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Anti-Depressant Activity of *Zizyphus xylopyrus*

Vimal Kant Sharma[1]*, Nagendra Singh Chauhan[1], Santram Lodhi[1], A.K. Singhai[1]

***Corresponding author:**

Vimal Kant Sharma

[1]Department of Pharmaceutical Sciences. Dr. H. S. Gour University, Sagar. (M.P.) India 470003

Email: VKS:

itsme_vimald2@yahoo.co.in

NSC:

chauhan.nagendra@gmail.com

Abstract

The present study was undertaken to investigate the effect of ethanolic extract (ext.), ethyl acetate (EA) fractions and precipitate fraction (ppt.) of total ethanolic extract of *Zizyphus xylopyrus* on depression in rats. In the present study, the antidepressant effect of *Zizyphus xylopyrus* was examined using two behavioral models, the forced swimming test (FST) in rats and tail suspension test (TST) in rats.

Ethanolic extract when administered at an acute dose of 50 mg/kg of body weight ($P<0.01$) reduced the immobility time by 10 and 15 seconds as compared to the immobility time of control in both the screening models. Similarly EA reduced latter by 30 and 35 secs. The ppt. fraction showed the best activity, reducing the immobility time by 50 and 60 secs. in both the tests. These results showed that after standard *i.e.* Imipramine HCl (30 mg/kg), the ppt. fraction is potent amongst all the studied drugs

The present study clearly demonstrated that *Zizyphus xylopyrus* exerts an antidepressant effect in these two behavioral models. It may be due to present of flavonoids.

Keywords: *Zizyphus xylopyrus,* Flavanoides, forced swimming test, tail suspension test.

Introduction

Antidepressant drugs such as tricyclic antidepressants, and selective serotonin reuptake inhibitors (SSRI) are used to treat depression showing various side effect and thus, the search for a new antidepressant herb without side effects is deemed important [1]. Herbal drug used in depression are *Centella asiatica , Hypericum perforatum, Rhodiola rosea, Pfaffia paniculata, Rauwolfia serpentine, Rhododendron molle, Shizandra chinesis, Thea sinensis, Uncaria tomentosa, Valeriana officinalis and Withania somnifera* [2].

Zizyphus xylopyrus (Retz.) Willd. (Family: Rhamnaceae) is found throughout North-Western India, Pakistan and China. A large, straggling shrub or a small three, armed with spines, up to 4 m. in height [3]. This plant is widely used in Turkish folk medicines as a potent Sedative. The leaves (2 1/2) are chewed for 15 days as well as fruit is used in urinary troubles [4] the roasted seed powder paste is applied over the chest for reliving pain after cough and colds [5]. The major chemical composition of *Z. xylopyrus* are Quercetin, Kempferol-4'-methylether and Kempferol , Cyclo peptide alkaloids Amphibine-H and Nummularine-K [6-.7].

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The other species of the same genera are being used as an anti-depressant, anti-ulcer, memory and learning enhancers, etc. The various medicated formulas for depression contain Zizyphus species. But till now there is no scientific works have been reported on its anti-depressant activity. Therefore the present study was aimed to explore this indigenous plant for anti-depressant activity.

Material and Methods

Plant Identification

Whole plant of *Zizyphus xylopyrus* (Retz.) Willd. was collected from the University campus, Sagar (M.P.) India in the months of January to March 2008. The herb (Leaves) was authenticated by Dr. P. Tiwari (**Herbarium no. Bot/413**) at the Department of Botany Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.) and preserved in the herbarium of the institute.

Preparation of extracts and fractions

Leaves of *Zizyphus xylopyrus* (Retz.) Willd. were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. The defatted marc of the drug was subjected to ethanolic extraction for a period of 6-7 days. The ethanolic extract obtained was dissolved in dist. water and kept overnight so as to settle down the undissolved matter, which was filtered off later. The supernatant was fractionated with ethyl acetate (400 ml) in separating funnel (250 ml) both fractions were dried at 40 ^{0}C in rotatory evaporator up to a semisolid consistency and were utilized for the antidepressant activity.

Administration of the extracts and fractions

Suspensions of ethanolic extract, ethyl acetate and ppt. fractions were prepared in distilled water using Tween-80 (0.2% v/v) as the suspending agent. The extract and fractions were administered in a dose of 2000 mg/kg to rats by oral route, 45 min before the test procedures for pre-pharmacological screening as per OECD guidelines. Control groups were given only the vehicle (0.2% v/v Tween-80 solution) in volume equivalent to that of the plant extracts and fractions.

Acute Toxicity Studies

Ethanolic extract and fractions in a dose of 2000 mg/kg is given orally for the assessment of acute toxicological studies [8]. All the parameters were thoroughly checked and dose for the further studies was calculated as per OECD. After the conduct of acute toxicological studies the dose of each extract and the two fractions were decided i.e. 50 mg/kg, 10 mg/ kg. oral route was selected for the administration of drugs.

Forced swimming test (FST)

Rats of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1 °C. All the rats of either sex were divided in five different groups. The first group assigned as control receiving only vehicle (NaCl 5ml/kg). The other three groups received acute dose of ext., EA and ppt. fraction (50, 10, 10 mg/kg). The fifth group recived standard drug Imipramine (30 mg/kg). The total duration of immobility was recorded during the last 6 min of the 10-min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect [9,10].

Tail suspension test (TST)

All the rats of either sex were divided in five different groups. The first group assigned as control receiving only vehicle (NaCl 5ml/kg). The other three groups received acute dose of ext., EA and ppt. fraction (50, 10, 10 mg/kg). The fifth group recived standard drug Imipramine (30 mg/kg). The total duration of immobility induced by tail suspension was measured according to the methods described by Steru *et al*., (1985) [11].

Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period [12]. Mice were considered immobile only when they hung passively and were motionless.

Statistical analysis

The immobility time in tail suspension test and forced swimming test was analyzed with ANOVA, further comparisons between vehicle and drug-treatment groups were performed using the Dunnett's t-test. Results are expressed as the means ± SEM. Analyses were performed using the software SPSS version 13 for windows. The level of statistical significance adopted was **P<0.01, when compared with the control group.

Results

The behavioral despair model was performed in order to investigate the ability of this herbal drug in the elevation of suppressed mood, which is quite common in today's scenario. The results obtained from FST and TST clearly revels the fact that this drug is potentially quite useful in cases of depression.

Table 1 : Effect of *Zizyphus xylopyrus* on Immobility time in FST

Group no.	Drug treatment	*Dose mg/kg*	Immobility period, mean ±S.E.M [n=6]
I	Contol	NaCl (5 ml/kg)	180 sec
II	Ethanolic extract	50	**160 sec.
III	Etylaceate fraction	10	**145 sec.
IV	Ppt. fraction	10	**130 sec.
V	Standard drug	30	**125 sec.

Values were mean ±S.E.M. for (n= 6 rats) expressed as the time (in seconds) of 6 animals in each group. Data analysis was performed using Dunnett's test.

**P < .01 vs. control

The present findings suggested that ethanolic extract when administered at an acute dose of 50 mg/kg of body weight (P<0.01) reduced the immobility time by 15 seconds as compared to the immobility time of control i.e. 190 sec. the time shown by animals treated with extract was found to be **170 sec. Ethyl acetate showed a reduction in immobilization time at an acute dose of 10 mg/kg (P<0.01) the mean immobility time of EA treated animals was **165 sec. The precipitate fraction of the drug shown the best results when it was compared with control and standard. The decrease in the immobility time was quite close to that of standard. The time of mobility was increased by ppt. fraction at a dose of 10 mg/ml, shown the immobility time **140 sec. (P<0.01) to that of standard **135 sec. (P<0.01). These results shows that after standard i.e. Imipramine HCl (100 mg/ml) the ppt. fraction is most potent amongst all the treated groups. (Table 1)

Table 2 : Effect of *Zizyphus xylopyrus* Immobility time in TST

Group no.	Drug treatment	Dose mg/kg	Immobility period, mean±S.E.M [n=6]
I	Control	0.2% v/v Tween-80 solution	195 sec.
II	Ethanolic extract	50	**170 sec.
III	Ethyl acetate fraction	10	**165 sec.
IV	Ppt. fraction	10	**140 sec.
V	Standard drug	30	**135 sec.

Values were mean ±S.E.M. for (n= 6 rats) expressed as the time (in seconds) of 6 animals in each group. Data analysis was performed using Dunnett's test.

**P < .01 vs. control

Findings on tail suspension test were quite comparable to the previous FS test. As shown in the observation table and bar graph, it is quite evident that none of the drug treated animals showed excellent results compared to the standard. The immobility of Imipramine HCL (P<0.01) 100 mg/ ml was came out to be **135

sec. In this test the time of animals treated with ethanolic extract was found to be **170 sec. ($P<0.01$) when it was compared to the control group of animals which was 195 sec. The immobility time of ethyl acetate and ppt. fractions when given an acute dose of 10 mg/kg each of body weight significantly reduced the time of immobility by **165 sec. ($P<0.01$) and **140 sec. The results clearly revels the fact that standard treated animals showed better response as compared to the plant extract and fraction treated groups but even though the ppt. fraction treated group showed better response as compared to the extract and EA fraction treated group of animals. (Table 2)

Discussion

For the purpose of investigation of antidepressant activity of this plant, we used two animal models, the forced swimming test (FST) in rats developed by Porsolt *et al.,* 1981 and tail suspension test (TST) in mice developed by Steru *et al*., 1985. The immobility displayed by rodents when subjected to unavoidable stress such as forced swimming is thought to reflect a state of despair or lowered mood, which are thought to reflect depressive disorders in humans. In addition, the immobility time has been shown to be reduced by treatment with antidepressant drugs. Moreover, a significant correlation was found between the clinical efficacy of antidepressant drugs and their potency in both models [10,11].

It has been recently shown that the regulation of α2-adrenergic receptor may be the major mechanism of this model [13]. The results indicate that *Z. xylopyrus* may have an antidepressant-like effect. However, further experiments evaluating the levels of noradrenaline and serotonin in different brain regions are necessary to confirm this hypothesis. Porsolt *et al.* proposed this behavioral model for the screening of new antidepressant compounds, concluded that the immobility time observed in the test reflected a state of lowered mood or hopelessness in animals, thus, this animal model is the most widely used tool for preclinical screening of putative antidepressant agents [14,15]. The FST shows a strong sensitivity to monoamine alterations and is a very specific cluster of stress-induced behaviors that are not related to depression symptoms in humans, but which are nonetheless exquisitely sensitive to monoaminergic manipulations [16]. It also provides a useful model to study neurobiological and genetic mechanisms underlying stress and antidepressant responses [17]. In both these studies, ppt. fraction significantly reduced the immobility time 145 and 140 sec. at a dose of 10 mg/kg, which was more, then the extract and EA fractions that reduced time by 160, 170 and 145 and 165 sec. at a dose of 50 and 10 mg/kg. Ethanolic extract is a complex product prepared from green leaves of the Ghont (*Z. xylopyrus*) plant. Major ingredients are polyphenols, especially flavonoids including quercetin, quercetrin and kaempferol. Recently, several studies have suggested the antidepressant effect of quercetin glycosides such as hyperoside, isoquercitrin and rutin using the positive results of FST [18-20]. Flavonoid glycosides are mostly hydrolyzed into their aglycons by mucosal and bacterial enzymes in the intestines, and then converted to conjugated metabolites during the absorption process [21,22]. This perhaps indicates that the active form for the antidepressant effect of quercetin glycosides is the conjugated form, not the glycoside form [23]. Additionally, the plant contains kaempferol and Rutin. These flavonoid glycosides seem to appear as conjugated forms in the blood stream as with quercetin glycosides. Transportation of these metabolites into the brain tissues via the blood brain barrier and their effect on the CNS system have been recently argued [24-25]. Moreover, quercetin metabolites were previously found in the brain tissues of rodents after oral administration [26]. Therefore, one of the antidepressant mechanisms of *Z. xylopyrus* is thought to involve flavonoid glycosides, which reach the brain tissues through to involve flavonoid glycosides, which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance,

and consequently, exerting an antidepressant effect. Our results confirm the traditional use of plant as antidepressant.

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