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Original Research Article

EVALUATION OF PSYCHOTROPIC EFFECT OF *ZIZIPHUS MAURITIANA* LEAVE EXTRACT

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Abstract: The *Ziziphus mauritiana* fruit are edible in nature and are being consumed worldwide. Researchers have isolated and identified a number of phytoconstituents within the different parts of the plant. At the same time many pharmacological values of the plant have also been reported, and these has been correlated with its phytoconstituents. The aim of present study is to evaluate *Ziziphus mauritiana* leaves for their effect on central nervous system. **Materials and Methods:** The aqueous and methanol extract was prepared and were analysed qualitatively for present phytoconstituents, followed by estimation of phenol and flavonoid contents. Subsequently; the extracts were evaluated for their effect on CNS using different preclinical screening models. **Results:** Total phenol contents in methanol and aqueous extract was found 42.13 and 13.32 (µg/mg of GAE), respectively. Total flavonoid contents were 7.25 and 4.97 (mg of rutin/100g extract) in respective extracts. The animal treated with extracts shown significant reduction in locomotion, muscle griping power, antianxiety, and antiepileptic property as compared to control group animals. **Conclusion:** The study results suggest that the leave extract of *Ziziphus mauritiana* possesses remarkable CNS depressant property. It was also postulated that the inhibition in CNS activity might be due to the present phenolic compounds; and more appropriately the flavonoid moieties.

Keywords: *Ziziphus mauritiana*, CNS depression, flavonoids, muscle relaxation

Introduction: *Ziziphus mauritiana* (family: *Rhamnaceae)* commonly also known as Indian jujube or chinee apple, is a spiny, evergreen shrub or small tree up to 15 m high, with trunk 40 cm or more in diameter. The fruit part is edible and can also be used in floury meal, candy making, pickling or as a condiment. When slightly under ripe, this fruit is bit juicy and has a pleasant aroma [Hussain *et al.*, 2011]. The leaves of *Ziziphus mauritiana* are ovate or nearly orbicular, about 3-8 cm long and 1.5-5 cm at the widest point. The apex is rounded, obtuse or sub acute to emarginated, the base

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rounded and mostly symmetrical [Azam *et al*., 2006]. The different parts of the plant have been reported presence of flavonoids, alkaloids, glycosides, saponins, resins, polyphenols, mucilage and vitamins, organic acids [Jarald *et al* ., 2009; Niamat *et al* ., 2012; Jain *et al* ., 2012]. Traditionally, the plant parts has been used as anodyne, sedative, tonic, anticancer, wound healer, antiulcer, antipyretic, mild laxative and also used in liver disorders and asthma [Perumal *et al* ., 2012; Raheman, 2012].

Materials and Methods:

Collection and authentification of plant material: The *Ziziphus mauritiana* leaves were collected from Jagatpura region of Jaipur (Rajasthan) in the month of November and were authenticated by, department of botany, University of Rajasthan. A voucher specimen viz. no. RUBL: 21186 was deposit in the herbarium, dept. of botany, University of Rajasthan, Jaipur (Rajasthan).

Extraction: The collected plant part was dried under shade, grinded manually and was passed through sieve no.40. Extraction was carried out using maceration process. 50 gm of powdered sample was kept for 72 hours with 200 ml of double distill water, and stirring was done, occasionally. The extract was filtered through whatman filter paper, and excess of solvent was evaporated. The collected brown colored mass was termed as aqueous extract of *Ziziphus mauritiana (AEZM)*. Similarly; the methanolic extract was also prepared and termed as methanolic extract of *Ziziphus mauritiana (MEZM)*. The percentage yield was 9.45 (w/w) and 8.92 (w/w) for aqueous and methanol extract, respectively.

Preliminary phytochemical screening [Agrawal, 1991; Vogel, 2004; Khandelwal, 2004]: The collected extracts were subjected to preliminary phytochemical screening for qualitative determination of phytoconstituents.

Determination of total phenol content: The total polyphenolic content of both extracts was measured by Folin- Ciocalteu reagent method [Maurya and Singh, 2010]. The absorbance was measured at 760 nm using UV spectrophotometer (Jasco V530 – UV/VIS/NIR) and was expressed as µg/mg of gallic acid equivalent.

Determination of flavonoid content: Total flavonoid content in both the extracts were estimated by aluminum chloride colorimetric assay method, and was expressed as milligrams of rutin equivalent per 100 gm of dry mass [Atanassova *et al* ., 2011]. The total phenol and flavonoid contents were found as given in table 1.

Pharmacological screening of *Ziziphus mauritiana* leave extracts:

Animals: Healthy albino rats and mice of either sex (150−200 g) were used in study. The animals were maintained under standard husbandry conditions of temperature (25 ± 2) °C, 12 h light/dark cycle in polypropylene cages and provided with standard pellet diet and water *ad libitum*. Animals were fasted overnight prior to the experiment and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

Evaluation of loco-motor activity using actophotometer: Thirty six rats were divided into 6 groups, each containing 6. Group I received normal saline at the dose of 10 ml/kg b.w. and served as control. Group II received diazepam (5 mg/kg b.w) and served as standard. Group III, IV, were treated with AEZM at the dose of 200 and 400 mg/kg b.w respectively. Group V and VI served as methanolic extract treated group at the dose of 200 and 400 mg/kg b.w respectivally. The spontaneous locomotor activity of each mouse was recorded individually by actophotometer for 10 minutes at the time interval of 30 min, 60 min, 120 min, and 180 min. All the treatments were made intraperitonially [Gangopadhyay *et al* ., 2012].

Evaluation of muscle relaxant property using rotarod: Six groups of animals containing 6 in each were created. Group I was treated with normal saline (control group). Group II was

given diazepam (3 mg/kg b.w.). Group III, IV, V and VI were treated with respective extracts as in above (loco-motor) method. The animals were placed at the horizontal rod, rotating at 25 rpm and the time to fell down for the animals from the rod was noted down. Experiment was done at the time interval of 30 min, 60 min, 120 min, and 180 min after treatments. All the treatments were made intraperitonially [Srikanth and Muralidharan, 2009; Jayasree *et al* ., 2012].

Evaluation of anti-anxiety effects using elevated plus-maze test: The grouping of animals was done as in earlier mentioned method. Group II was treated with diazepam (5 mg/kg b.w.) and served as control. All the treatments were made 30 minutes prior to placing the animal individually at the centre of the elevated plus-maze. The number of entries and average time spent (second) into open arms by the animals was recorded for 5 minutes [Anseloni *et al* ., 1997; Ronak *et al* ., 2012].

Evaluation of anti- epileptic activity:

Maximal electric shock model: The grouping of animals was done as mentioned in earlier model. Group II (standard) was treated with diazepam 5 mg/kg b.w. All other treatments were made orally. After 60 minutes of the respective treatments an electrical stimulus (150 mA for 0.2 seconds) was applied through ear-clip electrodes using electro convulsiometer. The hind limb tonic convulsion (HLTC), time to produce the convulsion and % protection was recorded [Castel *et al* ., 2009].

Chemically (Pentylenetetrazole) induced convulsion model: Thirty six healthy albino mice (weighing 25-30 gm), were randomly divided into 6 groups (n = 6 each). All the treatments were made as in maximal electric shock model. The convulsions were created in experimental animals by administrating pentylenetetrazole (80 mg/kg b. w.) I. P and observations were made for 30 minutes. The onset and duration of myoclonic jerks / hind limb tonic convulsion as well as the percentage of protection against mortality were recorded [Singh *et al.*, 2011].

Statistical analysis: Results are expressed as mean ± SEM. Statistical significance was determined using one way ANOVA followed by Dunnett's multiple comparison tests. $p < 0.05$ and $p < 0.01$ was considered significant and highly significant respectively.

Results and discussion:

Phytochemical evaluation of *Ziziphus mauritiana* leaf extracts: Preliminary phytochemical screening results indicate the presence for alkaloids, carbohydrates, saponins, phenol, flavonoids in both the extracts.

Table 1: Total phenol and flavonoid contents in AEZM and MEZM

S.NO	Extract	Total Phenol contents (µg/mg of GAE)	Total flavonoid content (mg of rutin/100g extract)
1.	Aqueous	13.32	4.97
2.	Methanolic	42.13	7.25

Pharmacological screening results:

Locomotor activity using actophotometer: The results of the locomotor activity were found as mentioned in Table 2. The study results revealed that the standard drug i.e. diazepam causes maximum inhibitory effect on locomotor activity within the experimental animals, which is a motor function of CNS. At the same time all the extracts were found to exhibit reduction in locomotion, yet it was maximum with the methanolic extract at the dose of 400 mg/kg b.w. After administration of standard drug the reduction in locomotion was observed about 92%, and with methanol extract (400) was 69% as compared to vehicle treated group.

Table 2 Effects of AEZM and MEZM on locomotor activity

Group	Dose (mg / k.g. b.w.)	Time (Min.)				
		0	30	60	120	180
I	Normal Saline (10 ml)	213±1.16	230±1.06	238±1.31	207±1.24	219±2.01
II	Diazepam (5)	205±2.02	24.5±1.08	18.75±1.36	16.25±1.11	19.75±1.01
III	AEZM (200)	208.25±2.1*	158.75±1.43*	144.25±1.27*	132.25±2.43 *	196.25±2.39 *
IV	AEZM (400)	209.25±2.09 *	132.25±2.14 *	113±1.39 *	107.75±2.16 *	172±1.21 *
V	MEZM (200)	201.75±1.10**	148.50±1.40**	109.50±1.47**	101.75±2.12**	163.75±1.35 **
VI	MEZM (400)	209±2.42 **	101±1.22 **	78.25±1.21 **	64.50±2.19 **	111±2.04 **

- Data are represented as Mean ± S.E.M, (n=6),
- *P<0.05 as compared to vehicle control, **P<0.01 as compared to vehicle control.

Rotarod test: The study results are given in table 3. The data indicates that all the treated group except the group I, increases the tendency of fall down of the animals from the rotating road, i.e. decreases the muscular griping power. With reference to the standard drug, methanolic extract at higher dose was found to exhibit maximum muscle relaxation.

Table 3. Effect of AEZM and MEZM on muscle relaxant activity by using rotarod apparatus

Group	Dose mg / k.g. b.w.	Time (Min.)				
		0	30	60	120	180
I	Normal Saline (10ml)	24.25±1.06	23.25±1.17	22.75±1.11	23.25±1.19	22.75±1.25
II	Diazepam (5)	24.25±0.98	3.5±0.58	2.50±0.37	1.75±0.19	2.50±0.48
III	AEZM (200)	23.25±1.21*	17.50±1.18*	15.50±1.29*	13.50±1.29 *	15.50±1.03*
IV	AEZM (400)	23±1.12 *	16.75±1.32 *	13±1.16*	12.75±0.95 *	14.50±1.38 *
V	MEZM (200)	24.25±1.00 **	16±1.13 **	13±0.81 **	12.15±0.97**	13.50±1.01**
VI	MEZM(400)	24.25±1.22**	12.25±0.95 **	10.25±0.95**	9.75±0.95 **	12.25±1.25**

- Data are represented as Mean ± S.E.M, (n=6),
- *P<0.05 as compared to vehicle control, **P<0.01 as compared to vehicle control.

Assessment of anti anxiety activity: Elevated plus maze in used to check the degree of anxiety in the animals. The animals treated with diazepam and both the extracts causes increased mean number of entries and mean time spent in open arms of elevated plus maze apparatus with respect to control, thereby producing anti-anxiety activity. The higher dose of methanol extract (400 mg/kg) causes maximum response. The study results were as depicted in table 4.

Table 4. Anti anxiety activity of AEZM and MEZM on rat by using elevated plus-maze model

Group	Dose (mg / k.g. b.w.)	Time spent (Sec.) in Open arm	No. of entries in Open arm
I	Normal Saline(5 ml)	19.50±1.21	3.50±0.57
II	Diazepam (5)	57.25± 1.12	17.25± 1.06
III	AEZM (200)	24.25±1.11*	5.32 ±0.49 *
IV	AEZM (400)	29.13±0.82 *	9.12 ±0.32 *
V	MEZM (200)	30.00±1.02 **	10.31 ±0.75 **
VI	MEZM (400)	38.48 ±0.81**	13.42 ±0.82 **

- Data are represented as Mean ± S.E.M, (n=6),
- *P<0.05 as compared to vehicle control, **P<0.01 as compared to vehicle control.

Assessment of anticonvulsant activity:

Effect of extracts on MES induced convulsion model: In MES induced convulsion method the animals treated with vehicle causes tonic convulsion 22.50±1.29 s, which exhibit upto 72.50±4.50 s. All the animals from this group showed 100 % mortality. The methanolic extract was found to produce better CNS depressant activity as compared to aqueous extract. Yet the methanol extract produced more prominent effect at higher dose. The methanolic extract also showed protection in about 68 % treated animals. (Table 5)

Pentylenetetrazole (PTZ) induced convulsion: In pentylenetetrazole-induced seizures, animals treated with vehicle, clonic convulsion appeared 54.75±1.70 s after PTZ administration and all animals died after seizures. *Ziziphus mauritiana* extracts significantly inhibited the onset and incidence of convulsions. The onset of convulsions in methanolic extract (400) treated group, was 87.25±1.25 s, which was about 78 % effective as compared to standard drug. At the same time % recovery of PTZ treated animals was also 62. (Table 6)

Table5. Anticonvulsant effect of *Ziziphus mauritiana* on MES -induced convulsions in animals

Group	Dose (mg / k. g., b.w.)	HLTC (sec)	Total duration (sec)	% protection
I	Normal Saline (5 ml)	22.50 ± 1.23	72.50 ± 2.09	-
II	Diazepam (5)	3.75±0.95	12 ± 1.01	83.44
III	AEZM(200)	16.50 ± 1.33*	39.50 ± 1.89 *	52.41
IV	AEZM(400)	14.12 ± 0.81 *	32.75 ± 1.25 *	54.82
V	MEZM(200)	13.21 ± 0.94 **	30.75 ± 1.07 **	56.93
VI	MEZM(400)	9.25 ± 0.67 **	19.25 ± 1.21 **	67.93

- Data are represented as Mean ± S.E.M, (n=6),
- *P<0.05 as compared to vehicle control, **P<0.01 as compared to vehicle control.

Table 6. Anticonvulsant effect of *Ziziphus mauritiana* on PTZ -induced convulsions in animals

Group	Dose (mg / k. g., b.w.)	Onset of convulsion (sec)	Total duration (sec)	HTLC (sec)	Protection (%)
I	Normal Saline (5 ml)	54.75±1.70	142.50±2.19	11.25±1.11	-
II	Diazepam (5)	111.75±1.82	24.40±1.15	2.25±0.50	82.87
III	AEZM (200)	62 ±1.12 *	98.25±1.99 *	9.12±0.81	36.92
IV	AEZM (400)	68.25±1.26*	84.75±1.30 *	8.31±0.92	48.50
V	MEZM (200)	71.25±1.22 **	78.75±1.41**	7.25±0.68	51.91
VI	MEZM (400)	87.25±1.08**	58.75±0.95 **	4.50±0.64	62.45

- Data are represented as Mean ± S.E.M, (n=6),
- *P<0.05 as compared to vehicle control, **P<0.01 as compared to vehicle control.

Conclusion: In the present research work, effects of methanol and water extracts of *Ziziphus mauritiana* was studied using many pharmacological models including, actophotometer for determination of locomotor activity, rotarod test for muscle relaxant activity, plus elevated maze test for anxiety and antiepileptic test against electrical and chemical induced convulsions. The result data of different screening methods revealed that both the extracts i. e. aqueous and methanol are significantly causing CNS depressant effect. Locomotor activity is directly correlated with alertness, and decrease in locomotor activity indicated sedative effect [Verma *et al.*, 2010]. Animals treated with extracts of *Ziziphus mauritiana* shows decreased locomotor activity, which indicate its CNS depressant potential. At the same time decreased griping power at rotarod, increased spent time by the animals at

open arm and prolongation of convulsion onset time, strongly established the muscle relaxing, anti anxiety and antiepileptic potential of the plant extract respectively.

Gamma-amino-butyric acid (GABA) is major inhibitory neurotransmitter of the central nervous system. It also have been proved that the CNS depressant activity of various drugs is through the GABA-A, therefore it is possible that extracts of *Ziziphus mauritiana* may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads decrease in firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts [Kolawole *et al* ., 2007].

The plant containing flavonoids, saponins and tannins have been found effective in CNS disorders [Bhattacharya and Satyan, 1997]. Phytochemical screening of the *Ziziphus mauritiana* extracts also showed the presence of alkaloids, flavonoids, saponins and steroids in the plant. From the study results it may be strongly quoted that the CNS depressant activity of the plant may be due to present flavonoid moieties. The previously reported data also support the conclusion. At the same time it may also be concluded that the higher CNS depressant property of the methanolic extract of the leaves as compared to aqueous extract is due to present higher concentration of the flavonoid contents. Still; further preclinical as well as clinical work is required to establish proper mechanism of action at molecular level, and to prepare suitable dosage form.

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