Research Article

Nepal Journal of Health Sciences

Submitted:31/01/2022
Accepted:08/06/2022
Published:25/06/2022

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

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ABSTRACT

Introduction: Depression disorder has significant potential morbidity and mortality, contributing to suicide, incidence and adverse outcomes of medical illness, disruption in interpersonal relationships, substance abuse, and lost work time.

Objectives: 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.

Methods: The animals were treated with the methanolic extract of seeds of Zanthoxylum armatum orally at two doses of 100, 200mg/kg body weight for eight days after CUMS induced depression in mice. The results demonstrate that Methanolic extract of seeds of *Zanthoxylum armatum* has got anti-depressant potential.

Results: The study showed that the extract of *Zanthoxylum armatum* had significant antidepressant activity. The Microsoft excel was used to calculate the mean ± SEM and one way ANOVA followed by turkey multiple comparison test were used to analyzed the results. The seeds extract presented significant antidepressant activity in mice ($p<0.05$).

Conclusions: This study was conducted to explore the antidepressant activity of seeds extracts of plant *Zanthoxylum armatum* in CUMS induced mice.

Keywords: Anti-depressant activity; imipramine; methanolic extracts; *zanthoxylum armatum.*

INTRODUCTION

Depression is a global mental disorder with a high incidence, recurrence rate, and rates of self-mutilation and suicide. It is characterized primarily 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.[2,3] Previous study reported that the people between 20 and 40 years with 8-12% prevalence rate.[4]

Although the different antidepressant drugs are available for the treatment, of depression.[5] The monoaminergic hypothesis, the depleted the level of serotonin, noradrenaline, and dopamine in the central nervous system which induces depression.[6] The 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.[6]

Plant species plays a very important role for the development of medicines. Native peoples had

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Citation

Yadav CK, Poudel K, Shrivastava A, Mehta R, Yadav T. Anti-depressant Activity of the Seeds of Zanthoxylum armatum on Swiss Albino Mice. Nepal J Health Sci. 2022 Jan-Jun; 2(1):98-103.

good knowledge about the medicinal herbs. In Nepal *Zanthoxylum armatum* called as Timur which is commonly used in Nepali culinary dishes and many more. It grows everywhere and it is mainly containing phenolic and glycoside derivative which shows variety of medicinal properties including diabetes, diarrhea, dyspepsia, asthma, convulsion etc.[2] This plant shows CNS effects thus we are selecting this plant for anti-depressant activity.

METHODS

This study was carried out from January 2020 to May 2020 at the department of pharmacology of Universal College of Medical Sciences and Teaching Hospital, Bhairahawa, Rupandehi, Nepal. After taking approval from Institutional Review Committee IRC No. (UCMS/IRC/230/19), UCMS, Bhairahawa, Nepal.

Animals

The experimental Swiss albino mice (28-30 days) of either sex weighing between 25-50 g were used in present study. The animals were housed in a propylene cage under standard conditions (25 ± 2 °C, 55 ± 5 % relative humidity, and 12 h light and dark cycles). The animals were allowed free access to tap water and standard laboratory mice food and acclimatized to laboratory conditions for 5 days before starting the behavioral studies. All the parameters was closely observed during the same time of the day i.e., between 10 a.m. and 4 p.m. The care and handling of mice accordance with the internationally accepted standard guidelines for use of animals.

Plant material and extraction

The seeds of *Zanthoxylum armatum* plant were collected from Parbat district of Nepal. The plant was shade dried under normal room temperature. After that the 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 (Registration No. 75/76). All other chemicals used in the study are of American Chemical Society (ACS) grade. 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 *Zanthoxylum 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]

Experimental Design

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

Group	Drug	Dose
Normal Control	Vehicle only	10 mL/kg[8]
Negative Control	CUMS Induced depression	Food and water Deprivation[9]
Test-1	*Zanthoxylum armatum*	100 mg/kg[10]
Test-2	*Zanthoxylum armatum*	200 mg/kg[10]
Standard Drug	Imipramine Hydrochloride	10 mg/kg[11]

Induction of Disease

For inducing depression in mice, Chronic Unpredictable Mild-Stress procedure was followed.[9] The all groups of animals except normal control were deprive food, water for 24 hours, and the alignment of propylene case was adjusted at 45º angle at same time frame after 24 hours of stress induced the animals were allowed for forced swim at 2-4 °C.

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.

Anti-depressant activity

Healthy mice weighing 25-50g (3- 4 weeks of age) 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 CUMS induced depression, Group 3 and 4 were treated with the seeds extract at the dose of 100 and 200 mg/kg body weight. The group 5 was treated with the standard drug (imipramine hydrochloride) at the dose of 10 mg/kg body weight. All extracts and the standard drug were administered orally.

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.[12] The volume of consumed sucrose solution and water was recorded and the sucrose preference ratio (SPR) was calculated according to the following equation;

$$SPR=\frac{\text{Sucrose intake (ml)}}{\text{[sucrose intake (ml) + water intake (ml)]}}\times 100\%$$

Forced Swimming Test

It is the most used behavioral model for screening anti-depressant activity in the rodents. In this, mice were forced to swim in the open glass chamber (25×15×25cm) 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 mouse is subjected to FST. The duration of immobility of mice was recorded during the last 4 minutes of the total 6 minutes testing period because the animal show vigorous movement during initial 2 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.[13]

Tail Suspension Test

Tail suspension test is also performed for screening the antidepressant like activity in mice, was done 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, 50cm above the floor by the adhesive tape placed approximately 1cm from tip of the tail. The total period of immobility was recorded for each mouse 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.[14]

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.

RESULTS

After 42 days of the treatment of varying concentration 100 and 200 mg/kg of Z. *armatum* extract showed that the high dose was effectively reduced the depressant activity 42.56 ± 3.67 as compared with standard drug 42.35 ± 4.44, represented in table 2.

The forced swim test was carried out and the immobility time was determined of selected groups. After 8 days of continuous treatment and observation the result showed that 200 mg/kg of *Zanthoxylum armatum* as compared with standard which was 33.78 ± 2.18 and 32.99 ± 2.85 showed significant result $p< 0.05$, represented in table 3.

The tail suspension test was performed. All the groups animal were treated individually. The negative control group which are induced with CUMS, showed the maximum immobility time as compared to the normal control group which was indicated the depressive effect. The standard drug (imipramine) decreased the immobility time compared with negative control group, showed antidepressant activity. The test-2 group showed significant decreased immobility time compared with test-1 and negative control group which was indicated that test-2 shoed better anti-depressant effect, table 4.

Table 2: Percentage Sucrose Preference of mice during Sucrose Preference Test.

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

Table 3: Effect of Zanthoxylum armatum extracts on the immobility time of mice during FST.

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

Table 4: Effect of Zanthoxylum 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	Control	10ml/kg BW	20.38 ± 3.48	19.69 ± 1.48
2	Negative Control	10ml/kg BW	90.99 ± 5.04	89.99 ± 3.24
3	Seeds Extract treated (low dose)	100mg/kg BW	48.14 ± 3.68	43.58 ± 3.81
4	Seeds Extract treated (high dose)	200mg/kg BW	33.76 ± 3.10	29.09 ± 2.74
5	Standard Drug treated (Imipramine)	10mg/kg BW	31.36 ± 1.82	26.94 ± 1.96

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 *Zanthoxylum 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 *Zanthoxylum 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.[15] 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.

The 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 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.[16, 17] 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.

CONCLUSIONS

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.

Conflict of Interest: None.

NJHS

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