Research paper

Gastroprotective activity of methanol leaves extract of *Barleria prionitis* Linn. on ethanol and indomethacin induced ulcer in rats

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Abstract

Aim: *Barleria prionitis* L. (Family Acanthaceae) is a medicinal plant found road side in India and whole plant or its various parts like leaves, root, bark, stem and flowers are used traditionally for various treatments like toothache, inflammation, boils, glandular swellings and ulcer. Leaf juice is useful in gastric ulcer. Here, we attempt to prove the use of this plant in gastroprotection.

Study design: This study was conducted to evaluate the antiulcer activity of methanol extract obtained from the leaves of *Barleria prionitis* Linn.

Place and duration of the study: The experiments were conducted at pharmacology lab of Institute of Pharmaceutical Sciences, Kurukshetra University during the period of July 2012 to December 2012.

Material and methods: Antiulcer activity was performed using the protocols of ulcer induced by ethanol and indomethacin at two different doses (250 and 500mg/kg). Parameters like volume of gastric juice, pH, free acidity, total acidity, aspartate amino transferase (AST) and alanine amino transferase (ALT) were also determined in ethanol induced ulcer model.

Results: The reduction in ulcer index in *Barleria prionitis* treated animals was found to be statistically significant (P=.05), when compared with control groups in both the models. Significant changes were observed in total acidity at dose 500mg/kg only and changes were significant in AST, ALT levels at both the doses. Other parameters showed non-significant results.

Conclusion: The results of the present study show that the methanols extract of *Barleria prionitis* possess antiulcer activity. This work supports the traditional use of this plant in treating gastric ulcer.

Keywords: *Barleria prionitis, G*astroprotective activity, Ulcer index, Methanol extract, Ethanol

1. Introduction

Gastric hyperacidity is a very common global problem that affects millions of people worldwide [1,2]. In gastric ulcer their occurs imbalance between aggressive (acid-pepsin secretions) and protective factors (such as mucus secretion, mucosal barrier, cell regeneration, blood flow and prostaglandins) [3,4]. The current treatment of peptic ulcer is mainly done with H_2 receptor antagonists, proton pump inhibitors, and antimuscarinics. But, most of these treatments produce adverse reaction like, hypersensitivity, arrhythmia, impotence, gynecomastia and hematopoietic disorders [5,6,7,8]. Therefore, there is requirement for new and safer treatment, with fewer side effects. Plants extracts are among the suitable treatments for the prevention of gastric ulcer [9].

Barleria prionitis L. (Family Acanthaceae; commonaly known as Vajradanti) is a medicinal plant found throughout South Africa, India, Sri-Lanka, and tropical Asia [10,11]. In India whole plant or its various parts like leaves, root, bark, stem and flowers are used traditionally for various treatments like toothache, inflammation, boils, glandular swellings, whooping cough etc [12,13,14,15]. The leaf juice is useful in stomach problems, ulcer, fever and urinary affections [16]. The whole plant including roots is used to induce diuresis. Plant is also useful in jaundice, hepatic problems and dropsy [17,18].

Phytochemical studies on hydro-methanolic extract of *B. prionitis* showed the presence of glycosides, steroids, tannins and flavonoids [19]. Iridoid glycosides, shanzhiside methyl ester, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside have been isolated from aerial parts [20]. None study was conducted scientifically to prove the gastroprotective effect of *B. prionitis* leaves. Hence the present study was conducted to evaluate the antiulcer properties of methanolic extract of *B. prionitis* Linn.

2. Materials and methods

Plant material

The leaves of *Barleria prionitis* were collected from Ashoka nursery Gharunda, Karnal, Haryana, India in the month of March, 2011. Then, collected leaves were positively identified by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum (RHMD), New Delhi. A voucher specimen of the plant (Ref. No. NISCAIR/RHMD/CONSULT/-2010-11/1497/95) has been preserved there for future references.

2.1. Extraction

The leaves were thoroughly washed under running tap water so as to remove any type of contamination. Then washed leaves were air dried in shade, powdered in grinder and passed through sieve of mesh size no-40. The dried powder was first defatted by petroleum ether and then successive extraction was done with chloroform and methanol by hot Soxhlet extraction method. The methanol extract was concentrated in a rotary evaporator under reduced pressure. The dried crude extract was collected and preserved in airtight glass container at 4°C - 8°C.

2.2. Preliminary phytochemical studies

To determine the chemical constituents, the methanol extract obtained was thus subjected to phytochemical analysis [21].

2.3. Antiulcer activity

2.3.1. Experimental animals

Healthy Wistar rats of either sex were obtained from a disease free animal house of Chaudhary Charan Singh, Haryana Agriculture University, Hisar, Haryana (India). The animals were housed in the animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana (India). Rats were fed with commercially available feed and were maintained under standard conditions of temperature (25°C ± 5°C), relative humidity (55 ± 10%), and 12/12 h light/dark cycle. They were housed in standard polycarbonate cages with wire mesh top and husk bedding.

2.3.2. Experimental design

Wistar rats weighing between 175-250 g of either sex were used for antiulcer study. All animals were divided into 4 groups of 6 animals. Before the experiments, animals were deprived of food but allowed free access to water.

2.3.3. Dose and route of administration

For experimentation 250mg/kg and 500mg/kg doses of BPM were used. Fresh drug solutions were prepared in sterile distilled water at the time of administration and were administered Per Oral (p.o.) so as to avoid any additional stress to the animals.

2.3.4. Group designing for ethanol and indomethacin induced ulcer models

Group I (Control): Animals received only distilled water; Group II (BPM 250): Animals received *B. prionitis* (250mg/kg, *p.o.*) I hr before the ulcerogenic procedure; Group III (BPM 500): Animals received *B. prionitis* (500mg/kg, *p.o.*) I hr before the ulcerogenic procedure; Group IV (Standard): Animals receivrd ranitidine (50mg/kg, *p.o*.) I hr before the ulcerogenic procedure.

2.3.5. Ethanol induced gastric mucosal lesions

This is a widely used model that seems to cause gastric ulcer. The activity was performed according to the slightly modified method of Mizui and Dotuchi [22]. Rats were fasted for 36 h before administration of absolute ethanol (1.0mL). The group I was given only distilled water. The extract (250 mg/kg, 500mg/kg, *p.o.*) and ranitidine (50mg/kg, *p.o.*) as standard drug, were given to Group II, III and IV respectively. One hour after treatment, all the rats received ethanol to induce gastric ulcer. Another one hour later, animals were sacrificed by cervical dislocation. The stomachs were removed, cut and opened along the greater curvature, washed with normal saline to remove the gastric contents and observed for the severity of the ulcers. The pH and volume of gastric juice was measured after centrifugation at 2000rpm for 10 min. From the supernatant, aliquots were taken for the determination of total and free acidity. The percentage protection was calculated using the following formula:-

% I = (UI of control- UI of test) ×100/UI of the control

Where I = Inhibition, UI= Ulcer index

2.3.6. Ulcer indexing

The mucosal layer of the stomach was observed under a magnifying lens and ulcers were checked. The area (mm^2) of all lesions was measured using digital callipers' to give a gastric damage score. The ulcer index was determined using the following formula [23].

UI =10/X

Where X= total mucosal area/total ulcerated area

2.3.7. Total acidity and free acidity determination

1.00mL of centrifuged and filtered gastric juice was taken in a conical flask. Two drops of 1% phenolphthalein indicator for total acidity and Topfer´s reagent for free acidity was added to it. It was titrated against 0.1mol/L sodium hydroxide until a permanent pink color (total acidity) or canary yellow colour (free acidity) was observed. The total/free acidity is expressed as meq./L by the following formula:-

Total/free acidity=n×0.01×36.45×1000

Where, n is the volume of NaoH consumed, 0.01 is normality of NaoH, 36.45 is molecular weight of NaoH, 1000 is the factor (to be represented in litre).

2.3.8. Indomethacin induced gastric ulcers

In this model, the gastric lesions are induced by the inhibition of prostaglandin synthesis. Activity was performed according to method of Djahanguiri [24] and 24 h fasted rats were used for study. Group I animals were treated orally with distilled water. The extract (250 mg/kg, 500mg/kg, *p.o.*) and ranitidine (50mg/kg, *p.o.*) as standard drug, were given to Group II, III and IV respectively. One hr. after the treatment, 20mg/kg of indomethacin (dissolved in 2% sodium bicarbonate) was administered orally. After 4 h, all animals were sacrificed by cervical dislocation. The stomachs were isolated, washed with normal saline and various parameters like ulcer index, free acidity and total acidity were measured as discussed above [25].

2.3.9. Serum biochemical parameters

Blood samples were analysed for AST and ALT level estimation in ethanol induced gastric lesions.

2.4. Statistical analysis

All the values were expressed as mean±standard error of mean. The statistical significance of difference among groups was analysed using one-way ANOVA. A value of $P<0.05$ was considered significant.

3. Results

3.1. Preliminary phytochemical screening

The percentage yield of petroleum ether, chloroform and methanol leaf extracts were found to be 4.9, 6.9 and16.7% (in weight). Preliminary phytochemical screening of methanol extract showed the presence of steroids, alkaloids, saponins, glycosides and flavonoids.

3.2. Antiulcer activity

3.2.1. Ethanol induced gastric ulcer

In this study, BPM was screened for gastroprotective activity against ethanol induced gastric ulcer in rats. The absolute ethanol administration (*p.o.*) induced severe ulceration. BPM and ranitidine groups showed the significant reduction in incidence and severity of ulceration. BPM and ranitidine showed a significant change in ulcer index when compared with the control group $P<0.01$ (Table 1). BPM and ranitidine showed slight changes in pH, volume of gastric juice, free acidity and total acidity but changes were not significant when compared with control group except total acidity in BPM (500mg/kg) treated group, $P<0.01$(Table 2).

Table 1. Ulcer index of ethanol and indomethacin induced gastric ulcers

Model	Group	Dose(mg/kg body weight)	Ulcer index	% Inhibition
Ethanol	Ethanol	--	0.90±0.01	-
	BPM	250	0.43±0.02**	52.2%
	BPM	500	0.29±0.04**	67.7%
	Ranitidine	50	0.22±0.02**	75.5%
Indomethacin	Indomethacin	20	1.35±0.15	-
	BPM	250	0.51±0.03**	62.2%
	BPM	500	0.40±0.02**	70.3%
	Ranitidine	50	0.51±0.03**	62.2%

*Results as mean±S.E.M. for six rats. Statistical comparison was performed using ANOVA followed by the Dunnett's test. **P<0.01 when compared with control group.*

Table 2. Volume of gastric juice, pH, free acidity and total acidity in ethanol induced gastric ulcer

Group	Dose(mg/kg)	Volume of gastric juice(ml)	pH	Free acidity(mmol/h)	Total acidity(mmol/h)
Ethanol	--	2.08±0.01	4.42±0.06	0.53±0.008	1.22±0.008
BPM	250	2.55±0.18	4.51±0.06	0.48±0.02	1.23±0.02
BPM	500	2.22±0.18	4.40±0.05	0.46±0.02	0.81±0.01**
Ranitidine	50	2.72±0.27	4.51±0.03	0.51±0.03	1.26±0.02

*Results as mean±S.E.M. for six rats. Statistical comparison was performed using ANOVA followed by the Dunnett's test. **P<0.01 when compared with control group.*

3.2.2. Biochemical parameters

Ethanolic group induced ulcer showed a increase in liver enzymes (ALT and AST) as shown in (Table 3). When rats were pretreated with BPM (250mg/kg and 500mg/kg) and ranitidine, there were significant reductions in serum concentration of these markers, $P<0.01$, $P<0.05$.

Table 3. Effect of BPM extract on liver functions tests in ethanol induced gastric ulcers

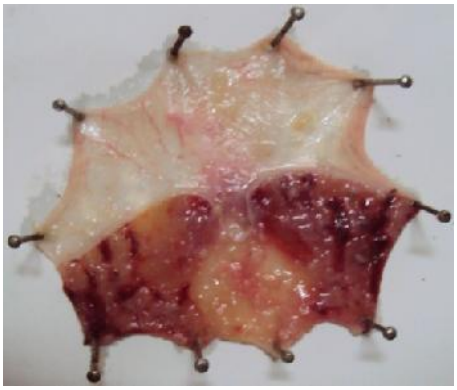
Group	Dose(mg/kg)	ALT(IU/L)	AST(IU/L)
Ethanol	--	65.98±2.5	353±2.1
BPM	250	56.08±2.1*	325±7.1**
BPM	500	45.44±2.0**	305±4.8**
Rantidine	50	55.18±3.5*	331±3.07*

*Results as mean±S.E.M. for six rats. Statistical comparison was performed using ANOVA followed by the Dunnett's test. **P<0.01, *P<0.05 when compared with control group.*

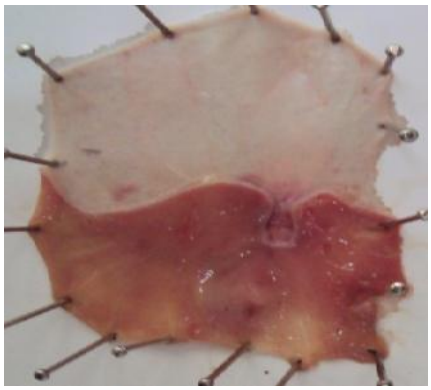
3.3.3. Indomethacin induced mucosal lesions

Indomethacin (20mg/kg, *p.o.*) administration induced severe gastric mucosal damage. BMP, at tested doses 250 and 500mg/kg, showed significant gastroprotective effect against gastric lesions, $P<0.01$. Standard drug ranitidine (50mg/kg, *p.o.*) included in the study as positive control also exhibited significant protection, $P<0.01$(Table1 and Figure 1).

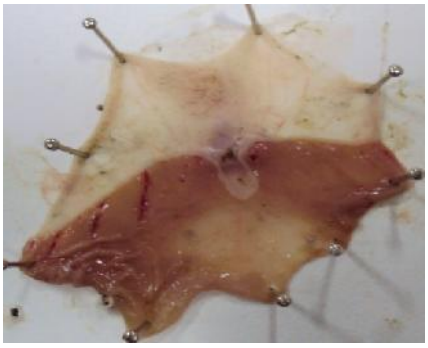
Figure1. Macroscopical view of rat stomach in indomethacin induced gastric ulcer

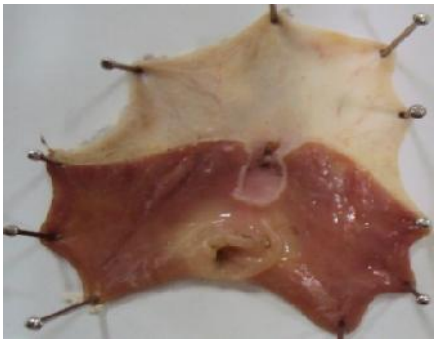


Ethanol Group



Ranitidine Group





BPM (250mg/kg) BPM (500mg/kg)

4. Discussion

Various noxious stimuli of endogenous (acid and pepsin) and exogenous (drugs and alcohol) origin, constantly comes in contact with gastric mucosa. Gastric mucosal defensive mechanism, like mucosal blood flow, bicarbonate and mucus secretion protect the gastric mucosa from damage. It is generally believed that it results from an imbalance between aggressive factor (acid, pepsin) and defensive factors (mucous secretions, prostaglandins) [26]. Therapeutic agents including different plant extracts are used to regain the balance by inhibiting the gastric acid secretion or by increasing the mucous production, stabilizing the surface epithelial cells. Herbs are the one of the most promising sources of new drugs as these are free of or having very less side effects and adverse reactions.

The methanol extract of *B. prionitis* was used to evaluate gastro protective activity by using ethanol and indomethacin induced gastric ulcers. Ethanol is considered very important in inducing gastric ulcers. Ethanol induced ulcer depends upon lots of factors. One reason may be its rapid penetration into gastric mucosa, which may cause more mucosal permeability and release of vasoactive factors that leads to gastric damage [27,28]. The other factor responsible may be formation of reactive oxygen species, which cause an imbalance between oxidant and antioxidant process, that results rupture of blood vessels, thus contributes to the haemorrhage, tissue necrosis and disrupting the protective mucosal barrier [29,30]. Indomethacin is considered to induce ulcers in the stomach mainly due to inhibition of prostaglandin synthesis [31]. To study the side effects of *B. prionitis* on liver, serum AST and ALT were determined in ethanol induced gastric ulcer model. Control group animals showed, increase of serum concentration of these enzymes that indicates hepatic injury since level of these enzymes increases in chemically triggered tissue injury. *B. prionitis* administration decreased the levels of AST and ALT that shows its tissue damage preventing action.

The preliminary phytochemical analysis indicated the presence of flavonoids, sterols, glycosides, saponins. Theses secondary metabolite classes are related to gastro protective activity. There are many studies related to the antiulcer genic properties of flavonoids [32,33]. Leaves of the plant also contain saponins. Saponins exhibit ulcer protective effect by selective inhibition of prostaglandin $F_{2\alpha}$ and by protection of gastric mucosa [29,34]. In view of this fact it is suggested that gastro protection elucidated by the methanol extract of *B. prionitis* may be related to the presence of these phytoconstituents.

5. Conclusions

The results provide support for the traditional use of this plant in the treatment of gastric ulcer. However, the data so far obtained do not indicate the specific mechanism(s) responsible for the antiulcer activity. Further studies are required to isolate the active components and to elucidate their mechanism of action. In conclusion, the results show that methanol extract of *Barleria prionitis* Linn. possess gastro protective activity.

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Competing interests

The authors declare that they have no competing interests.

Author's contribution

1st author wrote the protocol, performed the study and statistical analysis 2nd author designed the study, managed the analysis, write the first draft of manuscript, 3rd author helped in the literature study and finally all the authors read and approved the final manuscript.

Ethical Approval

All authors hereby declare that, Principles of laboratory animal care (NIH publication No.85-23, revised 1985) were followed. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (IAEC) (Register Number: 562/GO/02/a/CPCSEA) and were in accordance with the CPCSEA guidelines, Government of India.

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