ANTI-DEPRESSANT ACTIVITY OF THE LEAVES OF *ZANTHOXYLUM ARMATUM* ON SWISS ALBINO MICE

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ABSTRACT

INTRODUCTION

Depression is a global mental disorder that has high incidence, high recurrence, and high self-mutilation and suicide rates. Although the antidepressant drugs are available for the treatment, depression still continues to be a major medical problem. The present study was designed to study the anti-depressant activity of the leaves extract of *Zanthoxylum armatum* using forced swim test and tail suspension test (TST) on Swiss albino mice.

MATERIAL AND METHODS

The anti-depressant activity of the leaves of *Zanthoxylum armatum* was assessed using chronic unpredictable mild-stress (CUMS) induced depression in mice. The animals were treated with the methanolic extract of leaves of *Zanthoxylum armatum* orally at two doses of 100, 200 mg/kg body weight for eight days after CUMS induced depression in mice.

RESULTS

The data were analyzed by one-way ANOVA followed by tukey multiple comparison test. The leaves extract presented significant antidepressant activity in mice ($p<0.05$),

CONCLUSION

The results demonstrate that methanolic extract of leaves of *Zanthoxylum armatum* has got significant antidepressant activity.

KEYWORDS Anti-depressant activity, Imipramine, *Zanthoxylum armatum.*

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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.[1] The neurotransmitter levels in the brain such as norepinephrine, serotonin, and dopamine changes in case of the depression.[2] It has been most commonly found to affect the people between the ages of 20 and 40 with 8-12% prevalence rate.[3] 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.[4] As the monoaminergic hypothesis is unable to fully explain the depression complexity and delay in therapeutic effect of antidepressant drugs, other complementary theory such as neurotropic hypothesis is developed, which postulates that low levels of neurotropic factors mainly the brain derived neurotropic factor (BDNF) is associated with depression.[5]

This study was conducted to explore the antidepressant activity of leaves extracts of plant *Zanthoxylum armatum* in chronic unpredictable mild stress (CUMS) induced mice. Antidepressants are the drugs which act by elevating the mood in the depressive illness. They affect the monoaminergic transmission in the brain. Modified TST, which is an animal model for depression first proposed by Steru et al and adapted by Chermat et al for rats. It is based on the principle that suspending rat upside down leads to characteristic behavior of immobility after initial momentary struggle. The immobility displayed by these rodents reflects behavioral despair; which, in turn, reflects depressive disorders in humans. TST is simple, inexpensive, and reliable for screening of several different classes of antidepressant drugs. It has been reported that TST is less stressful and has higher pharmacological sensitivity than FST, the other commonly employed model to study antidepressant activity.[6]

MATERIAL AND METHODS

Animals

The experimental mice of either sex weighing between 25-50 g was used in present study. The animals were housed in cages under standard conditions (25±2°C, 55±5% relative humidity, and 12-hour light and dark cycle. The animals were divided into five groups and each group contain n=6 mice. The animals will be allowed free access to tap water and standard laboratory mice food and acclimatized to laboratory conditions for five days before behavioral studies. All the readings will be taken during the same time of the day i.e. between 10 AM. and 4 PM. 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".

Figure 1. Swiss albino mice used for anti-depressant activity

Plant material

The leaves 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, Institute of Agriculture and Animal Science (IAAS) Paklihawa, Rupandehi. All other chemicals used in the study are of AR grade.

Figure 2. *Zanthoxylum armatum* plant leaves

Preparation of extract

The leaves of *Zanthoxylum armatum* were collected, washed and air dried for a week at 35-40º C 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 extraction procedure the menstruum was collected and solvent was evaporated so as to obtain dried extract.[7]

Acute oral toxicity studies (oppts 870.1100)

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 leaves* was selected as low dose and high dose as per the acute oral toxicity studies.

Experimental design

Table 1. The mice were randomly divided into 5 groups (n=6 mice per group)

S.No	Groups	Dose
1	Normal control	Vehicle only (10 ml/kg)
2	Negative control	CUMS
3	Low dose (*Z. armatum*)	100 mg/kg
4	High dose (*Z. armatum*)	200 mg/kg
5	Standard (Imipramine)	10 mg/kg

Procedure

Healthy mice weighing 25-50 g were divided into five 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 leaves extract at the dose of 100 and 200 mg/kg body weight. The group 5 was treated with the standard drug at the dose of 10 mg/kg body weight. All extracts and the standard drugs were administered orally.[8]

Induction of depression in rats

For inducing depression in mice, chronic unpredictable mild-stress (CUMS) procedure was followed.[9] The stress method includes following;

a. Food deprivation for 24 hours
b. Drinking water deprivation for 24 hours with no drink bottle
c. Cage tilted at 45º angle for 24 hours
d. Placing together more than normal mice in a cage for two hours, and then individually separating them.
e. Lighting at night for 12 hours
f. Clamping of tail for 15 minutes
g. Forced swimming in cold water (4-8ºC) for 5 minutes
h. Empty drink bottle

The above stress method was randomly applied each day for six 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 hours 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 hours. 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.[10] 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% (Equ. 1)

Forced swimming test

It is the most commonly used behavioral model for screening anti-depressant activity in 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 ±1º C. 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 four minutes of the total six minutes testing period because the animal shows vigorous movement during initial two minutes of the test. The mice were considered immobile when they were ceased struggling and remained floating motionless in water, making only the movement to keep their head above water.[10,11]

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 laboratory to adapt the laboratory condition for one to two hours. In this test each individual animal was 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 mouse manually for six minutes. If the animals were completely passive and motionless then they were considered as immobile. For this test dim light room was preferred.[6,11]

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.

Table 2. Percentage sucrose preference of mice during sucrose preference test

S.No	Groups	Dose	Sucrose preference (%)	
			At day 1 of CUMS	At day 42 of CUMS
1	Control	10 ml/kg	67.10 ±2.12	59.96 ±6.01
2	Negative control	10 ml/kg	61.11 ±2.89	40.90 ±4.63
3	Low dose (*Z. armatum*)	100 mg/kg	60.98 ±3.38	39.12 ±4.07
4	High dose (*Z. armatum*)	200 mg/kg	54.82 ±3.76	37.62 ±3.08
5	Standard drug (Imipramine)	10 mg/kg	63.91 ±2.85	42.35 ±4.44

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

Table 3. Effect of *Z. armatum* extracts on the immobility time of mice during FST

S.No	Groups	Dose	Immobility time (SEC)	
			At day 1 of treatment	At day 8 of treatment
1	Normal control	10 ml/kg vehicle only	22.05 ± 3.00	20.69 ± 2.72
2	Negative control	10 ml/kg	87.18 ± 3.17	84.75 ± 3.16
3	Low dose (*Z. armatum*)	100 mg/kg	75.66 ± 2.58	72.35 ± 2.50
4	High dose (*Z. armatum*)	200 mg/kg	45.92 ± 3.82	40.59 ± 2.08
5	Standard drug (Imipramine)	10 mg/kg	33.19 ± 3.40	32.99 ± 2.85

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.

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

SN	Groups	Dose	Immobility time (SEC)	
			At day 1 of treatment	At day 8 of treatment
1	Normal control	10 ml/kg vehicle only	20.38 ±3.48	19.69 ±1.48
2	Negative control	10 ml/kg	90.99 ±5.04	89.99 ±3.24
3	Low dose (*Z. armatum*)	100 mg/kg	77.52 ±3.41	74.18 ±3.64
4	High dose (*Z. armatum*)	200 mg/kg	46.22 ±4.25	42.22 ±2.85
5	Standard drug (Imipramine)	10 mg/kg	31.36 ±1.82	26.94 ±1.96

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.

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 neurological disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this study, it was demonstrated that the different doses of the methanolic extract of *Z. armatum* when administered to the mice, showed anti depressant like effects.

The chronic stressor model CUMS for antidepressant effect of the leaves 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.[1]

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.[12] 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 norepinephrine almost equally. Furthermore, imipramine ameliorated depression-like behavior in animal decreased anhedonia, anorexia, reduced social, locomotors and exploratory behavior.[2] In this study we use similar model as mentioned by Liu Y, et al with some modification.[13] The depression in the animals was confirmed by CUMS procedure. The sucrose consumption was also tested. The sucrose consumption was measured prior to stress induction and post induction during our experiment. We observed that there was significant decrease (p 0.001) in the sugar consumption in the groups prior to stress induction compared to post CUMS induced mice.

In the previous study conducted in different plants, after one-week treatment, the plants extract 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.[14] In our study found significant decrease (p□0.05) 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

From the above study it can be concluded that the crude methanol extract of *Zanthoxylum armatum* leaves possesses significant antidepressant activity and appears to be attractive material for the further study and possible drug development.

LIMITATIONS OF THE STUDY

Preclinical study was conducted in Universal College of Medical Sciences, Bhairahawa, Nepal. Though there are some genetic similarities between experimental mice and humans, results cannot be extrapolated to humans until further studies are conducted. The animal behavioral study was carried thus the actual active phytoconstituents of methanolic extract of *Zanthoxylum armatum* leaves was not known.

RECOMMENDATIONS

The actual phytoconstituents and their mechanism of action responsible of antidepressant activity can be studied.

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