American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Neuropharmacological, Analgesic, Antidiarrheal and Antimicrobial Activities of Methanolic Extract of *Ziziphus mauritiana* Leaves (Rhamnaceae)

Md. Hamiduzzaman*[1], A.S.M. Moniruzzaman Sarkar[2], Mohammad Jamal Hossain[2], and Abdur Rashid[2]

[1]Dept. of Pharmacy, ASA University, Bangladesh
[2]Department of Pharmaceutical Chemistry, Dhaka University, Bangladesh

Date of Receipt- 17/02/2014
Date of Revision- 20/02/2014
Date of Acceptance- 25/02/2014

Address for Correspondence
Lecturer, Dept. of Pharmacy, ASA University, Bangladesh

E-mail:
hamid018@yahoo.com

ABSTRACT

The purpose of the present study was to evaluate the neuropharmacological, analgesic, antidiarrheal and antimicrobial activity of methanolic crude extract of *Ziziphus mauritiana* leaves in mice model. After collection of leaves it was washed, sun dried and made coarse powder. It was soaked in methanol for several days and extracted at room temperature. Dried methanolic extract was partitioned into pet ether, carbon tetrachloride, chloroform and aqueous soluble fractions. Among all the fractions, methanolic extract at a dose of 200 and 400 mg/kg body weight revealed 27.6 and 29.6 minutes of onset of sleeping; 79 and 89.8 minutes of total sleeping time where control group showed 15.8 minutes of onset of sleeping and 118.6 minutes of total sleeping time. Besides crude extract at a dose of 400 mg/kg body weight significantly inhibited the pain sensation at 48.55%, 57.77% and 61.44% after 30, 60 and 90 minutes with respect to standard morphine, revealed antidiarrheal activity by reducing 52.02% of diarrhea comparing with standard drug loperamide (50 mg/kg body wt) having 67.24% of reduction of diarrhea and crude extract and its different fractions inhibited the bacterial growth ranging from 6.5 to 18.8 mm against gram positive bacteria, 6.2 to 17.9 mm against gram negative bacteria and 7.4 to 14.7 mm against fungi compared with standard ciprofloxacin.

Keywords: *Ziziphus mauritiana*, Neuropharmacological activity, Analgesic activity, Antidiarrheal activity and Antimicrobial activity.

INTRODUCTION

Folk medicinal practices are very common in Bangladesh. Besides herbal medicine practice is also increasing day by day due to fewer side effects. Bangladesh is a good source for medicinal plants which is providing a reliable source of medicinally important secondary metabolites. Based on different traditional uses, one of the plant species of Rhamnaceae family was undertaken to evaluate different biological properties in laboratory. *Ziziphus mauritiana*, also known as Kul or Boroi in Bangladesh, Chinee Apple, Jujube, Indian plum and Masau is a tropical fruit tree species belonging to the family Rhamnaceae. It is a common plant in our country. Extensive investigation showed that this species revealed important biological activities such as antioxidant activity[1], antimicrobial activity, anti-inflammatory activity[2], anxiolytic property[3], antidiabetic activity[4].

MATERIALS AND METHODS

Collection of the plant sample

Leaves of *Ziziphus mauritiana* were collected from Gazipur district, Dhaka, Bangladesh in February, 2013. This plant was identified by botanists of the Botany Department of Dhaka University. The reference sample for the plant was DUSH, Accession Number 4257 and calls no 01.

Swiss albino mice of either sex, aged 4-5 weeks were the experimental animal and were obtained from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). Institution of Animal Ethical Committee which was maintained by Faculty of Biological Science, University of Dhaka gave approval for this project to collect and utilize Swiss albino mice as experimental animal. The approval reference number was Ref-DU/BD/IACE-A143. They were kept in standard environmental condition and fed ICDDR, B formulated rodent food and water. Experimental animals were collected, handled and kept by following standard protocol based on the ethical committee of our university.

Preparation of the plant material

After collection of leaves it was washed, sun dried and made coarse powder. Around 1 kg of powdered material was soaked in 2.5 litter of methanol for several days with occasional stirring. It was then extracted at room temperature and concentrated by evaporator. About 5 gm of dried extract was partitioned into pet ether, chloroform, carbon tetrachloride and aqueous soluble fractions by standard protocol by modified kupkan partitioning method[5]. The practical yield values of pet ether were 1.85 gm, chloroform 0.85 gm, carbon tetrachloride 1.07 gm and aqueous soluble fraction 0.65 gm respectively.

Evaluation of neuropharmacological activity

Methanolic crude extract of *Z. mauritiana* leaves was subjected for evaluation of neuropharmacological activity by phenobarbitone induced sleeping time test[6]. The study was carried out using Swiss albino mice (25-30 g) of either sex. Fifteen experimental animals were randomly selected and divided into three groups denoted as group-I (control group), group-II (A) and group-II (B) as experimental group consisting of 5 mice in each. The experimental groups were administered with test samples prepared with normal saline water and tween-80 at doses of 200 and 400 mg/kg body weight, while the control group was administered normal saline water containing 1% tween-80 solution. Thirty minutes later, phenobarbitone sodium (25 mg/kg body weight) was administered intraperitonially to all the groups

to induce sleep. The onset of sleep and total sleeping time were recorded for both control and experimental groups.

Preparation of the test samples

In order to administer the methanolic crude extract at doses of 200 and 400 mg/kg body wt of mice, 120 and 60 mg of dried extract were measured respectively and triturated unidirectionally by vortex mixture, adding of 2 to 3 drops of Tween-80 (a suspending agent) and 50-100 µl of DMSO. After proper mixing of extract, additional suspending agent, DMSO and normal saline were slowly added to make the final volume of the suspension up to 3.0 ml. Dose for crude extract was simulated with standard drug phenobarbitone (25 mg/kg body weight). They were 200 and 400 mg/kg body weight adjusted as 4 times and 8 times higher dose with respective to standard drug. For the preparation of phenobarbitone sodium (obtained from incepta Pharmaceuticals Ltd., Bangladesh) at the dose of 25 mg/kg-body weight, the supplied phenobarbitone injection (200 mg/ml) was diluted to 10 ml with saline water. 1 ml of the diluted solution was taken and was again diluted to 10ml. Thus 0.375 ml of solution contains 0.75 mg of phenobarbitone sodium. Table 1

Evaluation of analgesic activity

Methanolic crude extract of *Z. mauritiana* leaves was subjected for evaluation of analgesic activity by following radiant heat tail-flick method[7] using Swiss albino mice and analgesiometer. Following this method, basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) is taken as the end point. A cut off period of 15 second is observed to avoid damage to the tail. The measurements of withdrawal time using the tail flick apparatus was conducted at 30 min, 60 min and 90 min after administration of drugs. Group-I was served as normal control group and received 1% Tween-80 in saline water, group-II served as reference group and received morphine (10 mg/kg body weight, intraperitonially), group-III, and group-IV served as experimental groups and received crude extracts at a dose of 200 and 400 mg/kg body weight. The pain inhibition percentage (PIP) was calculated according to the following formula[8]:

Percent of pain inhibition (PIP) = $((T_1-T_0)/T_0) \times 100$

Where, T_1 is post-drug latency and T_0 is pre-drug latency.

Preparation of the test samples

In order to administer the crude extract at doses of 200 and 400 mg/kg body weight of mice, 50 and 100 mg of the dried extract were measured respectively and were triturated unidirectional way by the addition of small amount of suspending agents Tween-80. After proper mixing of extract and suspending agent, normal saline was slowly added and made 2.5 ml. Water for injection was added with morphine to dilute it so that 0.3 ml of the diluted solution will have 10 mg /kg body weight of morphine. Table 2

Evaluation of anti-diarrheal activity

The anti-diarrheal activity of the methanolic crude extract of *Z. mauritiana* leaves (at a dose of 200 and 400 mg/kg body wt) was evaluated by castor oil induced diarrhea in mice[9]. According to this model each mice was fed 1 ml of highly pure analytical grade castor oil to induce diarrhea. Twenty mice were taken and divided into four groups (Group I, Group II, Group III and Group IV). Group I was used as negative control, while Group II served as the positive control or standard group treated with Loperamide. Group III and Group IV were the test groups. Each mouse was fed with the test samples. Then thirty minutes later they

were given 1 ml of castor oil to induce diarrhea. The mice were kept under observation for the next four hours. For each mouse the number of times it defecated was recorded. The observation of the experimental groups was compared with the positive control to evaluate the anti-diarrheal activity of the samples.

% Reduction of number of defecation = [(NDC - NDT)/NDC] x 100

Where, NDC= Mean number of defecation of control group and NDT = Mean number of defecation of experimental group.

Evaluation of antimicrobial activity

Methanolic crude extract of *Z. mauritiana* leaves and its different fractions were subjected for the evaluation of antimicrobial activity against 5 gram positive, 7 gram negative and 3 fungi by following standard disc diffusion method (Table 3). In this classical method, antibiotics diffuse from a confined source through the nutrient agar media and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples at a dose of 400µg/disc were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (ciprofloxacin at a dose of 30 µg/disc) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media[10]. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter.

RESULTS AND DISCUSSIONS

Neuropharmacological activity

The methanolic crude extracts of *Z. mauritiana* leaves potentate the phenobarbitone induced sleeping time in a dose dependent manner. Methanolic extract at a dose of 200 and 400 mg/kg body weight revealed 27.6 and 29.6 minutes of onset of sleeping; 79 and 89.8 minutes of total sleeping time where control group showed 15.8 minutes of onset of sleeping and 118.6 minutes of total sleeping time. Table 4

Methanolic crude extract of *Z. mauritiana* leaves induced the sleeping time i.e. hypnotic effect induced by the phenobarbitone sodium in a dose dependent manner which suggests a profile of sedative activity. Crude extract probably possesses benzodiazepines and related compounds that bind to the receptors in the CNS to stimulate the sedative effect recorded here. This experimental findings from the study showed that the crude extract of *Z. mauritiana* leaves have moderate sedative activity in mice which suggests its central depressant activity.

Analgesic activity

Analgesic activity of crude methanolic leaves extract of *Z. mauritiana* was evaluated by following radiant heat tail-flick method. The crude extract effectively elongates the reaction time in a dose dependent manner. Methanolic crude extract at a dose of 400 mg/kg body weight significantly inhibited the pain sensation at 48.55%, 57.77% and 61.44% after 30, 60 and 90 minutes later in comparable with standard morphine at a dose of 10 mg/kg body wt. Table 5

It could be concluded that crude extract of *Z. mauritiana* leaves possesses important metabolites that could probably inhibit the formation of prostaglandins.

Antidiarrheal activity

Methanolic crude extract of *Z. mauritiana* leaves was subjected for the evaluation of antidiarrheal activity test by castor oil induced diarrhea in mice. Experimental data showed that methanolic crude extract at a dose of 400 mg/kg body weight revealed statistically significant antidiarrheal activity by reducing 52.02% of diarrhea comparing with standard drug loperamide (50 mg/kg body wt) having 67.24% of reduction of diarrhea. Table 6

In this experiment, castor oil causes diarrhea due to its active metabolite, ricinolic acid[11], which stimulates peristaltic activity in the small intestine leading to changes in the electrolyte permeability of the intestinal mucosa. It can also stimulate the release of endogenous prostaglandin, inhibit intestinal Na+ K+-ATPase activity, activate adenyl cyclase or mucosal cAMP mediated active secretion and platelet activating factor[12]. It could be concluded that crude extract of *Z. mauritiana* possesses such type of secondary metabolites which can manage diarrhea by inhibiting the mechanism by which castor oil cause diarrhea.

Antimicrobial activity

The antimicrobial potency of the test agents were measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale. Crude extract and its different fractions inhibited the bacterial growth ranging from 6.5 to 18.8 mm against gram positive bacteria, 6.2 to 17.9 mm against gram negative bacteria and 7.4 to 14.7 mm against fungi compared with standard ciprofloxacin. Table 7

CONCLUSION

Methanolic crude extract and its different fractions *Z. mauritiana* leaves revealed different biological activities due to having biologically important secondary metabolites. It could be concluded that further investigation should be performed to isolate and identify those secondary metabolites responsible novel pharmacological activities.

REFERENCES

1. Kayser O and Arndt SK. Antimicrobial activity of some Zizyphus species used in traditional medicine. *Pharmaceut and pharmacol Letter* 2000; 10:38-40.
2. Sharif M Al-Reza, Jung In Yoon, Hyo Jung Kim, Jong-Sang Kim and Sun Chul Kang. Anti-inflammatory activity of seed (essential oil) from Zizyphus jujube. *Food and Chem Toxicol* 2010; 48:639-643.
3. Peng WH, Hsieh MT, Lee YS, Lin YC and Liao J. Anxiolytic effect of seed of Ziziphus jujube in mouse models of anxiety. *J Ethnopharmacol* 2000; 72(3):435-41.
4. Cisse A. Antidiabetic activity of Zizyphus mauritiana Lam Rhamnaceae. *Dakar Med* 2000; 45 (2):105-7.
5. Van Wagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC and Swithenbank C. Ulosantoin, a potent insecticide from the sponge Ulosa ruetzleri. *J Org Chem* 1993; 58:335-337.
6. Williamson EM, Okpako DT and Evans FJ. Pharmacological Methods of Phytotherapy Research: Selection Preparation and Pharmacological Evaluation of plant Material. 1st ed., England: John Willet and sons; 1996:247-263.
7. Pizziketti RJ, Pressman NS, Geller EB, Cowan A and Adler MW. Rat cold water tail-flick: A novel analgesic test that

distinguishes opioid agonists from mixed agonist-antagonists. *Eur J Pharmacol* 1985; 119:23-29.
8. Christine C Wu, Michael J. Mac Coss, Kathryn E Howell and John R Yates III. A method for the comprehensive proteomic analysis of membrane proteins. *Nature* 2003; 21:32-38.
9. Chattergee TK. Handbook of Laboratory Mice and Rats. 1st ed., India: Jadavpur University; 1993:133-139.
10. Rizvi SMD, Biswas D, Arif JM and Zeeshan M. *In vitro* antibacterial and antioxidant potential of leaf and flower extracts of *Vernonia cinerea* and their phytochemical constituents. *Int J Pharm Sci Rev Res* 2011; 9:164-169.
11. Ammon PJ, Thomas, Philips S. Effects of oleic and recinoleic acids net jejuna water and electrolyte movement. *J Clin Investig* 1974; 53:374-379.
12. Meite S, N'guessan J D, Bahi C, Yapi H F, Djaman H A and Guede Guina F. Antidiarrheal Activity of the Ethyl Acetate Extract of *Morinda morindoides* in Rats. *Tropical J of Pharmaceut Res* 2009; 8 (3):201-207.

Table 1. Test materials used in the phenobarbitone induced sleeping time test

Test sample	Group	Identification	Dose (mg/kg b. w.)	R/A
1% Tween-80 in normal saline	I	Control group	0.1 ml/10 gm of body weight	Oral
Methanolic crude extract	II (A)	Test group	200	Oral
Methanolic crude extract	II (B)	Test group	400	Oral
Phenobarbitone	All	Sleep inducer	25	Intraperitoneal

Table 2. Test samples used in the evaluation of analgesic activity

Test samples	Group	Purpose	Dose (mg/kg)	Route of administration
1%Tween 80 in saline	I	Control Group	0.1 ml/10 gm of body weight	Oral
Morphine	II	Standard Group	10	Intraperitonial
Methanolic crude extract	III	Test sample	200	Oral
Methanolic crude extract	IV	Test sample	400	Oral

Table 3. List of microorganisms used in an antimicrobial activity test

Gram positive	Gram negative	Fungi
Bacillus cereus	*Escherichia coli*	*Candida albicans*
Bacillus megaterium	*Pseudomonas aeruginosa*	*Aspergillus niger*
Bacillus subtilis	*Salmonella typhi*	*Sacharomyces cerevacae*
Staphylococcus aureus	*Salmonella paratyphi*	
Sarcina lutea	*Shigella boydii*	
	Shigella dysenteriae	
	Vibrio mimicus	

Table 4. Effects of methanolic crude extract of *Z. mauritiana* leaves on phenobarbitone induced sleeping time

Experimental animal	Treatment	Time of onset of sleep (minute)	Total sleeping time (minute)
Group I (control)	0.1 ml/10 gm of b. w (1%Tween-80 solution)	15.8 ± 1.19	118.6 ± 2.81
Group II (A)	Methanolic extract at dose 200 mg/kg b. w	27.6 ± 1.76	79.0 ± 3.210
Group II (B)	Methanolic extract at dose 400 mg/kg b. w	29.6 ± 2.20	89.8 ± 2.85

Table 5. Effects of crude effects of *Z. mauritiana* leaves on pain sensation

Group	Dose (mg/Kg)	Latency period (sec)		
		30 min (%Pain inhibition)	60 min (%Pain inhibition)	90 min (%Pain inhibition)
Control	----	3.81	2.16	3.37
Morphine	10	52.77	71.49	84.78
Methanol extract	200	33.33	38.99	42.85
Methanol extract	400	48.55	57.77	61.44

Table 6. Effects of methanolic crude extract of *Z. mauritiana* leaves on castor oil induced diarrhea

Treatment	Dose	Number of diarrhoeal faeces (Mean ± SEM)	% Reduction of diarrhoea
Control (Saline)	10 ml/kg body wt	17.3	---
Standard (loperamide)	50 mg/kg body wt	5.67	67.24
Methanolic extract	200 mg/kg body wt	9.0	47.98
Methanolic extract	400 mg/kg body wt	8.3	52.02

Table 7. Antimicrobial activity of test samples of Z .mauritiana (leaves)

Test microorganisms	Diameter of zone of inhibition (mm)			
	PESF	CTSF	MCE	Ciprofloxacin
Gram positive bacteria				
Bacillus cereus	11.2	13.5	15.2	17.2
Bacillus megaterium	10.4	12.9	16.4	23.5
Bacillus subtilis	6.6	12.4	14.5	18.8
Staphylococcus aureus	6.5	14.8	18.8	21.6
Sarcina lutea	7.5	12.8	14.7	19.3
Gram negative bacteria				
Escherichia coli	7.2	6.2	-	20.1
Pseudomonas aeruginosa	8.2	14.7	16.9	19.6
Salmonella typhi	7.5	15.1	16.4	22.8
Salmonella paratyphi	8.2	13.9	17.4	23.4
Shigella boydii	7.2	14.2	15.8	22.7
Shigella dysenteriae	7.5	13.5	14.9	20.5
Vibrio mimicus	7.2	14.6	17.9	23.4
Fungi				
Candida albicans	-	10.2	-	24.4
Aspergillus niger	-	12.4	14.7	22.4
Sacharomyces cerevacae	-	7.4	-	20.4