_2 and occasionally dopamine D2.

Actions of imipramine

- **CNS**: Continuous treatment (2 - 3 weeks) gradually elevates mood and patient become more communicative.
- **ANS:** It acts as anti-cholinergic.

Materials and Methods

Animals

The experimental mice of either sex weighing between 25 - 50g will be used in present study. The animals will be housed in cages under standard conditions (25 ± 2°C, 55 ± 5% relative humidity, and 12h light and dark cycles). The animals will be allowed free access to tap water and standard laboratory mice food and acclimatized to laboratory conditions for 5 days before behavioral studies. All the readings will be taken during the same time of the day i.e. between 10 a.m. and 4 p.m. The care and handling of mice will be in accordance with the internationally accepted standard guidelines for use of animals and the protocol was approved by our "Institutional Review Committee, UCMS, Bhairahawa, Nepal".

Plant material

The seeds of *Zanthoxylum armatum* plant were collected from Parbat district of Nepal. Herbarium was made and sent for identification and was authenticated by Mr. Subodh Khanal, Assistant professor, medicinal and aromatic plants, Department of soil and environment science, IAAS Paklihawa, Rupandehi. All other chemicals used in the study are of AR grade.

Chemicals and drugs

SN	Particulars	Quantity
1	Normal saline	1 liter
2	Methanol	2 liters
3	Sucrose	100gm
4	Distilled water	Qs
5	Imipramine HCl	-

Table 1

Instruments/equipment's

S.N.	Particulars	Quantity
1	Mice holding cage	7
2	Grinding machine	1pcs
3	Feeding tube	1pcs
4	Whatman filter paper	1 set
5	Gloves	20-30 sets
6	Syringes (0.5 ml, 2 ml, 2.5 ml, 5 ml, 10 ml)	5 pcs of each
7	Forced swim test apparatus	1
8	Tail suspension test apparatus	1

Table 2

Preparation of extract

The seeds of *Zanthoxylum armatum* were collected, washed and air dried for a week at 35 - 40ºC and pulverized in a grinder. The preparation of methanolic extract of *Z. armatum* was done using continuous hot percolation (Soxhlet) extraction procedure and the procedure was conducted for about 8 - 10 cycles. After the ex-

traction procedure the menstruum was collected and solvent was evaporated so as to obtain dried extract.

Acute oral toxicity studies (oppts870.1100)

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides, and Toxic Substances) guidelines.

Methods

Dose selection: Lethal dose 2000 mg/kg selected and two doses of 100 mg/kg and 200 mg/kg body weight of methanolic extract of *Zanthoxylum armatum seeds* was selected as low dose and high dose as per the acute oral toxicity studies.

Experimental design

The mice were randomly divided into 5 groups (n = 6 mice per group):

- **Group 1:** Vehicle control and received saline (10 ml/kg BW)
- **Group 2:** Negative control and CUMS induced
- **Group 3:** Received low dose seeds extract (100 mg/kg BW)
- **Group 4:** Received high dose seeds extract (200 mg/kg BW)
- **Group 5:** Standard or positive control and received Imipramine hydrochloride.

Procedure

Healthy mice weighing 25 - 50g were divided into seven groups, each consisting of six animals. Group 1 received only saline and not depression induced while all other groups were depression induced following the CUMS procedure. Group 2 received saline, Group 3 and 4 were treated with the seeds extract at the dose of 100 and 200 mg/kg body weight. The group 7 was treated with the standard drug at the dose of 10 mg/kg body weight. All extracts and the standard drugs were administered orally.

Induction of depression in rats

For inducing depression in mice, Chronic Unpredictable Mild-Stress (CUMS) procedure was followed [19]. The stress method includes following:

- Food deprivation for 24 hr
- Drinking water deprivation for 24 hr with no drink bottle
- Cage tilted at 45º angle for 24 hr
- Placing together more than normal mice in a cage for 2 hr, and then individually separating them.
- Lighting at night for 12 hr
- Clamping of tail for 15 min
- Forced swimming in cold water (4 - 8ºC) for 5 min
- Empty drink bottle.

The above stress method was randomly applied each day for 6 weeks consecutively ensuring no same stress method was continuously applied. This did not allow mice to anticipate next type of stress method. The sucrose preference test was carried out on day 1 and day 42 of the CUMS procedure so as to confirm the depression in the mice.

Sucrose preference test

This test was performed to evaluate the anhedonia, the core symptom of depression. In this, the mice were allowed to drink sugar water 72 hour before the test. Two water bottles were kept simultaneously in each cage; one bottle filled with 1% sucrose solution while other with pure water. The bottle position was switched every 12 hr. After that, the test was conducted at 5:00 pm on days 1 and day 42 of the study. The mice were housed in individual cages and freed to access either of the two bottles containing 1% sucrose solution or water. The volume of consumed sucrose solution and water was recorded and the sucrose preference ratio (SPR) was calculated according to the following equation:

SPR = Sucrose intake (ml)/[sucrose intake (ml) + water intake (ml)] × 100%.

Forced swimming test

It is the most commonly used behavioral model for screening anti-depressant activity in the rodents. In this, mice were forced to swim in the open glass chamber (25 × 15 × 25 cm) containing fresh water to a height of 15 cm and maintained at 26 ± 1ºC. Here the animal cannot get support either from walls or bottom of the chamber. Water is changed after each mice is subjected to FST. The duration of immobility of mice was recorded during the last 4 min of the total 6 min testing period because the animal show vigorous movement during initial 2 min of the test. The mice were consid-

ered immobile when they were ceased struggling and remained floating motionless in water, making only the movement to keep their head above water.

Tail suspension test

Tail suspension test is also performed for screening the antidepressant like activity in mice, was first given by Steru., *et al*. Firstly prior to the laboratory test, animals were brought in the lab to adapt the lab condition for 1 - 2 hr. In this test each individual animal were suspended to the edge of table, 50 cm above the floor by the adhesive tape placed approximately 1 cm from tip of the tail. The total period of immobility was recorded for each mice manually for 6 min. If the animals were completely passive and motionless then they were considered as immobile. For this test dim light room was preferred.

Results

Statistical analysis

The results from the experiment are expressed as mean ± SD. The statistical analysis was performed by using one-way analysis of ANOVA followed by Tukey's Multiple Comparison test using graph and pad version 5.01. The values of $P < 0.05$ was considered as statistically significant.

SN	Groups	Dose	Sucrose Preference (%)	
			At Day 1 of CUMS	At Day 42 of CUMS
1	Control	10 ml/kg BW	67.10 ± 2.12	59.96 ± 6.01
2	Negative Control	10 ml/kg BW	61.11 ± 2.89	40.90 ± 4.63
3	Seeds Extract treated (low dose)	100 mg/kg BW	65.79 ± 2.19	44.36 ± 3.86
4	Seeds Extract treated (high dose)	200 mg/kg BW	63.52 ± 2.75	42.56 ± 3.67
5	Standard Drug treated (Imipramine)	10 mg/kg BW	63.91 ± 2.85	42.35 ± 4.44

Table 3: Percentage sucrose preference of mice during sucrose preference test.

All values are expressed as Mean ± SD. The differences are significant at $p < 0.05$ when analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test.

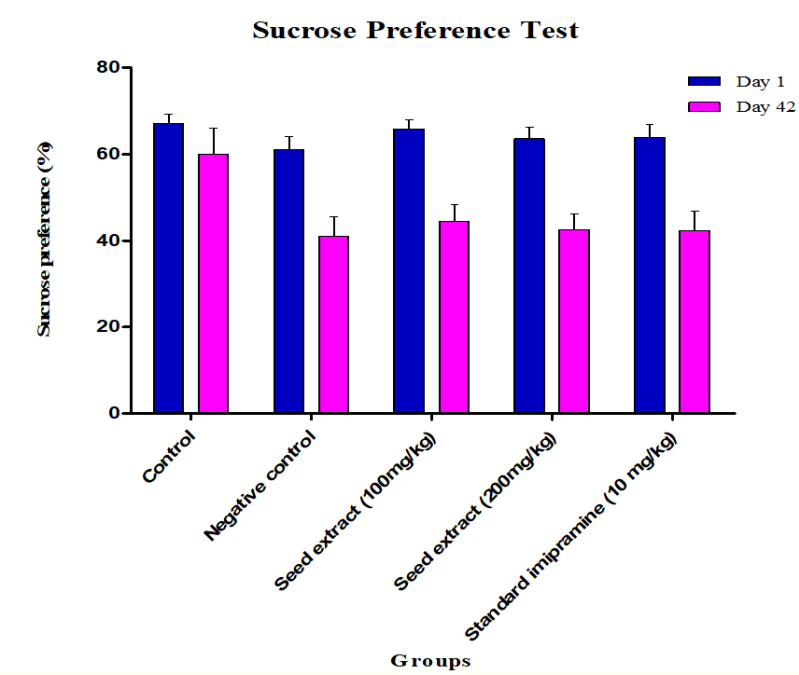

Figure 1: Bar diagram showing the effect of CUMS procedure on the sucrose preference ratio of the mice.

SN	Groups	Dose	Immobility time (sec)	
			At Day 1 of Treatment	At Day 8 of Treatment
1	Control (receive saline)	10 ml/kg BW	22.05 ± 3.00	20.69 ± 2.72
2	Negative Control (receive saline)	10 ml/kg BW	87.18 ± 3.17	84.75 ± 3.16
3	Seeds Extract treated (low dose)	100 mg/kg BW	46.25 ± 4.83	43.02 ± 3.62
4	Seeds Extract treated (high dose)	200 mg/kg BW	38.23 ± 4.53	33.78 ± 2.18
5	Standard Drug treated (Imipramine)	10 mg/kg BW	33.19 ± 3.40	32.99 ± 2.85

Table 4: Effect of *Z. armatum* extracts on the immobility time of mice during FST.

All values are expressed as Mean ± SD. The differences are significant at $p < 0.05$ when analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test.

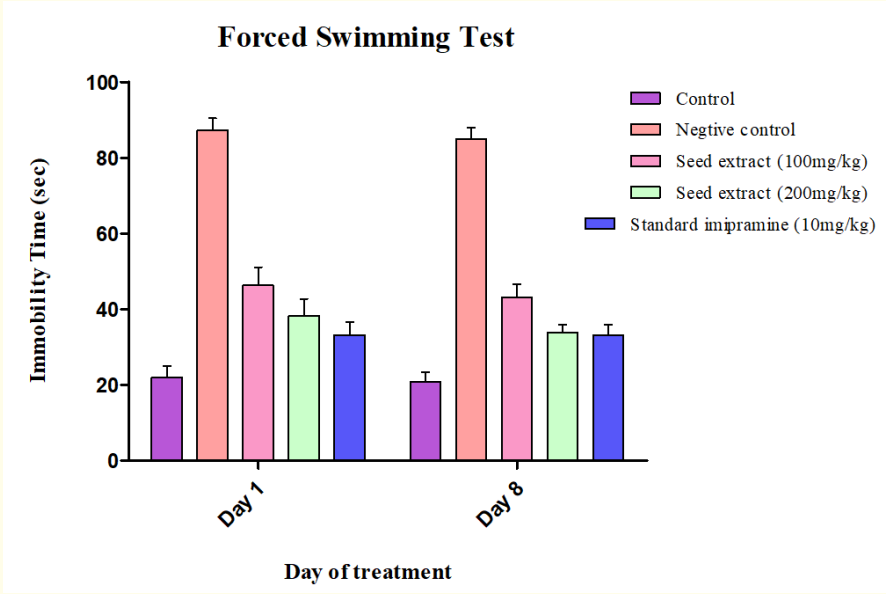

Figure 2: Bar diagram showing the effect of CUMS procedure on the forced swimming test.

Negative control group which are induced with CUMS, showed the maximum immobility time as compared to the vehicle control group which is the indication of the induction of depression. The standard drug (imipramine) showed the decrease in the immobility time than the negative control group, which is the indicative of the antidepressant activity. The group treated with 200 mg/kg seeds extract showed significant difference with the negative control group and hence showed to be more potent as antidepressant agent. The immobility time decreases from day 1 to day 8 which indicates the treatment is effective in the mice.

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SN	Groups	Dose	Immobility time (sec)	
			At Day 1 of Treatment	At Day 8 of Treatment
1	Control	10 ml/kg BW	20.38 ± 3.48	19.69 ± 1.48
2	Negative Control	10 ml/kg BW	90.99 ± 5.04	89.99 ± 3.24
3	Seeds Extract treated (low dose)	100 mg/kg BW	48.14 ± 3.68	43.58 ± 3.81
4	Seeds Extract treated (high dose)	200 mg/kg BW	33.76 ± 3.10	29.09 ± 2.74
5	Standard Drug treated (Imipramine)	10 mg/kg BW	31.36 ± 1.82	26.94 ± 1.96

Table 5: Effect of *Z. armatum* extracts on the immobility time of mice during TST.

All values are expressed as Mean ± SD. The differences are significant at $p < 0.05$ when analyzed by one-way ANNOVA followed by Tukey's Multiple Comparison Test.

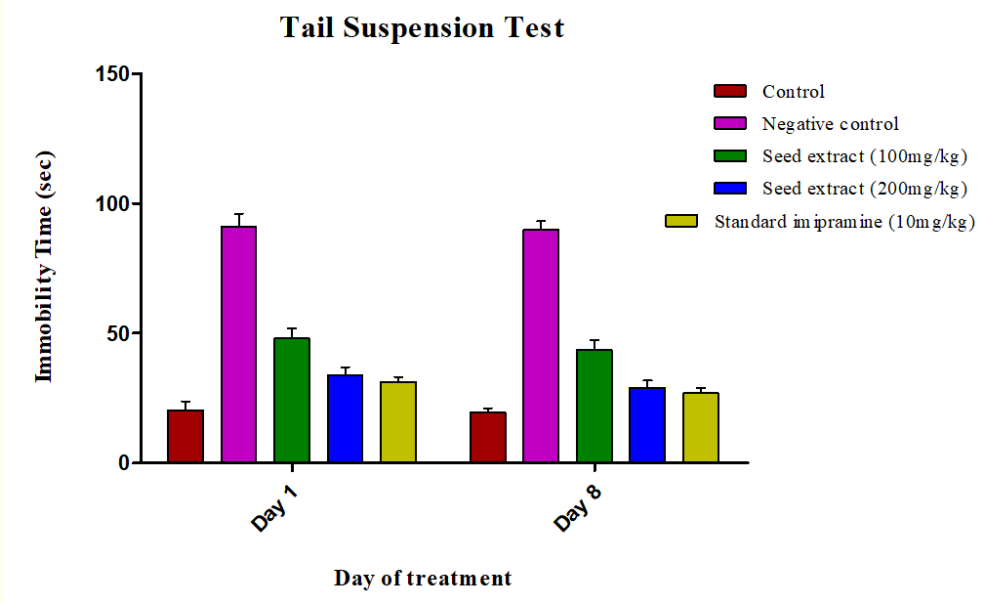

Figure 3: Bar diagram representing the effect of Methanol extracts of *Z. armatum* in immobility time in CUMS induced mice during TST.

Discussion

The incidence of depression in the community is very high and is associated with lots of morbidity. So, it is necessary to address these problems and find effective remedies. Despite the availability of several drugs for the treatment of depression in the market, all are associated with some limitations and hence there is an urgent need of the alternative medications for this disorder. Although the *Zanthoxylum armatum* is widely used for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the different doses of the methanolic extract of *Z. armatum* when administered to the mice, it was able to induce antidepressant effects.

In this study we employed a chronic stressor model CUMS to test the antidepressant effect of the seeds extract of *Z. armatum*. In this regard, the animal model of CUMS-induced depression has been developed to stimulate the pathogenesis of depression in humans. the validation of the CUMS procedure has been demonstrated in previously published reports [18].

In an attempt to mimic the excessive human day-to-day stress, several animal models have been developed. The tail suspension test and forced swimming test are the most common predictive test for screening of antidepressant-like activity of drugs. In both cases, animals are kept in unescapable situation and the antidepressant activity is expressed by the decrease in the immobility time as compared with the control groups [13]. In our study, we provided convincing evidence that the *Zanthoxylum* extract administered by oral route produces a specific antidepressant effects in FST and TST after one week of the treatment.

Imipramine is a tricyclic antidepressant; it blocks the reuptake of neurotransmitters serotonin and NE almost equally. Furthermore, imipramine ameliorated depression-like behaviour in animal decreased anhedonia, anorexia, weight loss, reduced social, locomotor and exploratory behavior [2,3]. This was also noticeable in our study.

Liu., *et al*. (2013) investigated for the confirmation of the depression in the animals following the CUMS procedure and tested for the sucrose consumption and found that the sucrose consumption significantly differs among the groups prior to the stress induction and post induction. Likewise, sucrose consumption was measured twice during our experiment.

There was significant difference ($p < 0.001$) in the sugar consumption in the groups prior to stress induction and post CUMS induced mice.

In the previous study conducted in the different plants, after one week treatment, the plants extracts as well as standard drug induced significant decrease in the immobility time during forced swimming test and tail suspension test when compared with the negative control group and the immobility time reduced as the treatment is prolonged [9,10]. In our study found significant decrease ($p < 0.001$) in the immobility time as compared to the negative control group in both FST and TST. Further, the significant differences between the extract treated group and standard drug treated group as compared to the negative control group indicates the antidepressant activity of the extract.

Conclusion

This study was conducted to explore the antidepressant activity of extracts of plant *Zanthoxylum armatum* in chronic unpredictable mild stress (CUMS) induced mice. The result of the study showed that the selected plant possesses significant antidepressant activity. The seeds extract presented significant antidepressant activity in mice, From the above study it can be concluded that the crude methanol extract of *Zanthoxylum armatum* possesses significant antidepressant activity and appears to be attractive material for the further study and possible drug development.

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