PHARMA RESEARCH BULLETIN
Journal Home Page : www.eduspread.com

Research Article

ISSN: 2582-676X

In-vivo Evaluation of Antidepressant Activity in *Ziziphus jujuba* on Albino Rats by Tail Suspension Test (TST) & Force Swim Test (FST) Methods

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Article History:

Received: 02 May 2019
Revised: 05 June 2019
Accepted: 10 June 2019

How to Cite:

Rani KN, Rao PV, Sirisha K, et al. *In-vivo* Evaluation of Anti-depressant Activity in *Ziziphus jujuba* on Albino Rats by Tail Suspension Test (TST) & Force Swim Test (FST) Methods. PRB, 2019;1(1):7-12.

Abstract:

The objective of the present study was to evaluate the anti-depressant activity of *Zizipus jujube*. *Ziziphus jujuba* (Rhamnaceae) is a traditional medicinal plant known as jujube. This plant has been used for the treatment of a variety of diseases. This study was done to evaluate the possible antidepressant effect of *Ziziphus jujuba* pulp extract (ZJPE) using tail suspension test (TST) & forced swim test (FST). Group-I (control) received normal saline (1ml/100gm), Group-II, III & IV received ZJPE in doses of 20, 30, 40 mg/kg orally (P.O.) respectively. Group V (positive control) received Imipramine at doses of 15mg/kg p.o. respectively. Drug treatment was given for seven & fourteen successive days and after 60 minutes interval last dose of drug or standard the immobility period was recorded. ZJPE produced significant antidepressant like effect at dose of 20, 30& 40 mg/kg administered for 7 & 14 consecutive days as indicated by reduction in immobility times of rat in TST & FST ($P<0.05$). The efficacy of ZJPE at 30mg/kg was found to be comparable to that of Imipramine at doses of 15mg/kg.

Keywords: *Ziziphus Jujuba*, Forced swim test, Tail suspension test, Antidepressants, Immobolity time.

Introduction:

Depression [1], officially termed major depressive disorder (MDD) ranks among the most prevalent diseases worldwide. According to the estimations of the World Health Organization, depression will be the second leading cause of disability in 2020. Depression, is a widespread incapacitating psychiatric ailment, imposes a substantial health burden on society. According to the most accepted hypothesis of depression, the monoamine [2-3] theory, patients with major depression have symptoms that are reflected changes in brain monoamine neurotransmitters, specifically norepinephrine (NE) and serotonin (5-HT). Clinical data suggests that dopamine (DA) is also involved in the pathophysiology and treatment of depression. Medications such as tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine-oxidise inhibitors (MAOIs), specific serotonin or epinephrine reuptake inhibitors (SNRIs), 5-HT24-6 receptor antagonists, and other heterocyclic's are clinically employed for drug therapy.

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However, these drugs can impose a variety of side effects including sedation, apathy, fatigue, sleep disturbance. According to the World Health Organization report, mood disorders are the second leading cause worldwide of disability adjusted life years and the leading cause of years lived with disability in all ages. Each drug used to treat this disorder has a success rate of about 60%. In addition, most therapies require several weeks of treatment before improvement of signs and symptoms is observed and there are numerous side effects caused by antidepressant [4-6]. Thus, the high prevalence of depression and the fact that a significant proportion of individuals do not respond well to any currently marketed antidepressants or treatments support the need for new therapeutics to treat depression. Numerous antidepressant compounds are now available, presumably acting via different mechanisms including serotonergic [7], noradrenergic [8] and/or dopaminergic systems.

Medical plant therapies may be effective alternatives in the treatment of depression, and has progressed significantly in the past decade. Hence, there remains a pressing need for new effective and better-tolerated antidepressants. *Ziziphus Jujuba* [9] (L) is a traditional medicinal plant known as jujube. It belongs to the family Rhamnaceae13 and is widely distributed throughout Southern Asia, between Lebanon, Northern India, and Southern and Central China. Depression is a major clinical illness affecting 9.5% of population. Changes in the monoamine neurotransmitters have been observed in patients of depression. The use of plant products for the treatment of human ailments has been a natural approach to health care since the beginning of civilization. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. It is reported to have for the treatment of a variety of diseases [10]. The roots, leaves and seeds are applied in wounds and ulcers. Fruits are useful in leprosy, skin diseases, pruritus, wounds and ulcers, haemorrhages and general debility. *Z. jujuba* have various activities like anti-inflammatory; sedative and hypnotic; anticancer, antiretroviral [3]; anticomplementary [11] and antioxidant [12] have been reported. Thus the present study has been undertaken to evaluate the antidepressant activity of *Ziziphus Jujuba* pulp extract (ZJPE) in rats employing tail suspension test (TST) & forced swim test (FST). Standard antidepressant drug such as Imipramine [13] (TCA) have been employed to standardize the animal models of depression.

Materials and Methods:

Collection of Plant Material and Extraction (ZJPE):

The fruits of *Ziziphus jujube* were collected from Guntur, a town in Andhra Pradesh. The plant material was authentified by a taxonomist, then they were dried in shade at room temperature and subjected to size reduction to a coarse powder using grinder/mixer. About 150 g of powdered material have to be soaked in 1000 mL distilled water at 25 ± 2 °C for 48 h in a beaker and mixture needs to be stirred every 10 h using a sterile glass rod. Filtrate was obtained 3 times with the help of Watsman No. 1 filter paper and sterilized cotton filter. The solvent was obtained. The extract was concentrated under pressure and then dried in air. The concentrated aqueous extract was suspended in polyethylene glycol. Freshly prepared solution was used for each experiment.

Preliminary Phytochemical Screening:

The aqueous extract of ZJPE was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, proteins and phenolic compounds (Kokate, 1986).

Test for Alkaloids:

The extract was treated with diluted HCl and filtered. The filtrate was treated with various alkaloidal agents.

- **Mayer's Test**: Sample was treated with mayer's reagent, appearance of cream colour indicates presence of alkaloids.
- **Dragendroff`s Test**: Sample was treated with dragendroff`s reagent, appearance of reddish brown precipitate indicates presence of alkaloids.
- **Hager's Test:** Sample was treated with hager's reagent, appearance of yellow colour indicates presence of alkaloids.

- **Wager's Test:** Sample was treated with wager's reagent, appearance of brown precipitate indicates presence of alkaloids.

Test for Phenols:

The extract was treated with neutral ferric chloride solution, appearance of violet colour indicates presence of phenols. The extract was treated with 10% sodium chloride solution, appearance of cream colour indicates presence of phenols.

Test for Flavanoids:

5ml of the extract solution was hydrolyzed with 10% sulphuric acid and cooled. It was then extracted with diethyl ether and divided in to 3 portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1 N sodium hydroxide and 1 ml of diluted ammonia solutions was added to the first second and third test tube respectively. Development of yellow color in each test tube indicates presence of flavonoids.

- **Shindoas test**: The extract was dissolved in alcohol, to which a piece of magnesium followed by drop wise addition of conc. HCL and heated. Appearance of magenta color indicates presence of flavonoids.

Test for Saponins:
Foam test: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes presence of saponins.

Test for Terpenes: The extract was treated with tin and thionyl chloride, appearance of pink colour indicates presence of terpenes.

Animals:

Male Swiss Albino rats weighing 150-250 gm were acclimatized to the experimental room at temperature 23 ± 2 °C, controlled humidity conditions (50-55%) and 12 h light and 12 h dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*. All the studies conducted were approved by the institutional animal ethical committee of Sri K.V.College of Pharmacy, Chickballapur, Karnataka, according to prescribed guidelines of CPCSEA, Government of India (Reg. No. 117/1998/ CPCSEA).

Acute Toxicity Studies:

Aqueous extract of *Z. jujuba* was studied for acute oral toxicity as per revised OECD (2002) guidelines No. 423. Animals were observed for four hours hourly for behavior changes and daily for fourteen days. The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 20, 30 & 40 mg/kg doses of extract were used.

Antidepressant Activity:

Experimental Design for Anti-depressant activity:

Rats were divided into five groups (n=6). Drugs/ vehicle were administered to the animals 60 min prior to study.
Group I: Negative control, administer saline 2 ml/kg orally.

Group II: Positive control and receive standard drug Imipramine (10 mg/kg orally).

Group III: Receive ZJPE 20 mg/kg orally

Group IV: Receive ZJPE 30 mg/kg orally

Group V: Receive ZJPE 40 mg/kg orally

Forced Swim Test:

For the forced swim 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. Treatment was given 60min prior to study as described by study design. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect (Porsolt et al.. 1977)

Tail Suspension Test:

The tail suspension method used in this study was similar to those described by Steru et al., (1985). Treatment was given 60 min prior to study as described by study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless.

Statistical Analysis:

All the values were expressed as Mean ± S.E.M. the results were analyzed statistically by one-way ANOVA followed by Dunett Multiple comparison test, $P<0.05$ was considered significant.

Results:

Preliminary Phytochemical Screening:

On preliminary phytochemical analysis of ZJPE showed the presence of flavonoids, saponins, glycosides, terpenoids amino acids, alkaloids, carbohydrates, phenolic compounds and proteins.

Acute Toxicity Studies:

Aqueous extract of *Zizipus jujuba* showed no behavioural changes nor mortality at dose 2000 mg/kg.

Antidepressant Activity:

The antidepressant effects of aqueous extract of *Zizipus jujuba* (20, 30 and 40 mg/kg) and Imipramine were studied by observing the changes in the duration of immobility in the two models: Forced swim test (FST) and Tail suspension test (TST). In both TST and FST, ZJPE 20, 30 and 40 mg/kg, p.o. produced significant reduction ($p<0.01$) in the immobility period when compared with that of control group animals that received only the vehicle. The results are tabulated in **Table 1**.

Table No. 1: Effect of ZJPE on duration of immobility in the FST and TST

Sr. No.	Groups	Dose	FST (Immobility)	TST (Immobility)
1	Group I – Control (Normal saline)	1mg/kg	190.6 ± 2.51	150.16 ± 10.30
2	Group II – Standard (Imipramine)	15mg/kg	25.06 ± 1.98	159.66 ± 05.57
3	Group III –ZJPE	20mg/kg	140.07 ± 2.78	130.16 ± 10.88
4	Group IV –ZJPE	30mg/kg	92.3 ± 2.78	153.31 ± 06.15
5	Group V –ZJPE	40mg/kg	50.5 ± 2.47	178.13± 05.21

Discussion:

Depression is an important psychiatric disorder that affects individuals' quality of life and social relations directly. Depression is characterized by emotional symptoms such as hopelessness, apathy, loss of self-confidence, sense of guilt, indecisiveness, and amotivation, as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances, and loss of appetite. When the symptoms are very severe, major depression is considered. The introduction of drugs like amitriptyline, fluvoxamine, imipramine, citalopram, venlafaxine and others have revolutionized the treatment of depression. The amazing efficacy of imipramine and fluoxetine in these depressive disorders has paved the way for the introduction and use of newer anti-depressant agents. However, the safety factor in respect of both the imipramine and fluoxetine anti-depressant drugs has been rather intriguing and hence a definite need is visualized for the introduction of safer antidepressant drugs having no troublesome adverse effects. The present study was selected to evaluate anti depressant activity of AEWS. The major biochemical constituents of *Ziziphus Jujuba* fruit are steroidal alkaloids and steroidal lactones in a class of constituents called with anolides. About 12 alkaloids, 35 with anolides, and several sitoindosides from this plant have been isolated and studied. A sitoindoside is a with anolide containing a glucose molecule at carbon 27. Much of *Ziziphus Jujuba's* pharmacological activity has been attributed to two main withanolides, withaferin A and with a anolide D. FST widely used to screen newer antidepressant drugs. This test is quite sensitive and relatively specific to all major classes of antidepressant drugs including tricyclics, selective serotonin reuptake inhibitors, monoamine oxidase (MAO) inhibitors and atypicals. Imipramine is a pre-synaptic uptake inhibitor of both nor-adrenaline as well as serotonin. Since catecholamines and 5-HT have been implicated in the aetiology of depression, the positive effect of these drugs in FST seems to be due to increased availability of these neurotransmitters at the postsynaptic receptor sites following their reuptake inhibition. In FST, mice were forced to swim in a restricted space, which induced a characteristic behavior of immobility. This immobility reflects a state of despair in animals and is claimed to reproduce a condition similar to depression in humans. Animals after anti-depressant treatment struggle more even in desperate situation, and they spend less time with immobility. In the present study, ZJFE in the dose of 40 and 50 mg/kg produced significant dose-dependent antidepressant-like effect in behaviour despair test/ Force swim test (FST), as they reduced the immobility time. In this study, we used two animal models, FST and TST.

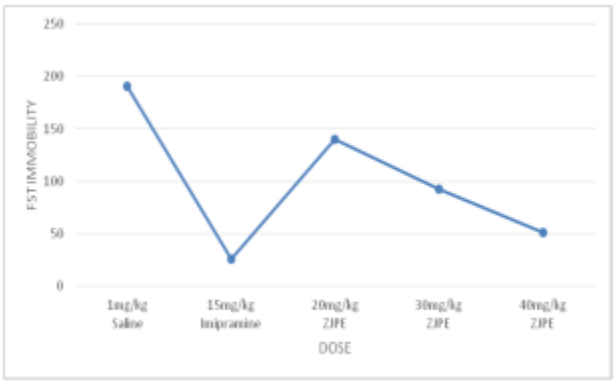

Figure 1 (a): FST Graph

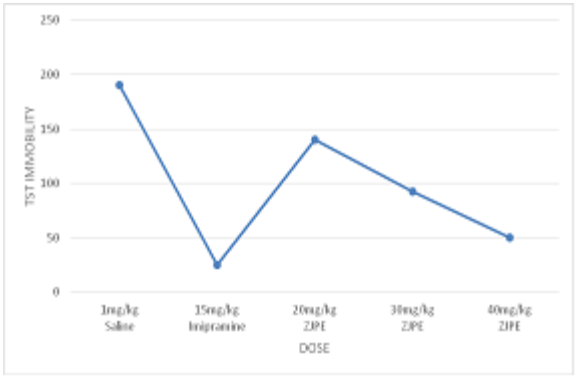

Figure 1 (b): TST Graph

Conclusion:

The present study on anti-depressant activity of aqueous extract of *Ziziphus jujuba* fruit (Pulp) revealed the presence of alkaloids, flavonoids, saponins, phenolic compounds & triterpinoides *etc.* The extract of *Ziziphus jujuba* fruit (Pulp) showed immobility time results increased and more than that of the standard imipramine, it indicates the test extract posses potent anti depressant activity. The investigations of aqueous extract of *Ziziphus jujuba* fruit (Pulp) (20mg/kg, 30mg/kg and 40mg/kg) in both FST& TST models in rats were showed *in-vivo* antidepressant activity. In this study the results concluded that the aqueous extract of *Ziziphus jujube* fruit (pulp) possess antidepressant activity.

Acknowledgement:

The authors are thankful to Rev Dr. K.V. K. Rao and Dr. P. Venkateswara Rao, Principal, for providing facilities to carry out this research work in St. Mary's Group of Institutions Guntur.

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