



Original article

Evaluation of antiulcer activity of *Boswellia serrata* bark extracts using aspirin induced ulcer model in albino rats

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Abstract

The effect of bark extracts of *Boswellia serrata* (Family Burseraceae) was evaluated in aspirin induced ulceration (200mg/kg) in albino rats. Antiulcer activity was evaluated by measuring ulcer index and percentage of ulcer healing. The petroleum ether (250mg/kg) and aqueous extracts (250mg/kg) of bark of *Boswellia serrata* plant showed significant antiulcer activity as evidenced by the data obtained. Histopathological findings also confirm the antiulcer activity of *Boswellia serrata* bark extracts in albino rats.

Key words: *Boswellia serrata*, antiulcer activity, aspirin

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Peptic ulcer disease is a very common global health problem today. Peptic ulcer is a lesion of gastric or duodenal mucosa. Duodenal ulcers are more common in adult males. Gastric ulcers occur commonly at old age and in lower socio-economic class of individuals. Although the exact cause of ulceration is not known, hydrochloric acid and pepsin are responsible for maintaining the lesion once it is produced. Peptic ulceration occurs only in areas which are bathed by the acidic gastric juice. Therefore, the term peptic ulcer refers to ulceration of the areas which might be acted upon by acid peptic juice namely the stomach and the first portion of duodenum¹.

Peptic ulcers also occur at the lower end of the

esophagus, on the jejunal side of a gastroenterostomy and in Meckel's diverticulum².

The current therapeutic approach to gastric ulceration is to achieve inhibition of gastric secretion, promotion of gastric protection, blockage of apoptosis, and epithelial cell proliferation for effective healing³.

In recent years, focus on plant research has increased worldwide and several studies had showed immense potential of medicinal plants⁴. Herbal medicines derived from plant extract, are increasingly being recognized in treating various clinical diseases, with relatively little knowledge of their modes of action⁵.

In ancient system of medicines, herbal preparations were used for treating duodenal ulcers⁶. In the last few years, efforts have been taken to identify new antiulcer drugs from natural sources like plants⁷. The plant *Boswellia serrata* (Family Burseraceae) is commonly known as Indian Olive in English. It is a deciduous medium sized tree with ash color bark, peeling off in thin flakes of 15-30 ft. This plant is considered to be a native of India especially available in Khandesh and Nagpur-Wardha division in Maharashtra and in Andhra Pradesh⁸. Traditionally the plant is reported to have antiulcer activity⁹. In the present study, an effort has been made to establish the scientific validity to the antiulcer property of the bark extracts of *Boswellia serrata* in aspirin induced ulcer in male albino rats.

Materials and methods

The bark of *Boswellia serrata* were collected from Narsapur forest of Medak district and authenticated by Central Research Institute of Unani Medicine, Hyderabad. The plant material was air dried at room temperature and powdered. Ranitidine (20 mg/kg) was used as standard drug.

Preparation of extracts

50 gm of powdered bark was extracted in soxhlet assembly¹⁰⁻¹² with petroleum ether. For aqueous extract, dried and coarsely powdered plant materials were extracted separately with distilled water for 48 hours by cold maceration at room temperature and filtered. The extracts were concentrated with the help of vacuum evaporator and kept in a dessicator¹³⁻¹⁵. The color and consistency of extracts are noted (Table I).

Qualitative phytochemical evaluation

Petroleum ether and aqueous bark extracts were screened for the presence of various secondary metabolites like tannins, alkaloids, glycosides, terpenoids, flavonoids, amino acids and proteins using standard methods¹⁶⁻¹⁸.

HPTLC profile

Chromatography was performed on high perfor-

mance thin layer chromatography (HPTLC) plates coated with 0.25mm layer of silica gel 60F254 (Merck, Munchen, Germany). The plates were first activated at 110°C for 5 min. Samples were applied as 4mm wide bands and 6mm apart by using of DESAGA Sarstedt Gruppe applicator equipped with 100µL syringe. A constant application rate of 5µL/second was used. The mobile phase was Toluene: Ethyl acetate (9:1v/v) and chromatograms were monitored at 366nm.

Experimental animals

Male albino rats weighing between 150-180gms were procured from Mahaveer Enterprises (Reg.No. 146/1999/CPCSEA) Amberpet, Hyderabad, India. They were maintained at standard housing conditions at a room temperature of 24±1°C, relative humidity of 45-55% with 12:12 hour light/dark cycle¹⁹⁻²¹. The feeding was done with commercially available rat feed pellets and water was given *ad libitum* during the experiment²². The was approved by Institutional ethical committee (Reg. No. 1330/ac/10/CPCSEA).

Acute toxicity studies

Acute toxicity was carried out as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Organization for Economic Co-operation and Development (OECD)/ Organization de coopération et de développement économiques (OCDE) guidelines. Group of 3 rats weighing between 22-30 gm were selected and kept for 3-4 hrs fasting with free access to water. Dose is calculated according to body weight and bark extracts were dissolved in rice bran oil and administered orally at a starting dose of 2000mg/kg and were observed for 24 hours.

Petroleum ether bark extracts (PBE)

After the dosing of 2000 mg/kg of petroleum ether bark extracts (PBE), it was observed that 1 rat died after 24 hours. Same dose was repeated again and one more rat died.

Table I. Percentage yield of petroleum ether and aqueous bark extracts of *Boswellia serrata*

S.No.	Extract	Color	Consistency	Wt. of sample before extraction	Wt. of sample after extraction	Theoretical Yield (gm)	Practical Yield(gm)	% yield
1	PBE	Golden	Semi solid	142	114	28	8.31	29.6
2	ABE	Light	Solid	73	50	23	5.61	24.3

According to CPCSEA rule, under category-5 of annex 2d (OECD/OCED rule) for petroleum ether bark extract lethal dose of 2500mg/kg body weight was determined. The 1/10th of the lethal dose was taken as an effective dose (E.D)²³⁻²⁵. Therefore the effective dose was found to be 250 mg/kg body weight.

Aqueous bark extracts (ABE)

Dose of 2000mg/kg was administered orally 15 rat, 1 rat died after 24hrs. Dosing was again repeated at 2000mg/kg, 1 rat died. According to CPCSEA rule, lethal dose falls under category-5 of annex 2d (OECD/OCED rule) with the lethal dose of 2500mg/kg body weight. The 1/10th of the lethal dose was taken as an effective dose i.e. 250 mg/kg body weight.

The animals were put in four groups, each containing 5 rats. The groups were as follows:

Group-I: Control (Aspirin 200mg/Kg)+Rice bran oil

Group-II: Aspirin (200mg/Kg) + Ranitidine (20 mg/kg)

Group-III: Aspirin (200mg/Kg) + Petroleum ether bark extract

Group-IV: Aspirin (200mg/Kg) + Aqueous bark extract

Control received 1ml/kg rice bran oil orally. Standard drug selected was Ranitidine 20mg/kg, orally. The remaining two groups received calculated effective dose of *Boswellia serrata* plant extracts orally according to body weight of animals. After 8 days of dosing, animals were fasted for 24 hours and later aqueous suspension of aspirin at a dose of 200mg/kg²⁶ was given orally. The animals were then sacrificed by euthanasia four hours later of Aspirin administration. The stomachs were dissected and examined for ulcers. Gastric tissues were used for histopathological studies.

Calculation of ulcer score – ulcer index

The stomach was opened along the greater curvature and washed slowly under running tap water. It was put on a glass slide and observed under 10X magnification for ulcers. The ulcers were scored as shown in Table II. Mean ulcer score in each group was calculated and was designated as ulcer index and percentage was calculated²⁷⁻²⁸ as

$$\% \text{ Protection} = (\text{C-T/C}) \times 100$$

Where C= ulcer index in control group

T= ulcer index in treated group

Statistical analysis

All the values were tabulated and presented in the tables and were expressed as mean \pm standard error mean (SEM) of five animals. Significant difference among the means were calculated at the level of $P^a < 0.001$, $P^b < 0.01$, $P^c < 0.05$ when compared with controls. The statistical significance was calculated using students 't' test.

Results

The results of preliminary phytochemical screening of plant extracts showed presence of tannins, alkaloids, glycosides and terpenoids (Table III). The presence of active phytoconstituents may be responsible for antiulcer activity.

Table II. Ulcer scoring

Observations on stomach	Ulcer score
Normal colored stomach	0.0
Red coloration	0.5
Spot ulcers	1.0
Hemorrhagic streaks	1.5
Ulcers $\geq 3 \leq 5$ mm	2.0
Ulcers > 5 mm	3.0

Table III. Phytochemical screening of petroleum ether and aqueous bark extracts of *Boswellia serrata*

S. No	Phyto-constituents	Petroleum ether bark extracts	Aqueous bark extracts
1	Tannins	+	+
2	Alkaloids	+	+
3	Cardiac glycosides	+	+
4	Steroids	+	+
5	Carbohydrates	+	+
6	Saponins	+	-
7	Flavonoids	-	+
8	Anthraquinones	+	-
9	Proteins	-	-
10	Terpenoids	+	+

(+) sign indicates presence of phytoconstituent in the extract and (-) sign indicates absence of phytoconstituent in the extract.

HPTLC analysis of *Boswellia serrata* extracts of sample solution was spotted as 8-10 mm on the precoated HPTLC silica gel 60F254 plates. The R_f value of the corresponding component as obtained by the software system attached with the

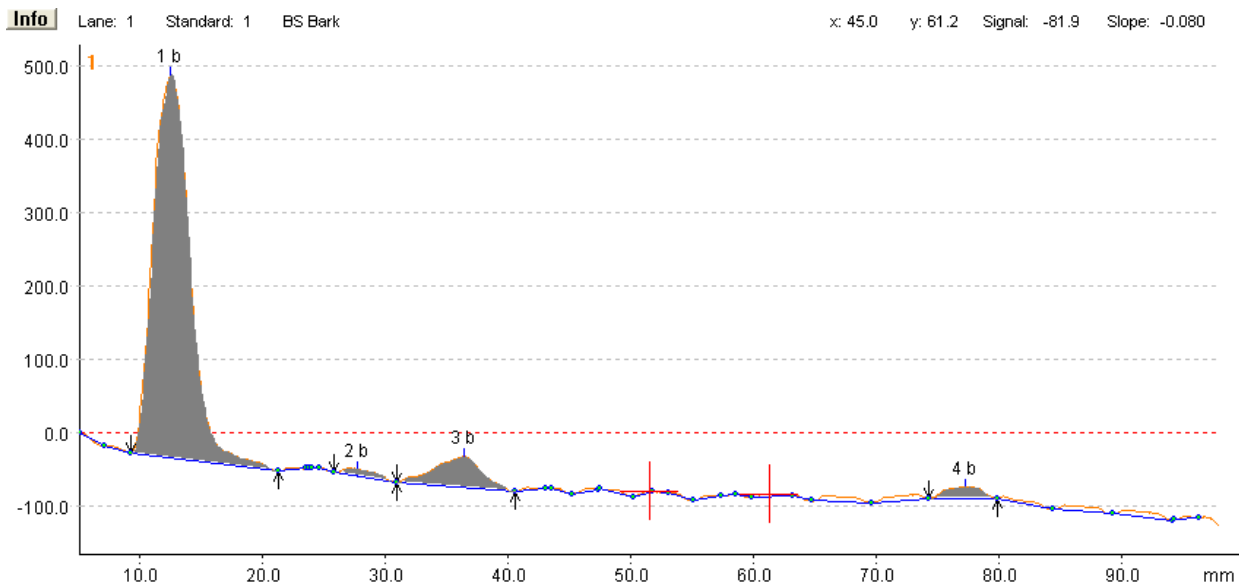


Fig 1. Chromatogram of petroleum ether extracts

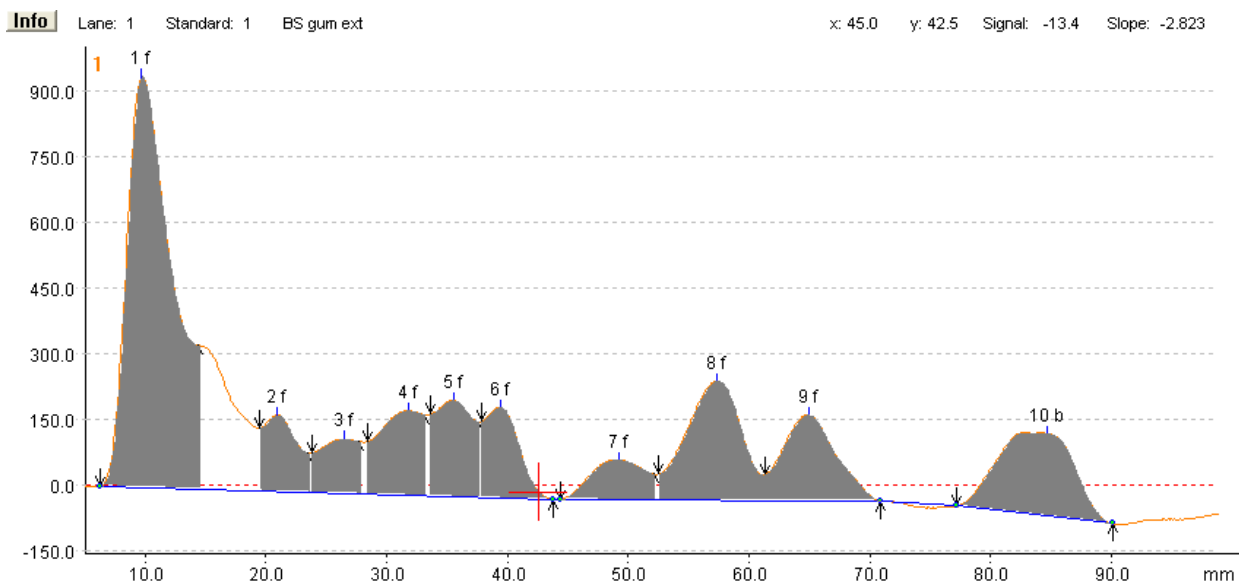


Fig 2. Chromatogram of aqueous extracts

instrument i.e., ProQuant 1.6 version are shown in the Tables IV and V. The area corresponds to each peak for the corresponding spot or component determines the concentration of the component in the solution.

The HPTLC finger printing of the extracts marked the presence of phytoconstituents (Fig 1 & Fig 2).

Aspirin induced ulceration was found to be (17.5 ± 1.1) in control group (Table VI). The standard drug Ranitidine showed significant activity i.e., $P < 0.001$ Vs control, as it reduced ulcer index to

(1.6 ± 0.8) . Percentage ulcer protection was found to be 90.8%. PBE has shown significant reduction of ulcer index (Fig 3) i.e., $P < 0.001$ Vs control (5.0 ± 1.08^a) . Percentage ulcer protection for PBE was found to be 71.4%. ABE has shown significant reduction of ulcer index i.e., $P < 0.01$ Vs control (7.0 ± 1.2^b) . Percentage ulcer protection for ABE was found to be 60%. In the histopathological examination, stomachs of control rat shows erosion in the upper part of epithelium and RBCs are seen in the eroded portion (Fig 4), stomachs of rats treated with standard drug (ranitidine) showed

Table IV. HPTLC profile of *Boswellia serrata* petroleum ether extract

Peak	y-Pos (mm)	Area	Area (%)	Height	Rf
1	9.7	3974.74	34.7	937.80	0.03
2	20.9	548.49	4.8	174.31	0.17
3	26.5	432.53	3.8	124.23	0.24
4	31.8	781.56	6.8	193.89	0.31
5	35.5	783.45	6.8	220.26	0.36
6	39.4	670.03	5.9	207.93	0.40
7	49.2	464.53	4.1	91.43	0.53
8	57.3	1421.96	12.4	272.28	0.63
9	64.9	991.58	8.7	195.89	0.72
10	84.7	1375.45	12.0	188.83	0.97

Table V. HPTLC profile of *Boswellia serrata* aqueous extract

Z	y-Pos (mm)	Area	Area (%)	Height	Rf
1	12.5	1773.81	87.2	516.76	0.03
2	27.7	27.84	1.4	8.90	0.24
3	36.4	181.59	8.9	41.54	0.35
4	77.2	50.29	2.5	15.05	0.90

Table VI. Effects of *Boswellia serrata* bark extracts in Aspirin induced ulcers in rats

Group	Ulcer index	% ulcer healing
Control	17.5±1.1	-
Ranitidine	1.6±0.8 ^a	90.8%
PBE	5.0±1.08 ^a	71.4%
ABE	7.0±1.2 ^b	60%

Values are mean ± SEM of five animals in each group P^a<0.001 Vs Control, ^b<0.01Vs Control, ^c<0.05 Vs Control, using students 't' test.

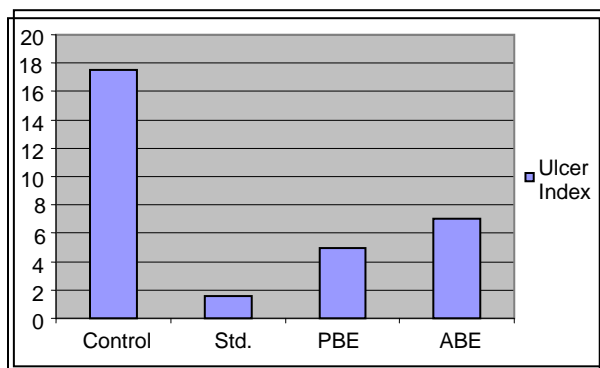


Fig 3. Effects of *Boswellia serrata* bark extracts, control and standard group on ulcer index in aspirin induced model.

small erosions with a minimal deviation from normal morphology, (Fig 5). Stomachs of rats treated with PBE extract showed small superficial erosion with minimal deviation from normal morphology, (Fig 6) and stomachs of rats treated with ABE extr-

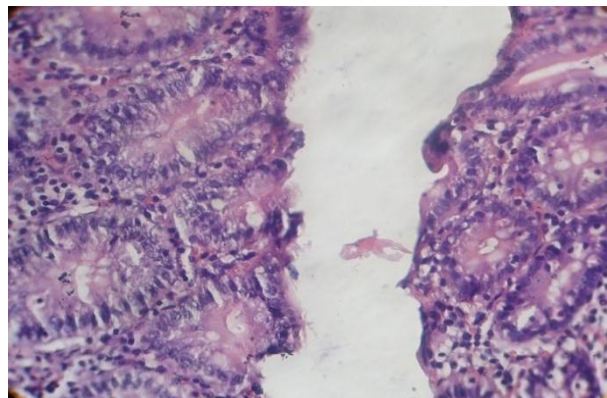


Fig 4. Stomach of control rat showing erosion in the upper part of epithelium with RBCs in eroded portion

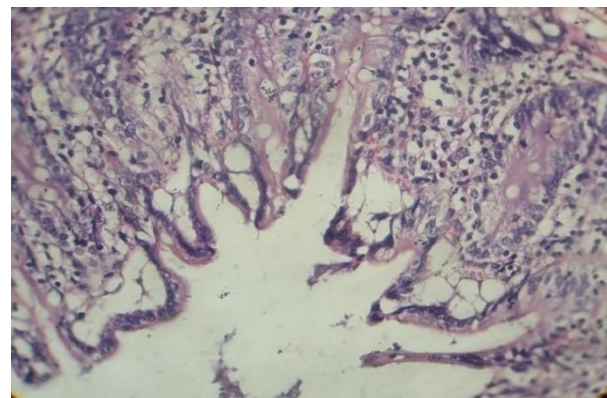


Fig 5. Stomach of rat treated with ranitidine showing small erosions with minimum deviation from normal morphology



Fig 6. Stomachs of PBE treated rats showing small superficial erosion with minimum deviation from normal morphology

act showed superficial erosions with minimum deviations from normal morphology (Fig 7).

Discussion

Peptic ulcer is one of the major ailments effecting humans and develops because of imbalance between aggressive factors (acid, pepsin, *H. pylori*, bile salts) and defensive factors (mucous, bicarbonate, blood flow, epithelial cell restoration and prostaglandins)²⁹.

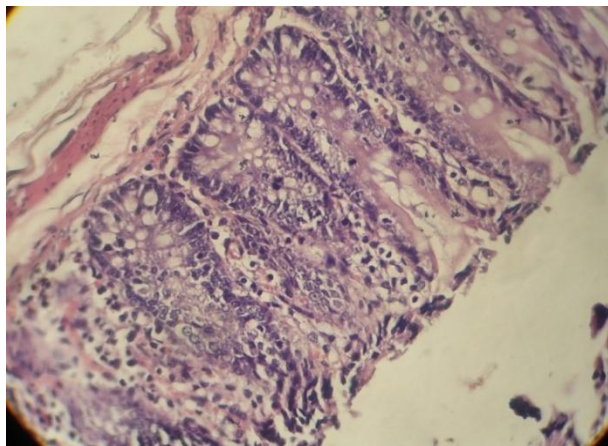


Fig 7. Stomach of rat treated with ABE showing superficial erosions with minimum deviations from normal morphology

There are several risk factors that may contribute to formation of ulcer in human beings such as stress, chronic use of anti-inflammatory drugs, continuous alcohol ingestion, *H. pylori* infection, Zollinger Ellison syndrome, etc. Although in most cases the etiology of ulcer is unknown. An effective antiulcer drug should act either by reducing the aggressive factors on gastroduodenal mucosa or by increasing mucosal resistance against them. The critical factors which maintain defense and integrity of gastric and intestinal mucosa include normal mucosal blood flow, local prostaglandins, mucous and bicarbonate secretion, epithelial proliferation and repair.

The treatment of peptic ulcer is mainly aimed at reducing the hydrochloric acid secretion, increasing gastric cytoprotection, eradication of *H. pylori* or curing Zollinger Ellison syndrome. The discovery of potential antiulcer agent from plants is a developing area. So far, several plants have been screened for antiulcer activity and many formulations have been developed by combining extracts of these plants³².

It was reported that Boswellic acid of *Boswellia serrata* is a known inhibitor of leukotriene by inhibiting 5-Lipo oxygenase (5-LOX). It may act by multiple mechanisms. The activity might be due to increasing the gastric mucosal resistance, local synthesis of cytoprotective prostaglandins and inhibiting the leukotriene synthesis³³. Since aspirin induces gastric ulceration by blocking prostaglandin production³⁰⁻³¹. In the present study treatment of *Boswellia serrata* extracts were able to prevent formation of ulcers.

It has also been reported that the presence of phytoconstituents tannins³⁴, terpenoids³⁵, sterols and flavonoids may be responsible for antiulcer

activity³⁶⁻³⁹ which is in agreement with our findings (Tables III-V and Fig 1 & 2).

Recent reports and extensive literature review indicated that flavonoids and tannins showed cytoprotective action by increasing mucosal content of prostaglandins and mucous in gastric mucosa⁴⁰.

Conclusion

The petroleum ether and aqueous extracts of bark of *Boswellia serrata* plant showed significant antiulcer activity which is evident by the data obtained.

Boswellia serrata having a tremendous potential deserves a special attention of the scientific fraternity to emerge as a milestone for medical science of this millennium due to its safety profile and can be a potent natural and safe alternative to conventional antiulcer treatment. However there is a shortage of clinical trial regarding its potency and efficacy.

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