J HerbMed Pharmacol. 2016; 5(3): 92-98.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Gastroprotective effect of plumbagin and ethanolic extract of plumbaginales in experimentally-induced ulcer

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 12 March 2015
Accepted: 3 June 2016

Keywords:
Anti-ulcer effects
P. zeylanica
P. indica
P. auriculata
Ulcer-induced model
Aspirin
Ethanol
Ulcer index

ABSTRACT

Introduction: Medicinal plants with phenolic compounds have been shown to have antimicrobial and antioxidant activities. The objective of the study was to evaluate the anti-ulcer effects of ethanolic extracts of Plumbaginales namely *P. auriculata*, *P. indica* and *P. zeylanica* and plumbagin in aspirin and ethanol induced gastric ulcer models.

Methods: In vivo studies including DPPH scavenging assay, lipid peroxidase inhibition assay, acid neutralizing capacity test, aspirin- and ethanol-induced ulcer models were performed to assess the antioxidant and antiulcer effects of plants. By using the models of Aspirin (200 mg/kg, 1 hour after the administration of last dose of the extract/ranitidine) and ethanol (1 mL/200 g, 90%) induced ulcer, animals were randomly divided into three groups of six animals each. Group I served as positive control, group II acted as standard and received ranitidine (20 mg/kg). The group III was treated with ethanolic extract by oral route in a dose of 300 mg/kg for a period of 5 days. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 mL of 0.9% NaCl and ulcers were scored.

Results: Both the aspirin- and ethanol-induced models of ulcer with various extracts of Plumbaginales showed significant acid neutralizing and antioxidant properties.

Conclusion: This study suggests that root extracts of *P. auriculata* may have good quality potentials for use in peptic ulcer diseases and that *P. auriculata* possesses an antiulcer effect.

Implication for health policy/practice/research/medical education:
This study belongs to a basic research program in the field of GIT pharmacology and herbal medicines. The main scope of this study was to explore the possible herbal medicines and to develop the herbal medicines for the experimental induced gastric ulcer model in albino Wistar rats. The outcome of this research will definitely help us to bring an effective herbal medicine with less toxic effects to counter the gastric ulcers.

Please cite this paper as: Ittiyavirah SP, Paul AS. Gastroprotective effect of plumbagin and ethanolic extract of plumbaginales in experimentally-induced ulcer. J HerbMed Pharmacol. 2016;5(3):92-98.

Introduction

The prevalence of peptic ulcer in India shows striking variation, the disease is common in some parts of the country and rare in others. India with extreme temperature has severe summers and winters. All year round, the south and coastal areas are warm and the hill districts are cold. In short, there is a correlation between ulcer prevalence and mean relative humidity as the incidence of ulcer being high in areas with high humidity. Ulcers have been ascribed in various parts of the India to hot foods, spices, fasting, overeating, excessive starch, alcohol, coffee and tea, and to smoking. India is still a developing country but people have to struggle for their day life; many of states and streets are best examples of these parasympathetic activities in human being (1). Another aspect of parasympathetic function is the physiological changes in anger, excitement, worry, suppressed emotions, and sexual arousal. Majority of these physiological changes are believed to be due to parasympathetic-related functions. The ulcers may lead to complicated stages such as intestinal bowel diseases (IBD), cancers, gastric upsets (2). The gastric problem is serious which affects the quality of life and a person's ability to carry out daily activities. According to the World Health Organization (WHO), ''Health is a state of complete physical, mental and social well-being and not merely an absence of disease or infirmity" (3). The rate of *Helicobacter pylori* infection are as high as 81% in people with peptic ulcer diseases (1). Due to limited resources, risk factor was excluded in this study. Univariate analysis suggested that male gender, age, lower socioeconomic sta-

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tus, cigarette smoking, family history of peptic ulcer and infection with *H. pylori* were all associated with increased risk of peptic ulcer. Separate analyses were performed by sex and occupational group to avoid confounding by cigarette smoking and age. Multivariate analyses showed that for all females and males, only family history was significantly predictive of peptic ulcer or duodenal ulcer. This study was designed to evaluate the anti-ulcer effects of ethanolic extracts of Plumbaginales namely *P. auriculata*, *P. indica* and *P. zeylanica* and Plumbagin in Aspirin and Ethanol induced gastric ulcer models.

Materials and Methods

Plant authentication

Samples of *P. auriculata*, *P. indica* & *P. zeylanica* were collected from different regions of Kerala. They were then authenticated (Voucher No. 1105, 1102, 1101) by School of Ecological Sciences, M.G. University, Kottayam, Kerala.

Plumbagin extract

Roots of *P. indica* were oven dried at 40˚C and powdered which was extracted with chloroform for 5 hours in a Soxhlet apparatus. The solution was evaporated to dryness. The extract obtained was dissolved in methanol and again evaporated to dryness to yield yellow crystals (4).

Preparation of Plumbagin free alcoholic extract

Roots of *P. auriculata*, *P. indica* and P. *zeylanica* (1 kg) were dried, coarsely powdered and soaked in limewater till red color of the lime water disappears. They were then dried again, finely powdered and extracted using Soxhlet's apparatus for 6 hours using ethanol as solvent. The extract obtained was concentrated and dried at room temperature.

The extraction efficiency was defined as follows:

$$\text{Percentage extraction (w/w)} = \frac{Mass\ of\ extracts}{Mass\ of\ dried\ root}$$

Estimation of the amount of Plumbagin in the extracts

One ml alcoholic KOH (10%) was added to 1ml of standard stock solution (1 mg/mL) of Plumbagin and the volume was then adjusted to the 5 ml with absolute alcohol. The absorbance of the colored solution was observed by UV/Visible spectrophotometer in the range of 400 to 800 nm against the reagent blank. The blank was prepared similarly in which volume of standard Plumbagin was replaced by an equal volume of absolute alcohol. The maximum absorbance was obtained at 520 nm. In a series of 5 mL volumetric flask, 0.2, 0.3, 0.4, 0.8 and 1.2 mL of standard stock solution of Plumbagin were mixed with 1 mL of 10% alcoholic KOH and volume was made upto the mark with absolute alcohol. The absorbance of colored solution was measured at 520 nm against a reagent blank. The absorbance was plotted against concentration of Plumbagin and the concentration of unknown solution was computed from the calibration graph or from the regression equation. 10 mg/mL of the four extracts were prepared as the standard sample solution with measuring absorbance at 520 nm (5).

In vitro Methods

DPPH assay

A stock solution of DPPH (1.3 mg/mL in methanol) was prepared such that 75 µl of it in 3 ml methanol gave an initial absorbance of 0.9. After 30 minutes, decrease in the absorbance in the presence of sample extract and standard at different concentrations was noted. A blank reading was taken using methanol instead of sample extract. Absorbance at 517 nm was determined after 30 minutes using UV-visible Spectrometer. The IC_{50} (Inhibitory concentration to scavenge 50% free radicals) was also determined. Lower absorbance levels of the reaction mixture indicate higher free radical scavenging activity. The IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The IC_{50} was calculated from equation of line obtained by plotting a graph of concentration vs. % inhibition. The ability to scavenge the DPPH radical was calculated using the following equation (6):

Percentage Inhibition = C-T/A × 100

Where C = Absorbance of DPPH alone, and T = Absorbance of DPPH along with different concentrations of extracts.

Lipid peroxidase assay

Lipid peroxidation product (i.e. malondialdehyde or MDA) was measured according to the method of Ohkawa et al (7). 1 mL of sample was mixed with 0.2 mL 4% (w/v) sodium dodecyl sulfate, 1.5 mL 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 mL of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated for 1 hour at 85°C in a hot water bath. The intensity of the pink color developed was read against a reagent blank at 532 nm (7).

Acid-neutralizing capacity

The acid-neutralizing capacity of the solute was carried out at a temperature of 37 ± 3°C. A pH meter was standardized using 0.05 M potassium biphthalate and 0.05 M potassium tetra oxalate standardized buffers. A magnetic stirrer was used to produce stirring rate of 300 ± 30 rpm. 0.5 g of the extract was transferred to a 250-mL beaker, and then 70 mL of distilled water added. Mixing with the magnetic stirrer continued for 1 minute. Then 30 mL 1 N HCl was added to the test solution with continuous stirring for 15 minutes. Excess HCl was titrated with 0.5 N NaOH to attain stable pH of 3.5. The number of mEq of acid consumed was calculated by the folowing formula (7):

Total mEq(ANC) = (30 × N of HCL) – (V of NaOH × N of NaOH)

N = Normality; V = Volume

Evaluation of ethanol-induced anti-ulcer activity

Samples of small intestine from goat were collected from a slaughterhouse. They were washed with the tyrode's solu-

tion to remove unwanted food materials and mesenteries. To keep the tissues active and live, they remained in the tyrode's solution supplied with air. The intestine samples were examined for any lesions or ulcers. Ethanol (80%) was used as an ulcerogenic agent. The goat small intestine was cut into six 1.5-2.0 cm long segments. The segments were opened upwards with mucous, washed and stretched on a dish containing the tyrode's solution. The dishes were supplied with air under constant temperature (8). The segments were divided into 2 groups, 3 segments each, and treated as following:

Group 1- control, treated with 1 mL of normal saline,
Group 2- treated with 1 mL of chloroform extract (Plumbagin),
Group 3- treated with 1 mL of *P. indica* extract,
Group 4- treated with 1 mL of *P. zeylanica* extract,
Group 5- treated with 1 mL of *P. auriculata* extract.

After 30 minutes of treatment of the groups, 1 mL of 80% of ethanol was added to each dish. Each intestinal segment was then examined for the presence of lesions and ulcers using a magnifying lens.

Statistical analysis

The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests with the normally-distributed data. Non-parametric tests were also used for the ordinal data. All the samples were compared themselves and statistical significance was noted.

In vivo Methods

Animals

The rats were housed in polyacrylic cages and maintained at 27 ± 2°C. They were fed with standard pellet diet (Hindustan Lever Ltd. Bangalore, India) and water *ad libitum*. All animal procedures were approved by the Animal Ethical Committee (No: 008/MPH/UCP/CVR/12) in endogenous defense mechanism

Aspirin-induced model

Animals were randomly divided into three groups, each with six animals. Group I served as positive control, Group II acted as standard and received Ranitidine (20 mg/kg). The group III was treated with ethanolic extract by oral route in a dose of 300 mg/kg for a period of 5 days in aspirin-induced ulcer model. On day 5, aspirin at a dose of 200 mg/kg was administered to the animals of all groups, 1 hour after the administration of last dose of the extract/ranitidine. After 4 hours, the animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 mL of 0.9% NaCl and ulcers were scored (9).

Ethanol-induced model

Animals were randomly divided into three groups, each with six animals.

- Group I: Untreated group received distilled water (p.o) for 9 days followed by ethanol (5 mL/kg, p.o) on 11th day.
- Group II: Ranitidine group (20 mg/kg, p.o) for 9 days followed by ethanol (5 mL/kg, p.o) on 11th day.
- Group III: Test group (300 mg/kg, p.o) for 9 days followed by ethanol (5 mL/kg, p.o) on 11th day.

All ethanol-treated animals were fasted for 36 hours before administration of ethanol. The animals in the standard drug group and aqueous extract (test drug group) were pretreated with respective drugs for 9 days. Later, food and water were withdrawn for 36 hours. The respective drugs were administered 1 hour before ethanol administration. Ethanol (90%) was administered to all animals at a dose of 1 mL/200 g. After 1 hour, the animals were sacrificed, stomach was removed slightly inflated by injecting 15% formalin solution for 10 minutes. The stomachs were then opened along the greater curvature with ulcer scoring and percentage inhibition (10).

Ulcer Score

The number of ulcers was noted and the severity recorded with the following scores (11):

Ulcer index

Ulcer index (UI) was calculated using the formula
$UI = US + UN + UP \times 10^{-1}$, where, US = Mean severity of ulcer score; UN = Average number of ulcers per animal; and UP = Percentage of animals with ulcer incidence.

Percentage protection

Percentage protection of ulcers = CUI-TUI/CUI, where CUI = Ulcer index of control groups, and TUI = Ulcer index of treated groups.

The mean scores for each group were then calculated with analyzing the results.

Histological studies

A portion of the ulcer region in the stomach was dissected out and fixed in 5% buffered neutral formalin solution for histological observations. Following fixation, tissues were embedded in paraffin and sections with a 5 µm thickness were cut from the paraffin block and then stained with hematoxylin and eosin (H & E). The sections were examined with the help of a light microscope and photomicrographs were taken and scored (12).

Statistical analysis

The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dennett multiple comparisons for the data which were normally distributed. For the data of ordinal type, a non-parametric test was used. Statistically significant results were noted.

Results

Plumbagin extraction

A yellow crystal of Plumbagin was obtained. The amount of Plumbagin obtained was 12.6 g with a percentage yield of 1.26%. The percentage yield of Plumbagin in *P. zeylanica* extract was found to be 1.37%. That in *P.auriculata* extract, and *P.indica* was 0.78% and 1.5%, respectively.

DPPH assay

The obtained percentage inhibition of different concentrations of Plumbaginales is shown in Table 1. The IC_{50} value of Plumbagin, *P. indica, P. zeylanica* and *P. auriculata* was found to be 33.94, 20.93, 29.03 and 20.11 µg/mL, respectively.

Table 1. Average percentage inhibition of *P. indica, P. zeylanica, P. auriculata* and Plumbagin in DPPH assay and lipid peroxidase assay

	Assay method	Control	10	20	30	40
Plumbagin	DPPH assay	-	18.75± 0.79	34.32±1.26	39.12±0.79	60.94±1.41
	Lipid peroxidase	-	6.64± 2.50	23.31±1.57	34.14±1.96	49.98±2.32
Plumbago indica	DPPH assay	-	31.24±1.09	53.13±0.76	65.71±0.70	71.87±1.21
	Lipid peroxidase	-	16.91±2.14	36.65±2.12	49.98±1.37	62.46±2.86
Plumbago zeylanica	DPPH assay	-	21.25±0.93	33.12±0.67	51.87±1.02	68.12±0.60
	Lipid peroxidase	-	8.26±3.62	33.27±3.49	42.48±2.09	64.40±10.46
Plumbago auriculata	DPPH assay	-	37.19±0.30	51.24±0.87	62.5±0.05	71.24±1.39
	Lipid peroxidase	-	24.10±3.78	47.52±0.69	57.79±3.04	68.30±2.06

Table 2. Number of ulcer spots in ethanol induced in vitro models

Sl No.	Group	Ulcer No.	Average Ulcer No (Mean ± SD)	% Protection
1	Positive	8		
2	Control	9	8.33 ± 0.58	
3		8		
1		4		
2	Plumbagin	6	4.67 ± 1.54	43.94
3		4		
1		3		
2	*P. indica*	5	4.33 ± 1.54	48.02
3		5		
1		3		
2	*P. zeylanica*	2	2 ± 1.0	75.99
3		1		
1		0		
2	*P. auriculata*	0	0.33 ± 0.58	96.04
3		1		

Lipid peroxidase assay

The obtained percentage inhibition of different concentrations plumbaginales is shown in Table 1. The IC_{50} value of Plumbagin, *P. indica, P. zeylanica* and *P. auriculata* was found to be 40.26, 30.68, 32.27 and 25.41 µg/mL, respectively.

Acid neutralizing capacity

P. auriculata extract showed maximum of the total mEq of acid consumed (23.6) as compared to the other extracts. It was followed by *P. zeylanica* (21.2), *P. indica* (20.3) and Plumbagin (16.7).

Evaluation of ethanol- induced anti-ulcer activity

This novel in vitro ulcer model gave a percentage protection of 43.94, 48.02, 75.99 and 96.04 for Plumbagin, *P. indica, P. zeylanica* and *P. auriculata*, respectively, with a *P* value of <0.0001 (F: 31.81 and df: 4,2). The results were shown in Table 2.

In vivo methods

Aspirin-induced model

In this model, treatment with a standard drug ranitidine (30 mg/kg) significantly reduced ulcer number (0.83 ± 0.40) ulcer score (1.92 ± 0.55), ulcer index (5.13 ± 0.05). A significant rise in ulcer number (7.67 ± 0.61), ulcer score (25.83 ± 0.91), ulcer index (11.41 ± 0.08) was noted in the positive control group. The extract of *P. auriculata* at a dose of 300 mg/kg significantly reduced the ulcer number (0.83 ± 0.40), ulcer score (1.92 ± 0.55) and ulcer index (5.13 ± 0.05). The extracts produced a significant (P < 0.0001, df: 2,5 and F: 2207) anti-ulcer activity with a percentage protection of 40.45% and that of the standard was 70.57%. Table 3 depicts the results.

Ethanol-induced model

In this model, treatment with a standard drug ranitidine (30 mg/kg) significantly reduced ulcer number (0.33 ± 0.21) ulcer score (1.92 ± 0.24), ulcer index (3.42 ± 0.03). A significant rise in ulcer number (10.50 ± 0.76), ulcer score (22.58 ± 0.90), ulcer index (11.62 ± 0.10) was noted in the positive control group. The extract of *P. auriculata* at a dose of 300 mg/kg significantly reduced the ulcer number (1.17 ± 0.40), ulcer score (5.25 ± 0.81) and ulcer index (6.92 ± 0.05). The extracts produced a significant (P < 0.0001, df: 2, 5 and F: 4025) anti-ulcer activity with a percentage protection of 39.35% that of the standard was 55.04%. Table 4 depicts the results.

Histological studies

The results on aspirin-induced histopathological investigations revealed that the pretreatment with *P. auriculata* extract absolutely prevents the aspirin-induced congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes erosions and ulceration in the gastric mucosa of rats, whereas the positive control showed severe ulceration and inflammatory changes in the gastric mucosa. The standard group showed normal gastric histology.

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Discussion

Oldest medicinal systems in the world can lead to find therapeutically useful compounds from plants. Therefore, ethnomedicinal knowledge supported by modern science is necessary to isolate, characterize, and standardize the active constituents from herbal source. This combination of traditional and modern knowledge can produce better antiulcer drugs with fewer side effects. Research on natural products often is guided by ethnomedicinal knowledge, and has made substantial contributions to drug in-

Table 3. Antiulcer effect in aspirin induced ulcer models in rats

Sl No.	Group	Ulcer number	Average ulcer number (Mean ± SEM)	Ulcer score	Average ulcer score (Mean ± SEM)	Ulcer index	Average ulcer index (Mean ± SEM)
1		8		26		11.45	
2		5		23.5		11.09	
3	Control	9	7.67 ± 0.61	29.5	25.83 ± 0.91	11.64	11.41 ± 0.08
4		7		26		11.35	
5		8		26.5		11.46	
6		9		23.5		11.49	
1		2		4		5.30	
2		2		0.5		5.26	
3	Standard	0	0.83 ± 0.40	3	1.92 ± 0.55	5.03	5.13 ± 0.05
4		0		1		5.03	
5		0		1		5.03	
6		1		2		5.15	
1		4		8		7.27	
2		1		5		6.89	
3	Test	2	1.33 ± 0.61	4.5	4.58 ± 0.87	6.98	6.92 ± 0.08
4		1		1.5		6.81	
5		0		3.5		6.76	
6		0		5		6.79	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 4. Antiulcer effect in Ethanol induced ulcer models in rats

Sl No.	Group	Ulcer number	Average ulcer number (Mean ± SEM)	Ulcer score	Average ulcer score (Mean ± SEM)	Ulcer index	Average ulcer index (Mean ± SEM)
1		8		20.5		11.31	
2		10		22.5		11.56	
3	Control	13	10.50±0.76	26.5	22.58 ± 0.90	11.96	11.62 ± 0.10
4		9		20.5		11.41	
5		11		23		11.68	
6		12		22.5		11.76	
1		0		2		3.38	
2		0		1		3.36	
3	Standard	0	0.33±0.21	1.5	1.92 ± 0.24	3.37	3.42 ± 0.03
4		0		2		3.38	
5		1		2.5		3.50	
6		1		2.5		3.50	
1		2		7		7.04	
2		2		7		7.04	
3	Test	2	1.17±0.40	5	5.25 ± 0.81	6.99	6.92 ± 0.05
4		1		2		6.82	
5		0		4		6.77	
6		0		6.5		6.83	

*P<0.05,**P<0.01,***P<0.001

novation by providing novel chemical structures and/or mechanisms of action. Large numbers of herbal extracts are used in folk medicine to treat various gastrointestinal disorders. Different varieties of Plumbago species can be found in different countries. The present folklore-based studies focused on the certain herbs of Plumbaginales, which are traditionally mentioned as an antiulcer in a folk literature of *Materia medica*, screened for their anti-ulcer activities. Reviews based on the traditional knowledge and the use of plants also focus the antibacterial activity of the plants, hence the future prospect could focus on the field of gastroprotective effects of herbal medicine.

Soxhlet was the most efficient extraction technique however, prolonged heating time promoted Plumbagin degradation and *P. indica species* has a maximum of Plumbagin content. So in the present study *P. indica was* used for extraction of Plumbagin with a yield of only 1.26%. Constricted results may due to the climatic conditions during extraction.

The extent of decrease in the absorbance of DPPH in the presence of antioxidants is associated with the free radical scavenging potential of the antioxidant. These scavenging activities might be due to the presence of different phytochemical constituents. Color formation with DPPH is indicative of a balance between antioxidants and free radicals. The scavenging ability of *P. auriculata* extract is higher as compared to the other extracts. Analytical investigations revealed that extract of *P. auriculata* is an excel-

lent antioxidant. The results of different extracts were of the same magnitude. All the extracts show a positive scavenging capacity with DPPH, but the their quantities differ. Antioxidant activities parallel with those of the previous works (13-17).

Among the cellular molecules, lipids containing unsaturated fatty acids and with more than one double bond are particularly susceptible to action of free radicals. The resulting reaction, known as lipid peroxidation, disrupts biological membranes and is thereby highly deleterious to their structure and function. Lipid peroxidation is being studied extensively in relation to disease, modulation by antioxidants and other contexts. During this process, a large number of by-products are formed which affect at a site away from area of their generation. Hence they behave as toxic 'second messengers'. Oxygen dependent deterioration of lipids, known as rancidity, is the after effect of the oxidation. Malondialdehyde are formed as a result of the fission of cyclic endoperoxidases. With thiobarbituric acid, MDA readily forms an addict. This proves that the selected plants possess antioxidant activities to give a ground for anti- free radical mechanism.

Thus, extracts could pose antioxidant activities against excessive formation of reactive oxygen species (ROS). This is due to the impaired antioxidant defense system in ulcerative conditions that cause membrane damage, leading to deleterious effects. Studies show that plants with high antioxidant activity are able to inhibit free radicals produced in biomolecules. Thus this species with an antioxidant activity showed a type of prevention intervention. The stomach's acidic interior is generated by "stomach acid" (essentially 0.1 M hydrochloric acid) (18). This acid is necessary for digestion but too much stomach acid can cause discomfort. One way of relieving excess acidity in the stomach is to neutralize some of the acid with a weak base or "antacid". Acid neutralizing capacity (ANC) is a measure of the ability to neutralize acid inputs. The present study aimed at comparing the ANC of extracts. All the extracts showed good acid neutralizing capacity, especially *P. auriculata.* This study can be compared with other medicinal plants used in Ayurvedic therapeutics (19).

In vitro ethanol ulcer model in goat ileum is a novel approach to check if the targeted drug or extract has an ulcer protecting effect. This easily-used method does not require animal sacrifice. By this method, the action of drugs alone can be studied with exclusion of indirect actions of drugs via other sites in the body. The results of the current study show an antiulcer activity of the extracts against ulcers. The plant shows an anti-ulcer activity as compared to the results obtained for ranitidine

A possible explanation of these variations could have been due to the differences in chemical constituents of each plant species. The dose selection for the current study (300 mg/kg) was based on the on the acute toxicity studies that were previously conducted for the anti-inflammatory and analgesic screening of the selected plants (19,20).

When aspirin is in its lipid soluble undissociated form, it can damage the gastric mucosa. After aspirin-induced ulcers,, the rate of acid secretion varies with prostaglandin deficiency as ulcer is mediated through tissue damaging free radicals. Ethanol-induced ulcers are widely used for the evaluation of gastroprotective activity. Ethanol can produce free radicals through stimulating the formation of leukotrienes and mast cell secretary products. In fact, incidence of ulcer is predominant in the glandular part of the stomach. From this it is clear that free radicals play a role e in implication of mucosal ulceration scavenging of and consequently in healing ulcer. In the present study led to significant reduction of ulcers in the test group as compared to the controls. The protection against ulcers in both models indicates cytoprotective activity of the herbal extract. This may have been done through an antioxidant activity of the prostaglandin produced by the gastric mucosa, which helps to scavenge the generated free radicals. The current study suggest that the plant extract has a significant antiulcer activity comparable to control.

The data obtained were subjected to statistical analysis with one-way ANOVA followed by Dunnett and Tukey post-tests. In vitro results were caparisoned using Tukey test to compare the most effective antiulcer herb among the selected plants of Plumbago species. From the In vitro studies *P. auriculata* was selected for in vivo studies; the percentage protection of the plant was compared to that of positive control and standard.

The extract of *P. auriculata* and deprived of Plumbagin has shown a beneficial anti-ulcer activity. Factors such as oxidative stress, acidity, NSAIDs, and ethanol were evaluated for Plumbaginales undertaken in this study. It is obvious that this plant may have an anti-ulcer activity. On the basis of in vitro studies, antiulcer activities of *P. zeylanica* and *P. indica* cannot be neglected. Plumbagin with antioxidant and acid neutralizing capacities can be used against ulcers. Therefore, attention has been focused on studying the various plants of Plumbago species as a whole for its gastro protective effects especially antiulcer activity.

Conclusion

It is concluded that the Plumbago species play a protective effect on GIT ulcers. Various herbal plants extracts, *P. auriculata, P. indica* and *P. zeylanica* have significant antiulcer activities in animal models. *P. auriculata* shows significant antioxidant and acid neutralizing capacities. Novel in vitro antiulcer method using goat ileum indicates that all plants represent an antiulcer activity. The present study explores the plant's mechanism of action in experimentally -induced gastric ulcers. Among the various Plumbaginales, *P. auriculata* could able to show a gastroprotective activity as compared to Ranitidine. In sum, the results of this study indicate that root extracts of *P. auriculata* may have good quality potentials for use in peptic ulcer diseases and that *P. auriculata* possesses an antiulcer effect.

Acknowledgements

The authors like to acknowledge Mahatma Gandhi University for providing all support and basic facilities for the success of this project. We have not availed any grant either

from funding agencies or from any institutions.

Authors' contributions

Authors have equal responsibility for the success of this project work. However, the experimental design and drafting of the paper is under the direct responsibility of the corresponding author.

Conflict of interests

The authors declared that there is no conflict of interest.

Ethical considerations

The authors like to declare that all the experiments were conducted by following the ethical guidelines drafted by CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) a body constituted to safeguard to safeguard the ethical issues in animal studies in India. The present study was conducted after getting prior approval from the Institutional Animal Ethical Committee (IAEC) bearing the approval (No: 008/MPH/UCP/CVR/12). The IAEC is constituted as per the provision of CPCSEA.

Funding/Support

Not availed any grant either from funding agencies or from any institutions.

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