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Research Article

EVALUATION OF CNS DEPRESSANT AND BEHAVIORAL ACTIVITY OF AN ETHANOL EXTRACT OF ZIZIPHUS *JUJUBA* (BER) IN MOUSE MODEL

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ABSTRACT

Objective: *Ziziphus jujuba*, known as Ber (Hindi), is an indigenous herb found in India. *Z. jujuba* has been used to treat various diseases such as respiratory system diseases (asthma, cough, and laryngitis), gastrointestinal problems (constipation, colitis, and liver diseases), as well as cardiovascular and genitourinary system diseases. Seeds and fruit have been also reported to have central nervous system (CNS) depressant effect. Hence, in this study, neuropharmacological effects of leaves are studied which are not reported previously. *Z. jujuba* extract's potential to depress the CNS and affect behavior was examined in this study, along with the phytochemicals that may be responsible for these effects.

Methods: 400 mg/kg of the extract was administered intraperitoneally. As a benchmark, diazepam (2 mg/kg body weight i.p.) was used. ANOVA and Dunnett's tests were used to evaluate the data. The results were all presented as Mean (SEM) values. $p>0.05$ was regarded as meaningful.

Result: The outcome of the study demonstrated that ethanol extract of *Z. jujuba* (400 mg/kg *i.p.*) reduced locomotion, caused muscle relaxation, and had anti-anxiety effects.

Conclusions: Ethanols extract of *Z. jujuba* leaves exhibits CNS depressant action and significant anxiolytic activity comparable to diazepam.

Keywords: *Ziziphus jujuba*, Open field test, Rotarod, Behavioral, Ber.

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INTRODUCTION

Ziziphus jujuba belongs to family *Rhamnaceae*, grows in Europe, Southern, and Eastern Asia. It is use in traditional and folklore medicine since ancient time. It is known as *jujuba* in China and Ber in India [1].

Z. jujuba is used traditionally as tonic and aphrodisiac and sometimes as hypnotic-sedative and anxiolytic, anticancer (melanoma cells), antifungal, antibacterial, antiulcer, anti-inflammatory, cognitive, antispastic, antifertility/contraception, hypotensive, antinephritic, cardiotonic, antioxidant, immunostimulant, and wound healing properties [2].

As seeds of *Z. jujuba* are reported to have anxiolytic activity, so, in the present study, leaves of *Z. jujuba* were evaluated for anxiolytic effects [3].

METHODS

This study was conducted in the Department of Pharmacognosy at School of Pharmaceutical Sciences, IFTM University, Moradabad. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Plant material

Z. jujuba leaves were gathered from outskirts region of Moradabad city. Leaves were authenticated by Dr. Ashok Kumar, School of Sciences, IFTM University. The leaves were washed under running water, shade dried [4]. For 10 days at room temperature, 95% ethanol was periodically extracted from the dried leaves. The extract was then concentrated by evaporation after being filtered through Whatman filter paper. The refrigerator was used to keep the dried extract. Weighing the crude extract, a percentage yield was computed.

Phytochemical study

It was evaporated, then diluted HCl was added, the mixture was well-shaken, and the residue was filtered. The subsequent tests were carried out with filtrate for the detection of various elements utilizing traditional protocols, namely: (A) The Mayer's, Wagner, Hagner's, and Dragendorff's test for alkaloids, (B) Foam and hemolytic tests for saponins, (C) Salkowski's test and Lieberman–Buchard test for steroids/triterpenoids, (D) Gelatin test, ferric chloride test for tannins, (E) Shinoda test for flavonoids, and (F) Molisch's test for carbohydrates [5].

Experimental animals

Wistar albino mice of either sex, weighing 40–55 g, were utilized for this experiment. They were inbred at the IFTM University's Animal House facility. The animals were housed under standard laboratory conditions, maintained on natural light and dark cycle and had free access to pellet diet and water. They were acclimatized to laboratory conditions before the experiment. Each animal was used once in every experiment and all experiments were carried out in daylight [6].

Acute toxicity study was carried out refering to the (Organization for Economic Co-Operation and Development) Guidelines No. 423 [7].

Test methods

Animals were divided into various groups such that six animals were in each group. There were animals in each group. Animals treated with 5% Gum Acacia Suspension (0.1 ml p.o.) served as the test group's control, while those treated with Diazepam (2 mg/kg i.p.) served as the standards and test with *Z. jujuba* ethanolic extract (200 mg/Kg i.p.) and (400 mg/kg i.p.), respectively. Each animal received the appropriate medication 30 min before; the following is the details of experiments performed:

Rotarod performance

Rotarod apparatus (Dolphin make) is a four panel techno device with timer. Four animals at a time were put on a rod that was rotating at a speed of 20–25 rpm. Only the mice that showed the capacity to stay on the rotating rod (20–25 rpm) for 5 min following training sessions were chosen for the investigations. Before and 30 min after the delivery of the

medicine, the fall off time was observed in all groups. Reduced fall-off time is indicative of central nervous system (CNS) depression [8].

Open field test

Open field apparatus with few modifications. Dimensions were 50 cm × 50 cm × 40 cm a 25 equal square surface made of plywood that was open on the top and bottom, with 9 core squares and 16 periphery squares. 1 h before the experiment, samples of 5% gum acacia suspension, EEAA, and diazepam were administered to the animals. During 5 min session of observation, each animal was placed in the corner of open field apparatus and behavior of animal as determined by ambulation (number of squares entered with both forelimbs), rearing, preening, and defecation was recorded [9].

Light and dark arena test

The light/dark test (LDT) is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, novel environment and light. The test apparatus consists of a small dark safe compartment (one-third) and a large illuminated aversive compartment (two-thirds). The test was developed with male mice. The strain, weight, and age may be crucial factors. The extent to which an anxiolytic compound can facilitate exploratory activity depends on the baseline level in the control group. Differences between the type and severity of external stressors might account for the variable results reported by different laboratories. The LDT may be useful to predict anxiolytic-like or anxiogenic-like activity in mice [10].

Elevated plus maze test (EPMT)

Effect of ZJE extract in EPMT

In this test, animal treated with ZJE (200 mg/kg) did not show significant effect, ZJE (400 mg/kg) shows the more significant effect, and standard drug diazepam (2 mg/kg) shows highly significantly increased time spent in open arm on EPMT compared to the control group [11]. The results are shown in (Fig. 2).

Bioactive extract was analyzed by HPTLC using marker compound Quercetin.

RESULTS AND DISCUSSION

Phytochemical screening

The ethanolic extract of *Z. jujuba* has flavonoids, terpenoids, flavonoids, alkaloids, and tannins.

Rota rod test

Effect of ZJE extract on rotarod

In this test, animal treated with ZJE (200 mg/kg) did not show significant effect, ZJE (400 mg/kg) shows the more significant effects, and diazepam (2 mg/kg) showed highly significant effect reduced time spent on revolving rod compared to the control group as the results are shown in Table 1.

LDT

Effect of ZJE in bright and dark arena test

In bright and dark chamber, animal treated with ZJE (200 mg/kg) did not show significant effect, ZJE (400 mg/kg) showed more significant effect, and standard drug diazepam (2 mg/kg) highly significantly increased the time spent in light chamber compared to the control group [12]. As the results are shown in (Fig. 1).

EPMT

In this test, it was shown that with increasing dose of *Z. jujuba* extract mouse increase time in open arm. Hence, it has anti-anxiety effect as compared to diazepam.

HPTLC profiling of extract

HPTLC is a major advancement of TLC principle with short time duration and better resolution ten compound is found by the toluene:ethylacetate:Formic acid=5.5:4.0:0.5 ratio and maximum R*f* value is (0.01).

TLC Plate Development –Pre-Saturated Camag Twin through Chambar.

HPTLC is a major advancement of TLC principle with short time duration and better resolution ten compound is found by the chloroform and methanol solvent system (8.5:1.5) ratio and maximum Rf value is (0.01). The marker compound found in leaves was quercetin.

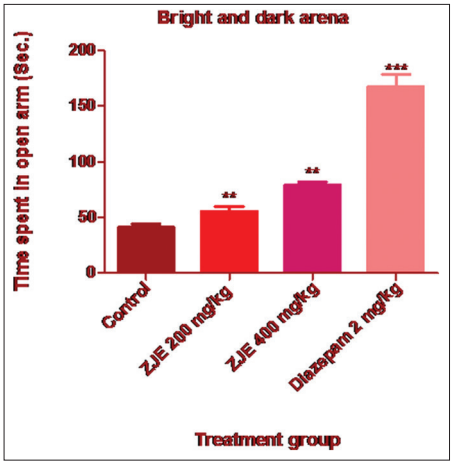

Fig. 1: Effect of ZJE in bright and dark arena test. Data were analyzed using one-way ANOVA followed by Dunnett test value, which are expressed as Mean±S.E.M. n=6, **p<0.01, ***p<0.001, (ns): Non-significant

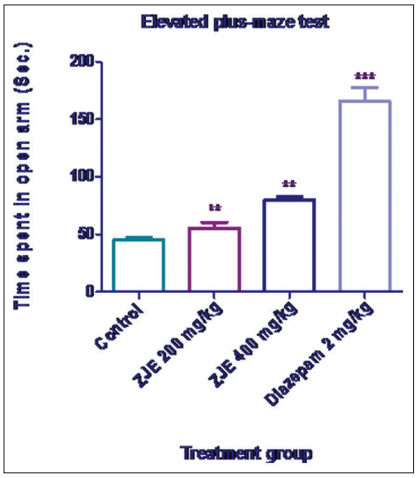

Fig. 2: Effect of ZJE extract in elevated plus-maze test Note: Data were analyzed using one-way ANOVA followed by Dunnett value, which are expressed as MEAN±S.E.M. n=6,*p<0.05, **p<0.01, ***p<0.001 when compared to the control group.

Table 1: CNS depressant activity of ZJE extract

Groups	Dose (mg/kg)	Time spent on revolving rod (In sec)
Control	Vehicle, 5 ml	18.33±1.382
Ethanolic extract *Z. jujube*	200	7.50±0.763^{ns}
Ethanolic extract *Z. jujube*	400	4.33±0.614**
Standrad (Diazepam)	2	2.000±0.258***

Data were analyzed using one-way ANOVA followed by Dunnett test value, which are expressed as MEAN±S.E.M. n=6, (ns): non-significant**p<0.01, ***p<0.001 is considered as highly significant, *Ziziphus jujube: Z. jujube*, CNS: Central nervous system

Fig. 3: HPTLC profile of *Ziziphus jujuba* leaves extract

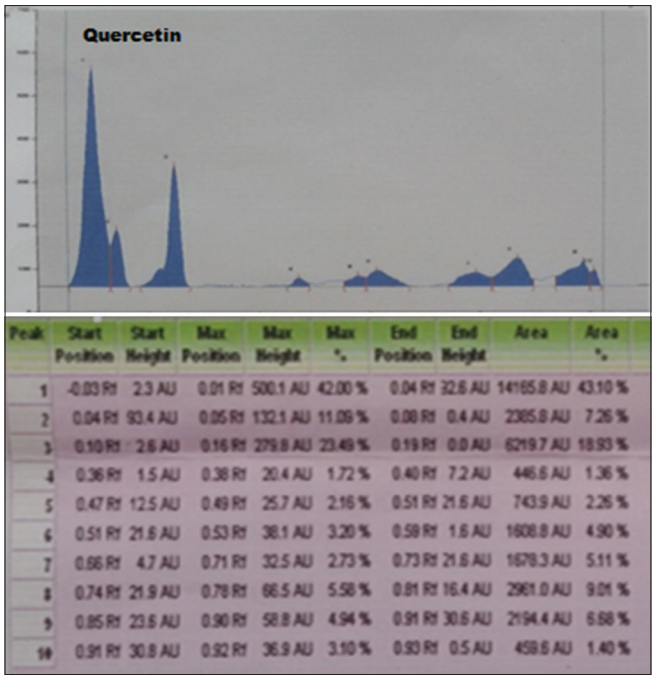

Fig. 4: HPTLC profile of *Z. jujuba* leaves extract

Table 2: Activity of ZJE extract on anxiety

Groups	Dose (mg/kg)	Time spent in each arm (in seconds)
		Open arm (OA)
Control	Vehicle, 5 ml	41.00±3.235
Ethanolic extract *Z. jujuba*	200	155.50±4.759^{ns}
Ethanolic extract *Z. jujuba*	400	179.17±2.522**
Standard (Diazepam)	2	167.2±11.42***

Data were analyzed using one-way ANOVA followed by Dunnett Test value, which are expressed as MEAN±S.E.M. n=6, **p<0.01, ***p<0.001, *Ziziphus jujube: Z. jujube*

DISCUSSION

The findings of present study suggest that ethanolic extract of *Z. jujuba* leaves was bioactive [13] as it contain various secondary metabolites such as flavonoids, alkaloids, and saponin. Study revealed CNS depressant effect of ethanolic extract of *Z. jujuba* as observed by rotarod, open field, light, and dark and EPMT [14] as compared to standard drug diazepam, so this ethanolic extract may be used in CNS-related disorders as anxiety. As HPTLC studies shows presence of quercetin, so it may be responsible for its CNS depressant effects.

AUTHORS CONTRIBUTIONS

Author has contributed in research work.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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