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Research Article

Antiulcer Activity of Methanolic Extract of *Ziziphus mauritiana* Stem Bark

Panchal Siddharth*[1], Panchal Kailash[2], Vyas Niraj[3], Modi Karuna[1], Patel Vimal[1], Bharadia P[1], Pundarikakshudu K[4]

[1]B. S. Patel Pharmacy College, Saffrony Institute of Technology, Linch, Mehsana, Gujarat, India.
[2]Torrent research centre, Bhat, Gandhinagar, Gujarat, India.
[3]Ramanbhai Patel Pharmacy College, Changa, Gujarat, India.
[4]L. J. Institute of Pharmacy, Sarkhej-Gandhinagar highway, Ahmedabad, Gujarat, India.

ABSTRACT
The methanolic extract of *Ziziphus mauritiana* stem bark was evaluated for its antiulcer activity using two models. Models are ethanol induced gastric ulcers model and aspirin induced gastric ulcer model in mice. It was found that the methanolic extract of stem bark have significant antiulcer activity in dose dependent manner where 3 different oral doses prepared (100 mg/kg of body weight, 250 mg/kg of body weight and 500 mg/kg of body weight). Evaluation was done on both models comparing with reference standard ranitidine (80 mg/Kg/ p. o.). The compounds like alkaloids, carbohydrates, saponins, phytosterols, flavanoids and tannins were detected by usual chemical test in methanolic extract. The above result shows that *Ziziphus mauritiana* stem bark probably contains some active ingredients that could be developed for above mentioned abnormal condition as have been claimed by traditional system of medicine.

KEY WORDS
Ziziphus mauritiana, gastric ulcer, antioxidant activity, ethanol, aspirin.

Introduction

Ziziphus mauritiana a plant belonging to the family Rhamnaceae is one of those plants which have been used in many disorders since long time in many parts of India as well as other countries like Burma, Iran, Syria, Afghanistan, Europe, Australia, America and Africa as a traditional system of medicine. In India commonly it is known as a ber in Hindi and Badrah in Sanskrit. It is distributed originally from the Middle East or the Indian subcontinent, but now cultivated throughout the tropics and subtropics for its nutritious. In India it is found throughout the country to an altitude of approximately 1500 m in the Himalayas, typically on or near old village sites, and very commonly on black cotton soils in the central India. Traditionally, various parts of the plant is useful in variety of disease conditions like, Roots are useful in vitiated conditions of pitta, fever, wounds, ulcer and cephalalgia. Bark is useful in dysentery, diarrhea, gingivitis and boils and ulcers. Leaves are useful in stomatitis, wounds, syphilitic ulcers, asthma, leucorrhoea, typhoid fever, diarrhea and obesity. Paste of leaves is applied on wounds, cuts and boils, etc. Fruits are useful in vitiated condition of pitta, burning sensation, hyperdipsia, consumption, vomiting, constipation, flatulence, dyspepsia, nausea, leprosy, thirst, anorexia, fatigue, leucorrhoea, pruritis, wounds and ulcers. Seeds are useful in encephalopathy, ophtalmopathy, cough, and asthma, vitiated condition of pitta, burning sensation, diarrhea, vomiting, leucorrhoea, general debility and insomnia. [1], [2], [3], [4], [5], [6], [7] Looking to the very vast uses of the amazing traditional uses we decided to evaluate antiulcer activity of the stem bark.

Materials and methods

Collection of plant material and processing

Ziziphus mauritiana stem bark was collected from north Gujarat. It was authenticated by ethanobotanist. The plant material was dried in a hot air oven below 50^0 C for 24 hours. It was then stored into air tight glass bottles and powdered to 40 mesh when required. The voucher specimen of powdered material was preserved in the department of Phytopharmaceuticals and Natural Products, Institute of Pharmacy, Nirma University (Specimen No.-MPH/08/03/043). Powdered material was stored in airtight glass containers at 10^o C till further use.

Preparation of methanolic extract

20 grams of powder of *Z. mauritiana* was macerated with methanol (4×200 ml) for 2 days and filtered. The solvent was removed under reduced pressure and residue was dried to constant weight. (Yield- 4.859 g)

Chemicals

Methanol AR grade and Aspirin (O-acetyl salicylic acid) AR grade reagent were purchased from Central Drug House (P) ltd. New Delhi, Ethyl alcohol was procured

*Author for Correspondence: siddh2109@gmail.com

Table 1: Effect of methanolic extract of *Ziziphus mauritiana* (100, 250, 500mg/kg/p.o. for 14 days) and ranitidine (80mg/kg/p.o. for 14 days) in absolute ethanol induced gastric mucosal damage in mice

Treatment group	Dose (mg/kg,p.o.)*14days	Ulcer index [a]	Protection (%)
Control	-	0.75 ± 0.01	-
Standard	80mg/kg	0.03 ± 0.0**	96.00
Methanolic extract	100mg/kg	0.69 ± 0.02**	8.00
Methanolic extract	250mg/kg	0.25 ± 0.02**	66.67
Methanolic extract	500mg/kg	0.13 ± 0.05**	82.67

[a] Results represents mean ± S.E.M., n=6.
Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test.
** Significantly different from control group. (P< 0.01)

from Baroda chemicals industries ltd, Baroda, Gujarat. Ranitidine procured from Cadila Healthcare Limited, Changodar, Gujarat. All the reagents used for the experiments were of analytical grade.

Table 2: Effect of methanolic extract of *Ziziphus mauritiana* (100, 250, 500mg/kg/p.o. for 14 days) and ranitidine (80mg/kg/p.o. for 14 days) in aspirin induced gastric mucosal damage in mice

Treatment group	Dose (mg/kg,p.o.) * 14days	Ulcer index [a]	Protection (%)
Control	-	0.82 ± 0.01	-
Standard	80mg/kg	0.03 ± 0.0**	96.34
Methanolic extract	100mg/kg	0.50 ± 0.01**	39.03
Methanolic extract	250mg/kg	0.45 ± 0.01**	45.12
Methanolic extract	500mg/kg	0.07 ± 0.01**	91.46

[a] Results represents mean ± S.E.M., n=8.
Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test.
** Significantly different from control group. (P< 0.01)

Measurement of antiulcer activity by using ethanol induced gastric ulcer model

Intragastric application of absolute ethanol is a reproducible method to produce gastric lesion in experimental animals. Ethanol produces necrosis of gastric mucosa and produces gastric ulcer. [8]

Healthy albino mice of either sex weighing 20-30 gm were selected for the antiulcer activity. The animals were housed and fed with standard mice diet and water ad libitum. The animals were acclimatized for 5 days before starting the experiment. All the experiments were conducted in accordance with the CPCSEA guidelines (Rule 5 (a) of the "Breeding of and Experiments on animals) Rules 1998 (Project no-IPS/PCOG/MPH08/002)" Government of India. Animals were deprived from foods at least 24 hr before start of the experiment but allowed free access of water. Coprophagy was prevented by keeping animals in cages with grating as the floor. The mice were divided into six groups with 6 mice each. Group 1 served as induced control. Group 2 served Reference standard drug group and received Ranitidine (80 mg/kg/day p.o.) for 14 days. Group 3 served as test drug group and received *Ziziphus mauritiana* (100 mg/Kg/day p.o) for 14 days. Group 4 and Group 5 received other two doses 250mg/Kg/day p.o. and 500 mg/Kg/day p.o of the same test drug for the 14 days Group 6 served as normal control.

On the day 14, absolute ethanol (0.1 ml, p.o.) was administered 1 hr after respective treatments. After 2 hr of absolute ethanol administration the animals were sacrificed using diethyl ether as anesthesia and cutting the neck blood vein. Stomachs were removed and opened along the greater curvature and examine for the lesion severity. [8]

Lesion severity was determined by measuring ulcer index. It was calculated as follows,

Ulcer Index = 10/X

Where X is total mucosal area/total ulcerated area.

Measurement of antiulcer activity by using Aspirin induced gastric ulcer model

Non steroidal anti-inflammatory agents, like indomethacin and acetyl-salicylic acid, induce gastric lesion in man and in experimental animals by inhibition of gastric cyclo-oxygenase resulting in less formation of prostacyclin, which is predominant prostanoid produced in the gastric mucosa. The ulcer produced by NSAIDs can be prevented by exogenous PGE_2 and PGI_2. [9]

Healthy albino mice of either sex weighing 20-30 gm were selected for the antiulcer activity. The animals were housed and fed with standard mice diet and water ad libitum. The animals were acclimatized for 5 days before starting the experiment. All the experiments were conducted in accordance with the CPCSEA guidelines (Rule 5 (a) of the "Breeding of and Experiments on animals) Rules 1998 (Project no-IPS/PCOG/MPH08/002)" Government of India. Animals were deprived from foods at least 24 hr before start of the experiment but allowed free access of water. Coprophagy was prevented by keeping animals in cages with grating as the floor. The mice were divided into six groups with 8 mice each. Group 1 served as induced control. Group 2 served Reference standard drug group and received Ranitidine (80 mg/kg/day p.o.) for 14 days.

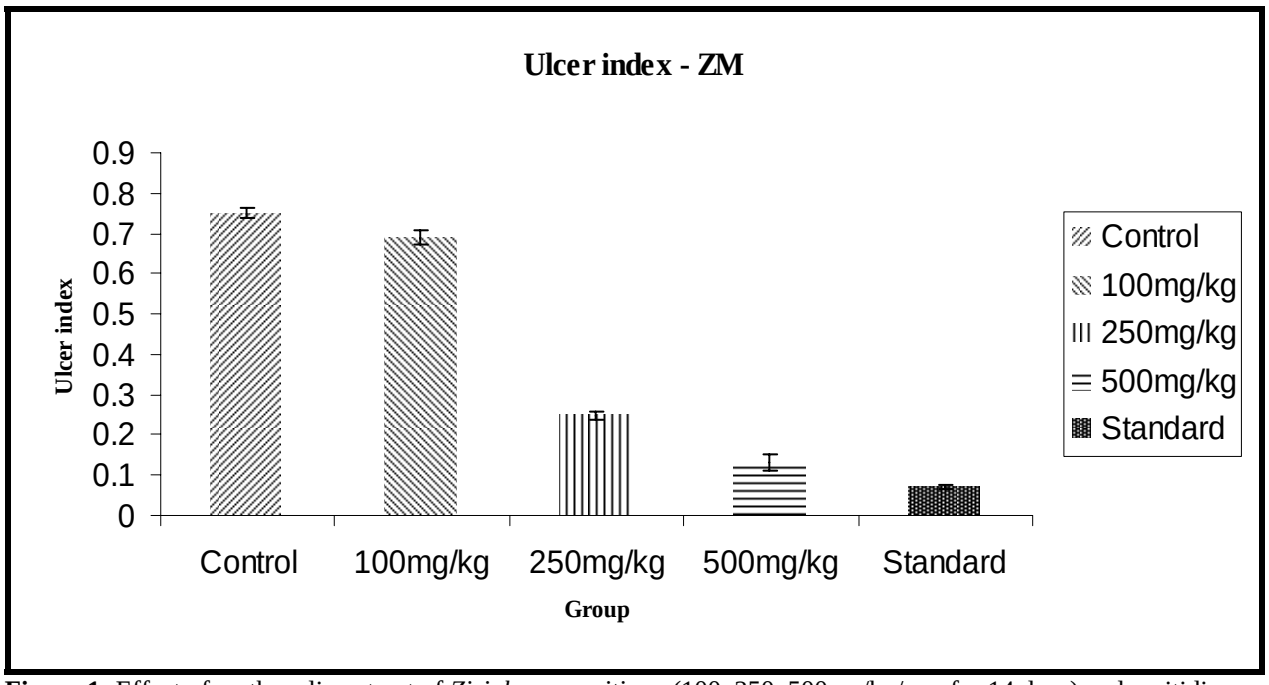


Figure 1: Effect of methanolic extract of *Ziziphus mauritiana* (100, 250, 500mg/kg/p.o. for 14 days) and ranitidine (80mg/kg/p.o. for 14 days) in absolute ethanol induced gastric mucosal damage in mice. Results represents mean ± S.E.M., n=6. ** Significantly different from control group. (P< 0.01)

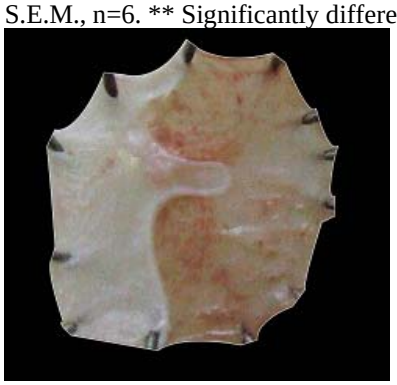


Fig.2

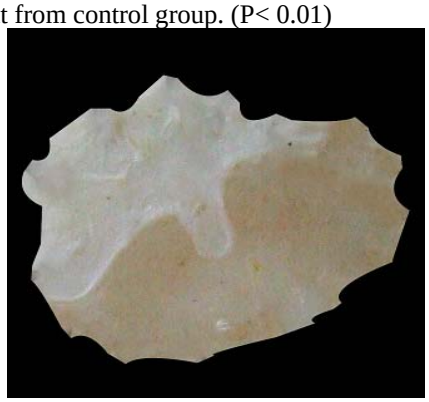
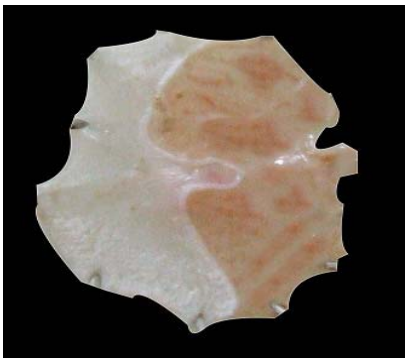


Fig.3



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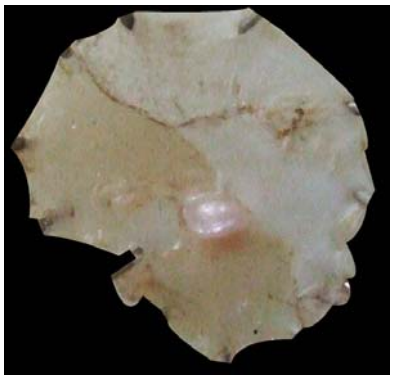

Fig.5

Fig.6

Figure 2: Illustrative picture showing severe ulcers in opened stomach of control ethanol induced model in mouse.

Figure 3: Illustrative picture showing open stomach of mice pretreated with ranitidine (80 mg/Kg, p.o., 14 days). The severity of ulceration is reduced by ranitidine pretreatment when compared to control ethanol induced mouse.

Figure 4: Illustrative picture showing open stomach of mouse pretreated with methanolic extract (100 mg/Kg, p.o., 14 days) of stem bark of *Ziziphus mauritiana.*

Figure 5: Illustrative picture showing open stomach of mouse pretreated with methanolic extract (250 mg/Kg, p.o., 14 days) of stem bark of *Ziziphus mauritiana.*

Figure 6: Illustrative picture showing open stomach of mouse pretreated with methanolic extract (500 mg/Kg, p.o., 14 days) of stem bark of *Ziziphus mauritiana.*

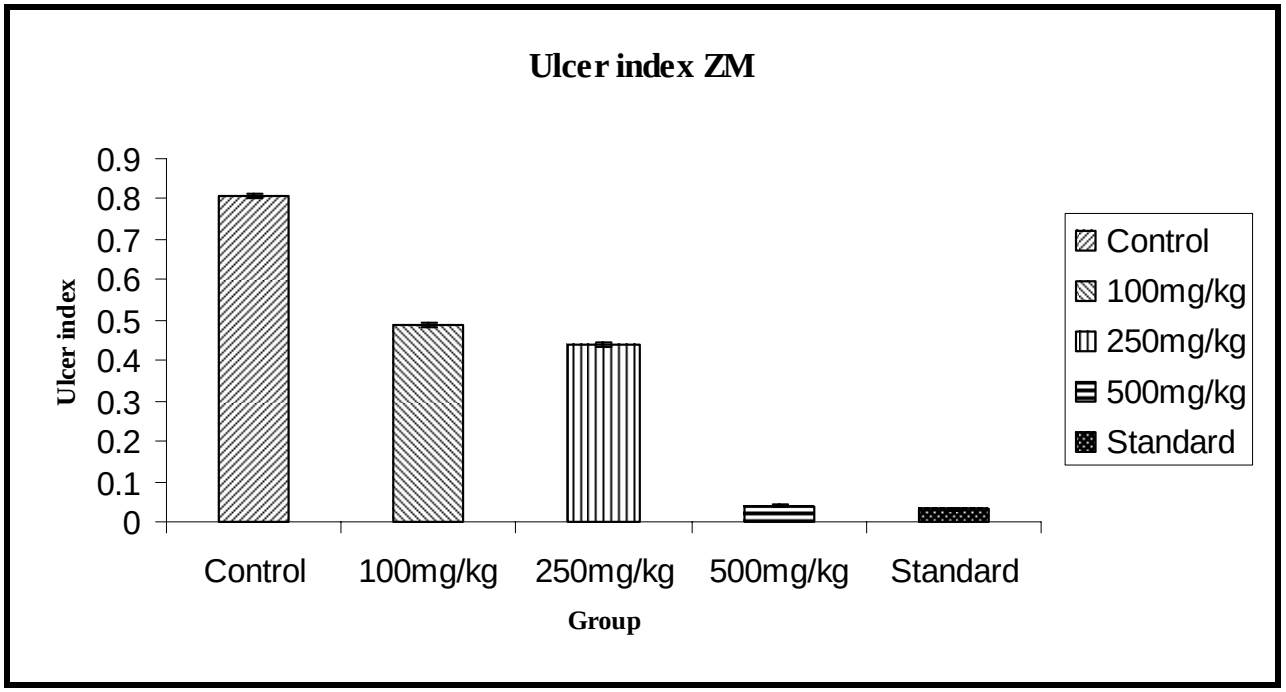

Figure 7: Effect of methanolic extract of *Ziziphus mauritiana* (100, 250, 500mg/kg/p.o. for 14 days) and ranitidine (80mg/kg/p.o. for 14 days) in aspirin induced gastric mucosal damage in mice
** Significantly different from control group. (P< 0.01)

Group 3 served as test drug group and received *Ziziphus mauritiana* (100 mg/Kg/day p.o) for 14 days. Group 4 and Group 5 received other two doses, 250mg/Kg/day p.o. and 500 mg/Kg/day p.o of the same test drug for the 14 days. Group 6 served as normal control.

After the period of 14 days, all animals were fasted for 24 hr following the dose of Aspirin (500mg/Kg/p.o.). After 4 hrs of Aspirin administration animals were sacrificed using diethyl ether as an anesthesia and cutting the neck blood vein. Stomachs were removed and opened along the greater curvature and examine for the lesion severity. Lesion severity was determined by measuring ulcer index. It was calculated as follows,

Ulcer Index = 10/X

Where X is total mucosal area/total ulcerated area. [10], [8]

Results

Ethanol induced gastric ulcer model

Treatment with absolute ethanol produced linear and scattered dot-like haemorrhagic lesions on the glandular portion of the stomach. Some of the linear lesions had fused together to form large haemorrhagic patches. The treatment with methanolic extract of *Ziziphus mauritiana* at various concentration showed significant (p<0.01) reduction in the number and length of the haemorrhagic gastric lesions induced by ethanol. The percentage reduction in the total ulcerated area by 100, 250 and 500 mg/kg of methanolic extracts of stem bark of *Ziziphus mauritiana* were 8%, 66.67% and 82.67% respectively.

Followings are the figures showing gastric mucosal damage in control group and protection in treated groups in absolute ethanol ulcer model.

Aspirin induced gastric ulcer model

The result of the present investigation demonstrated that methanolic extract of stem bark of *Ziziphus mauritiana* has anti ulcer activity in mice. The extract is having significant effect (p<0.01) at the concentration of 250 and 500mg/kg but the extract at 100mg/kg concentration is not having anti ulcer activity (p>0.05). Thus, methanolic extract of stem bark of *Ficus religiosa* possesses powerful gastro protective properties.

Administration of aspirin to mice produced dot like scattered lesions on the glandular portion of the stomach. Methanolic extract of the stem bark of *Ziziphus mauritiana* showed significant effect (p<0.01) at concentration of 250 and 500mg/kg**.** The methanolic extract (100, 250 and 500mg/kg) reduced the gastric lesions at 9.76%, 68.29% and 86.58% respectively with respect to the control group.

Ulcer produced in control group and reduction in ulcer index in mice pretreated with methanolic extract (100, 250 and 500mg/kg) is shown in the figures below.

Discussion

In both models microscopic examination of the stomach of control mice revealed disruption of the integrity of the mucosal surface, with deep necrotic penetrations in the mucosa and prominent congestions. In contrast, the methanolic extract (100, 250mg/kg and 500mg/kg) treated mice, the integrity of the epithelium was preserved and neither necrotic damages nor congestions were observed. In control, dilated capillaries filled with red blood cells were observed. From all results and analysis it was clear that methanolic extract contains

Fig.8

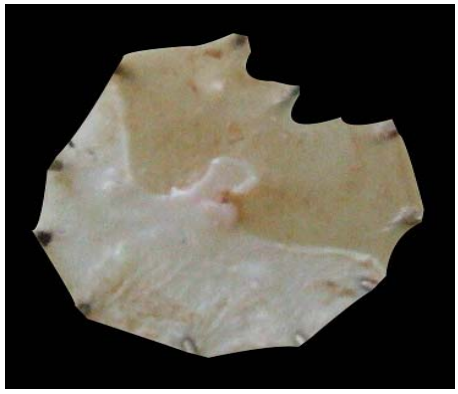

Fig.9

Fig.10

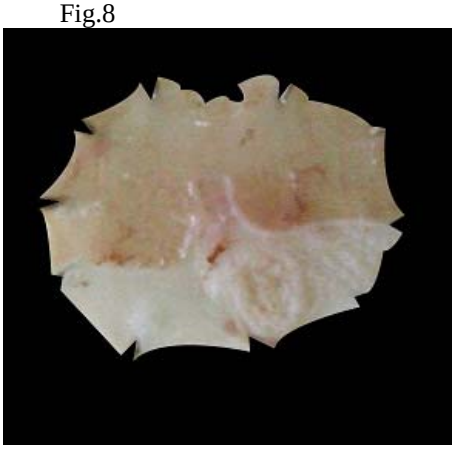

Fig.11

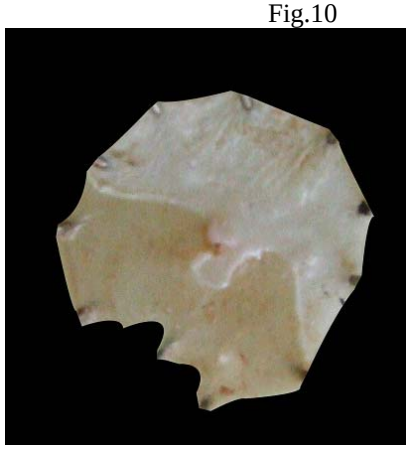

Fig.12

Figure 8: Illustrative picture showing severe ulcers in opened stomach of control aspirin (500 mg/Kg, p.o., 14 days) induced model in mouse.

Figure 9: Illustrative picture showing open stomach of mice pretreated with ranitidine (80 mg/Kg, p.o., 14 days). The severity of ulceration is reduced by ranitidine pretreatment when compared to control aspirin induced mouse.

Figure 10: Illustrative picture showing open stomach of mouse pretreated with methanolic extract (100 mg/Kg, p.o., 14 days) of stem bark of *Ziziphus mauritiana.*

Figure 11: Illustrative picture showing open stomach of mouse pretreated with methanolic extract (250 mg/Kg, p.o., 14 days) of stem bark of *Ziziphus mauritiana.*

Figure 12: Illustrative picture showing open stomach of mouse pretreated with methanolic extract (500 mg/Kg, p.o., 14 days) of stem bark of *Ziziphus mauritiana*

certain phytochemicals which are useful in the treatment of ulcers and lesions. Further researches and Future studies at molecular level may be helpful for designing proper drugs and formulation with the incorporation of plant *Ziziphus mauritiana* methanolic extract.

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