academicJournals

Vol. 10(40), pp. 849-853, 29 October, 2016
DOI: 10.5897/AJPP2015.4423
Article Number: 27DF36B61419
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Analgesic and central nervous system depressant activities of methanol extract of *Ziziphus rugosa* Lam. Leaves

Israt Jahan Bulbul[1], Mohammad Firoz Khan[2] and Mohammad A. Rashid[3]*

[1]Department of Pharmacy, Southeast University, Dhaka-1213, Bangladesh.
[2]Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh.
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

Received 6 August, 2015; Accepted 3 September, 2015

The present investigation was designed to evaluate the analgesic and central nervous system (CNS) depressant activities of the methanolic extract of *Ziziphus rugosa* Lam. in rat model. The analgesic activity of the extract was examined using acetic acid-induced writhing test (chemically induced pain) at the doses of 200 and 300 mg/kg body weight. Further, the CNS depressant activity of the plant extract was evaluated by open field and hole cross tests at the doses of 300 and 500 mg/kg body weight. In the analgesic activity test, the methanolic extract of *Z. rugosa* showed significant ($p < 0.01$) antinociceptive activity in a dose dependent manner. The extract at the dose of 300 mg/kg exhibited 51.87% inhibition of writhing response which was comparable to the reference drug Indomethacin (55.20%). On the other hand, the plant extract also demonstrated significant ($p < 0.01$) dose-dependent reduction of locomotor and exploratory activities in the open field and hole cross tests. This analgesic and CNS-depressant activity of the extract might be due to the presence of biologically important chemical compounds.

Key words: Central nervous system (CNS)-depressant, antinociceptive, hole cross tests, writhing, locomotor activity.

INTRODUCTION

Pain is the part of a defensive response against dysfunction of an organ or imbalance in its functions towards potentially dangerous stimulus. Pain management has become the focus of global scientific research because of its inference in virtually all human and animal diseases (Sarker et al., 2012). Many drugs are used to relieve the pain and most of them produce some side-effects on the physiology of the body (Devraj and Karpagam, 2011). As a result, searching of new analgesic drugs from traditionally used plant species as pain killers should still is a rational strategy. The plant *Ziziphus rugosa* Lam. is a large straggling armed shrub with large elliptic usually subcordate leaves and belongs to the family Rhamnaceae. The flowers are paniculated and fruits are small drupe, glabrous and become white when ripe. Traditionally bark is used as astringent and antidiarrhoeal while flowers are used for menorrhagia and hemorrhage. Stem and fruit are hypotensive. Fruit is used

*Corresponding author. E-mail: rashidma@du.ac.bd.

in the treatment of rheumatism and hypotension. The root bark of the plant have been reported to exhibit analgesic, anti-inflammatory, antibacterial and antifungal activities (Abhimany and Pratiksha, 2010; Rambabu et al., 2011). The seed extract has been reported for anxiolytic and cytotoxic properties (Prashith et al., 2011; Gawande et al., 2011). Cytotoxicity, antimicrobial and antioxidant properties (Sazzad et al., 2013) have also been reported from leaves. The fruit pericarp is known to have antibacterial, insecticidal and free radical scavenging properties (Kekuda et al., 2011). Rugosanine-A, a cyclopeptide alkaloid (Pandey et al., 1988) and three flavonoids - kaempferol-4'-methylether, luteolin and luteolin-7-O-glucoside (Singh et al., 2009) have been isolated from the stem bark of the plant.

A new glycoside zizyphoside has been isolated along with betulic, oleanolic, alphitolic and 2-α-hydroxyrusolic acids; zizyphoside on hydrolysis yielded altered aglycone, ebelin lactone (Bakshi et al., 1999). Due to the absence of report of the *Ziziphus rugosa* leaves on central nervous system (CNS) activity, an effort has been made as part of our regular research program (Rahman et al., 2008; Ferdous et. al., 2012; Khan et al., 2015) to scientifically evaluate the CNS depressant and analgesic effects of methanolic extract of *Z. rugosa* leaves in model rat.

MATERIALS AND METHODS

Plant

The leaves of plant Z. *rugosa* were collected from Gazipur in the month of March, 2011 and identified in the Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. DACB-34479) has been mentioned for future reference.

Preparation of extract

The leaves were first washed with running water to remove adhering dirts, then dried in an electric oven at 45°C and powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The powdered material (1 kg) was taken in a clean, flat bottomed glass container and soaked in methanol for seven days. The whole mixture was then filtered through a piece of clean, white cotton bed followed by Whatman filter paper number 1. The total filtrate was concentrated, *in vacuo* at 40°C to render brownish red colored mass (390 g).

Drugs and chemicals

The active drugs Indomethacin and Diazepam were obtained from Square Pharmaceuticals Ltd., Bangladesh. Acetic acid was obtained from Merck, Germany. Tween-80 was procured from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. All chemicals used were of analytical reagent grade.

Animals

Long-Evan's rats of either sex weighing about 120 to 170 g were used for the experiment. The rats were purchased from the Animal Resources Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 24.0 ± 0°C temperature, 55-65% relative humidity and 12 h light/12 h dark cycle) for one week for acclimation after their purchase and were fed ICDDR, B formulated rodent food and water *ad libitum*. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines and recommendations were followed to reduce the pain and stress of the experimental rats and were approved by the institutional animal ethical committee.

Acute toxicity study

The median lethal dose (LD_{50}) of the extract in rat was estimated by the up and down method (Bruce, 1985). Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.

In vivo analgesic activity

The analgesic activity of the methanolic extract of Z. *rugosa* leaves was evaluated using acetic acid-induced writhing method in rat (Sharma et al., 2010). At first, twenty four animals were divided into four groups with six rats in each.

Group I: Treated with vehicle (1% Tween 80 in water, 10 ml/kg body weight p.o.)
Group II: Received Indomethacin (10mg/kg) body weight (p.o.)
Group III: Treated with 200 mg/kg body weight (p.o.) of the extract
Group IV: Treated with 300 mg/kg body weight (p.o.) of the extract.

The test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% v/v acetic acid but Indomethacin (reference drug) was administered orally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min. Complete writhing was not always accomplished by the animal; this incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhing in each treated groups was compared to that of a control group. Samples having analgesic activity will reduce number of writhes of treated rats. The percent inhibition (% analgesic activity) was calculated by:

$$\% \text{ inhibition} = \frac{A-B}{A} \times 100$$

Where A = average number of writhing of control group; B = average number of writhing of test group.

CNS depressant activity

Open field test

The open field test was carried out to evaluate the effect of extract on locomotor activity of rats by the method described by Gupta et al. (1971). Here, rats were divided in to 4 groups of 6 rats each. The control group received 1% Tween 80 in water (10 ml/kg body weight), the standard group received Diazepam (1 mg/kg body weight) and the experimental groups received crude extract at 300 and 500 mg/kg body weight. The floor of an open field was divided into a series of squares each alternatively colored black and white. The apparatus had area of half square meter and a wall of 40 cm height. The number of squares visited by the animals was counted

Table 1. Analgesic effect of methanolic extract of *Z. rugosa* leaves in **acetic acid-induced writhing test.**

Group	Dose (mg/kg b.w.)	No. of writhing	Percent of inhibition (%)
1% Tween 80 in water (Control)	10 ml/kg	15±.894	
Indomethacin (Standard drug)	10	6.75±.524**	55.20
Leaves extract of Z. *rugosa*	200	9.92±.585*	33.87
	300	7.22±.785**	51.87

All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P < 0.05, ** P <0.01, significant compared to control.

Table 2. CNS depressant activity of methanol extract of *Z. rugosa* leaves in open field test in rats.

Treatment	Doses	No. of movement				
		0 min	30 min	60 min	90 min	120 min
1% Tween 80 in water (Control)	10 ml/kg	118.4±2.70	118±2.91	115.4±1.14	117.4±2.61	118±1.58
Diazepam (Standard drug)	1 mg/kg	117.2±2.59	77.6±2.21**	41.8±2.30**	21.8±1.32**	11.6±1.04**
Leaves extract of Z. *rugosa*	300 mg/kg	118.2±1.92	80.8±2.32**	53.2±0.48**	37.4±1.08**	22.6±2.42**
	500 mg/kg	117.3±2.09	66±1.53**	41±1.10**	19.4±0.14**	12.4±2.00**

All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. **P < 0.01, significant compared to control.

for 3 minute, on 0, 30, 60 and 120 min during the study period.

Hole cross test

The method described by Takagi et al. (1971) was implemented for this study. Again 24 rats were equally divided into 4 groups. The control group received 1% Tween 80 in water (10 ml/kg body weight), the standard group received Diazepam (1 mg/kg body weight) and the experimental groups received crude extract at 300 and 500 mg/kg body weight. A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the partition. The number of passages of rats through the hole from one chamber to other was counted for a period of 3 minute after 0, 30, 60 and 120 min of oral administration of the extract.

Statistical analysis

All the values in the test are expressed as mean ± standard deviation (SD). The data were statistically analyzed by ANOVA (Analysis of variance) and post-hoc Dunnett's tests with the statistical package for social sciences (SPSS 16.0, USA) program. Dissimilarity between the means of the various groups were measured significantly at $p < 0.05$ and $p < 0.01$.

RESULTS

Acute toxicity

Oral administration of graded doses of the methanol extract of *Z. rugosa* leaves (500 to 5000 mg/kg, body weight) did not cause any death in different dose groups. The LD_{50} value for oral administration of the plant extract was found to be greater than 5000 mg/kg body weight.

In vivo analgesic activity

In acetic acid induced writhing test, the methanol extract of *Z. rugosa* significantly ($p < 0.05$ and $p < 0.01$) reduced the number of writhing movements induced by intraperitoneal administration of acetic acid solution. The dose-dependent inhibition (Table 1) of abdominal constrictions by the methanol extract indicates anti-nociceptive potential of the plant. The exerted inhibition of writhing at a dose 300 mg/kg body weight was close to the standard non-narcotic analgesic drug, Indomethacin.

CNS Depressant Activity

Open field test

The extract was evaluated by open field test to determine the decreasing capability of CNS-locomotor activity in rat model. The extract significantly decreased the locomotor activity in a dose dependant manner (Table 2) and this effect was evident from the initial observation (0 min) period and continued up to 5th observation period (120 min).

Table 3. CNS depressant effect of methanol extract of *Z. rugosa* leaves on hole cross test in rats.

Treatment	Doses	No. of movement				
		0 min	30 min	60 min	90 min	120 min
1% Tween 80 in water (Control)	10 ml/kg	13.67±1.37	14±1.09	13.33±1.03	11.33±1.86	9.17±1.60
Diazepam (Standard drug)	1 mg/kg	10.67±1.03*	5.83±1.33**	5.171±1.52**	4.5±1.52**	3.33±1.03**
Leaves extract of Z. *rugosa*	300 mg/kg	13.83±1.47	11.5±1.05	9.67±0.816**	8.17±1.47**	7.67±1.03**
	500 mg/kg	11±1.26	9.17±2.56*	7.33±2.42**	5.83±1.72**	3.83±1.17**

All values are expressed as mean ± SD, (n=6); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P < 0.05, ** P <0.01, significant compared to control.

Hole cross test

In this test, the extract showed a decrease in locomotion in the test animals. The number of crossing hole from one chamber to another by rat of the control group remained almost steady to slight decrease from 0 minute to 120 minute (Table 3). But the extract at 300 and 500 mg/kg dose showed significant (indicate p-value) gradual decrease of movement from 0 to 120 min. The extract displayed dose dependent activity and maximum depressive effect was observed at fifth (120 min) observation period. Depression produced at 500 mg/kg body weight was found to be close to that of standard drug, Diazepam.

DISCUSSION

The present study was conducted to elucidate analgesic and CNS depressant activities of the methanol extract of *Z. rugosa* leaves. The relatively high oral median lethal dose (LD_{50}) in rat suggests that the extract is relatively non toxic when taken orally (Lorke, 1983; Magaji et al., 2008). Acetic acid-induced abdominal constriction test is used for the evaluation of pheripheral analgesic activity (Gene et al., 1998; Faruk et al., 2015). The extract showed significant analgesic activity in acetic acid-induced writhing test in rats. This indicates that the extract possessed peripheral mediated analgesic activity. The abdominal constriction response is thought to involve, in part, the local peritoneal receptors (Bentley et al., 1983; Sani et al., 2013), so the extract may have interfered with these peritoneal receptors to bring analgesia. Acetic acid-induced writhing test has been associated with increase in the levels of prostaglandins E2 and F2α in peritoneal fluid (Deradt et al., 1980) as well as lipooxygenases (Levini et al., 1984; Sani et al., 2013). So the mechanism of activity of the extract may be linked to cyclooxygenases and/or lipooxygenases.

CNS depressing agents are gaining importance in treating mental disorders like anxiety, dizziness and restlessness. So, we carried out locomotion test to confirm the CNS activity of the methanol extract of the plant. Increased locomotor activity (by excitatory neurotransmitter) is considered as alertness while decreased locomotor activity (due to inhibitory neurotransmitter) indicates sedative effect (Verma et al., 2010). Our extract had decreased locomotor activity indicating its CNS depressant activity. The CNS depressant activity is observed when the GABAA receptor is activated either by Gamma-amino-butyric acid (GABA) or any other chemical substances. Different anxiolytic, muscle relaxant and sedative-hypnotic drugs exert their action through GABAA receptor. Therefore it is possible that the extract of *Z. rugosa* leaves may act either by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization or may be due to direct activation of GABA receptor (Kolawole et al., 2007). Many research results showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders and many flavonoids and neuroactive steroids were ligand capable for the GABAA receptors in the central nervous system. This led to the assumption that they can act as benzodiazepine like molecules (Bhattacharya and Satyan, 1997).

Conclusion

Based on the results of the present study, we conclude that the methanolic extract of *Z. rugosa* leaves possesses remarkable dose dependent analgesic and CNS depressant activity. However, further studies are indispensable to examine the underlying mechanisms of such CNS effects and to isolate the active compounds responsible for these pharmacological activities.

Conflict of interest

The authors have not declared any conflict of interest

REFERENCES

Abhimany Y, Pratiksha S (2010). Analgesic and anti-inflammatory activities of *Zizyphus rugosa* root barks. J. Chem. Pharmaceu. Res. 2: 255-259.

Bhattacharya SK, Satyan KS (1997). Experimental methods for evaluation of psychotropic agents in rodents: I--Anti-anxiety agents. Indian. J. Exp. Biol. 35: 565-575.
Bruce RD (1985). An up- and down procedure for acute toxicity testing. Fundam. Appl. Toxicol. 5: 151-157.
Devraj A, Karpagam (2011). Evaluation of anti-inflammatory activity and analgesic effect of *Aloe vera* leaf extract in rats. Int. Res. J. Pharma. 2(3):103-110.
Faruk MA, Khan MF, Mian MY, Rahman MS, Rashid MA (2015). Analgesic and anti-diarrheal activities of *Aganosma dichotoma* (Roth) K. Schum. in swiss-albino mice model. Bang. Pharm. J. 18(1): 15-19.
Gawande RK, Tare HL, Shende VS, Bongirwar AA, Deore SR, Dama GY (2011). Anxiolytic and CNS depressant activity of extracts obtained from seeds of *Ziziphus rugosa*. Int. J. Cur. Pharmaceu. Clin. Res. 1:21-32.
Gene RM, Segura L, Adzet T, Marin E, Inglesias J (1998). Heterotheca inuloides: anti-inflammatory and analgesic effects. J. Ethnopharmacol. 60: 157-162.
Bakshi GDN, Sensarma P, Pal DC (1999). A lexicon of medicinal plants in india. 1st Edition, Kolkata, India Naya Prokash. 1: 205-206.
Bentley GA, Newton SH, Starr J (1983). Studies on the anti-nociceptive action of agonist drugs and their interaction with opiod mechanisms. Br. J. Pharmacol. 32: 295-310.
Deradt R, Jougney S, Delevalcee F, Falhout M (1980). Release of prostaglandin E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 51:17-24.
Ferdous MR, Rashid RB, Sikder MA, Aktar F, Rashid MA (2012). Preliminary in vitro biological and phytochemical screenings of *Parmentiera cereifera* Seem. Bang Pharm. J. 15(2): 103-106.
Gupta BD, Dandiya PC, Gupta ML (1971). A psychopharmacological analysis of behavior in rat. Jpn. J. Pharmacol. 21:293-298.
Khan MF, Khan ZI, Uddin MR, Rahman MS, Rashid MA (2015). n vivo hypoglycemic and alloxan induced antidiabetic activity of *Xeromphis uliginosa* Retz. Afr. J. Pharm. Pharmacol. 9(11): 363-366.
Kekuda TR, Vinayaka KS, Mallikarjun N, Bharath AC, Shailendra B, Rakesh MC, Vinod HR (2011). Antibacterial, insecticidal and free radical scavenging activity of methanol extract of *Ziziphus rugosa* Lam. fruit pericarp. Pharmacog. J. 2: 65-69.
Kolawole OT, Makinde JM, Olajid OA (2007). Central nervous depressant activity of *Russelia equisetiformis*. Nig. J. Physiol. Sci. 22: 59-63.
Lorke D (1983). A new approach to acute toxicity testing. Arch. Toxicol. 54:275-287.
Magaji MG, Anuka JA, Abdu-Aguye I, Yaro AH, Hussaini IM (2008). Preliminary studies on anti-inflammatory and analgesic activities of *Securinega virosa* (Euphorbiaceae) in experimental animal models. J. Med. Plant. Res. 2(2):039-044.
Pandey VB, Tripathi YC, Devi S, Singh JP, Shah AH (1988). A cyclopeptide alkaloid from the bark of *Zizyphus rugosa*. Phytochem. 27(6):1915-1918.
Prashith Kekuda TR, Raghavendra HL, Vinayaka KS (2011). Evaluation of pericarp and seed extract of *Zizyphus rugosa* Lam. for cytotoxic activity. IJPBA. 2:887-890.
Rahman MS, Begum B, Chowdhury R, Rahman KM, Rashid MA (2008). Preliminary cytotoxicity screening of some medicinal plants of Bangladesh. Dhaka Univ. J. Pharm. Sci. 7(1):47-52.
Rambabu P, Venkata Ramana K, Ganapaty S (2011). Anti bacterial and anti fungal potential of roots of *Zizyphus rugosa*. IJPRD, 3: 85-93.
Sani YM, Musa AM, Yaro AH, Amoley A, Magaji MG (2013). Phytochemical screening and evaluation of analgesic and anti-inflammatory activities of the methanol leaf extract of Cissus polyantha. J. Med. Sci. 13(8): 824-828.
Sarker M, Das SC, Saha SK, Mahmud ZA, Bachar SC (2012). Analgesic and anti-inflammatory activities of flower extracts of *Punica granatum* Linn. (Punicaceae). J. Appl. Pharma. Sci. 2(4): 133-136.
Sharma A, Bhatial S, Kharyaz MD, Gajbhiye V, Ganesh N, Namdeo AG (2010). Anti-inflammatory and analgesic activity of different fractions of *Boswellia seratta*. Int. J. Phytomed. 2:94-99.
Singh A, Pandey MB, Singh S, Singh AK, Singh JP (2009). Flavonoids of *Zizyphus rugosa*. J. Indian. Chem. Soc. 86(2):177-178.
Takagi K, Watanabe M, Saito H (1971). Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethane, its acylates on the central nervous system. Jpn. J. Pharmacol. 21:797-810.
Verma A, Jana GK, Sen S, Chakraborty R, Sachan S, Mishra A (2010). Pharmacological evaluation of *Saraca indica* leaves for central nervous system depressant activity in mice. J. Pharm. Sci. Res. 2(6):338-343.