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EVALUATION OF ANTI-ULCER ACTIVITY OF PERGULARIA EXTENSA LINN IN ALBINO RATS

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Abstract

The present study was undertaken to determine the antiulcer activity of the methanolic extract from the barks of Pergularia extensa Linn. The preliminary phytochemical investigation showed the presence of alkaloids, saponins, flavonoids, terpenoids, tannins, cardiac glycosides, gums andphytosteroids. The pharmacological and acute toxicity studies of Methanolic extract was performed by following, OECD-423 guidelines. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants. Similarly, the Methanolic extract of Pergularia extensa Linn.,, showed the presence flavonoids and their glycosides, tannins, triterpenoids and saponins. These phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by aspirin. Pergularia extensa Linn., showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 27.18, 45.47 and 61.28 at doses of 100, 200 and 400mg/kg doses respectively. The ulcer protective action of extracts at 400mg/kg was good to that of standard drug.

Keywords: Anti-Ulcer Activity, Aspirin Induced Ulcer, Pantoprazole, Pergularia Extensa Linn.

Introduction

The stomach lies between the oesophagus and the duodenum (the first part of the small intestine). It is on the left upper part of the abdominal cavity. The top of the stomach lies against the diaphragm. Two sphincters keep the contents of the stomach contained. They are the esophagealsphincter dividing the tract above, and the Pyloric sphincter dividing the stomach from the small intestine. The stomach is surrounded by parasympathetic (stimulant) and ortho sympathetic (inhibitor) plexuses (networks of blood vessels and nerves in the anterior gastric, posterior, superior and inferior, celiac and meiotic), which regulate both the secretions activity and the motor (motion) activity of itsmuscles. In adult humans, the stomach has a relaxed, near empty volume of about 45 ml. The stomach of a newborn human baby will only be able to retain about 30ml. Peptic ulcer disease is one of several disorders of the upper gastrointestinal tract that is caused, at least partially, by gastric acid. Patients with peptic ulcer disease may present with a range of symptoms, from mild abdominal discomfort to catastrophic perforation and bleeding.[1]

Materials & Methods

Plant Profile

Plant name :Pergularia extensa
Family :Malvaceae
Drugs&Chemicals: Freund's Adjuvant injection, Diethyl ether, Indomethacin, Distilled water, surgical spirit, Carboxy methyl cellulose, Formaldehyde and Ethanol

Procedure

Determination of LD_{50} value of Pergularia extensa linn

Acute Oral Toxicity Study

The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion. All the experimental procedures were reviewed and approved by Institutional Animal Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals. The method uses defined doses (5, 50, 300, 2000 mg / kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized system (GHS) for the classification of chemical which cause acutetoxicity.

Procedure

Six animals (Albino mice, 25-75gm) were selected for studies. The methanolic extracts of Pergularia extensa Linn., was administered through oral route. Most of the crude extracts possess LD_{50} value more than 2000 mg. /kg of the body weight of animal used. Dose volume was administered 0.1 ml / 100 gm body weight to the animal by oral route. After giving the dose the toxic signs

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were observed within 3-4 hours. Body weight of animals before and after administration, onset of toxicity and signs of toxicity like changes in skin and fur, eyes, and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern, signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was also to be noted, if any, wasobserved.[3,4]

Pharmacological evaluation

Evaluation of anti-ulcer activity

Animals

Male Albino rats, weighing 150-200g were used in the present study. All the rats were kept at room temperature (22°C) in the animal house. All the animals were housed and treated as per the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food and were acclimatized to laboratory conditions. All the experimental procedures were reviewed and approved by Institutional Animal Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals.[5]

Aspirin induced ulcer

Male Albino rats were divided in to six groups of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy. Six groups were made as below.

Group I - received 1% Methyl cellulose (1.0ml/kg p.o) as normal control.
Group II - received 1% Methyl cellulose (1.0ml/kg p.o) as vehicle control.
Group III - received (100mg/kg, p.o) Methanolic extract of Pergularia extensa Linn.,
Group IV - received (200mg/kg, p.o) Methanolic extract of Pergularia extensa Linn.,
Group V - received (400mg/kg, p.o) Methanolic extract of Pergularia extensa Linn.,
Group VI - received (20mg/kg, p.o) Pantaprazole as standard.

One hour after the drug treatment the animals were treated with absolute aspirin [500mg] to induce ulcers. The animals were sacrificed after 1hr and stomach was opened and percentage inhibition of ulcer was determined.[6]

Biochemical parameters

The stomach was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The gastric contents were analyzed for gastric juice volume, pH. The results are given intable No.1

Measurement of gastric juice volume and pH :

Gastric juice was collected from aspirin induced ulcer rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100g body weight. The pH of the supernatant was measured using digital$P_{H.}$ [7]

Ulcer index (UI)

The mucosa was flushed with saline and stomach was pinned on frog board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined. [8]

- 0– Normal colored stomach
- 0.5 – Redcoloration
- 1– Spotulceration
- 1.5 – Haemorrhagicstreak
- 2–ulcers
- 3–Perforations.

Percentage inhibition:

Percentage inhibition was calculated using the following formula.

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$$\% \text{ inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{ulcer}}}{UI_{\text{ulcer control}}} \times 100$$

Statistical Analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison test. The values are statistically significant at three levels, ***p<0.001. **p<0.01. *p<0.05. But ns if p > 0.05. [9, 10]

Results & discussion

Pergularia extensa Linn., was subjected for hot continuous extraction using methanol as solvent. The yield for methanolic extract was found to be 12.75% w/w.

S.NO	PHYTOCHEMICAL CONSTITUNTS	METHANOLICIC EXTRACT
1	Alkaloids	++
2	Saponins	++
3	Tannins	++
4	Terpenoids	++
5	Flavonoids	++
6	Carbohydrates	_ _
7	Cardiac glycosides	++
8	Phytosteroids	++
9	Amino acids	_ _
10	Gums	++

Table-1: Phytochemical screening of Pergularia extensa Linn.,

Pharmacological studies

Acute oral toxicity studies

The acute oral toxicity of the methanolic extract of Pergularia extensa Linn., was carried out as per OECD 423 – guidelines (Acute toxic class method). The acute toxicity studies revealed that LD_{50}> 2000mg/kg for the extract. Hence, the biological dose was fixed at 100, 200 and 400mg/kg of body weight for the extract.

Anti-ulcer screening

Aspirin induced ulcer

Effects of Methanolic extract of Pergularia extensa Linn., on ulcer index induced by aspirin in rats are shown in Table No 2. Aspirin induced gastric damage showed gross mucosal lesion, including long haemorrhage bands and petechial lesion. Animals pretreated with Methanolic extract of Pergularia extensa Linn., and standard drug Pantaprazole showed very mild lesions and sometimes no lesion at all, when compared to ulcer control group. Pergularia extensa Linn., showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 23.16, 43.93 and 63.01 at doses of 100, 200 and 400mg/kg doses respectively. The ulcer protective action of extracts at different doses was better as that of standard drug, pantaprazole, which exhibited an inhibition percentage of 78.73. Aspirin produces severe gastric haemorrhagic lesions. The pathogenesis of aspirin induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leucotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage. So, the cytoprotective mechanism of the Pergularia extensa Linn., extract may therefore include mechanisms other than simple acid neutralization.

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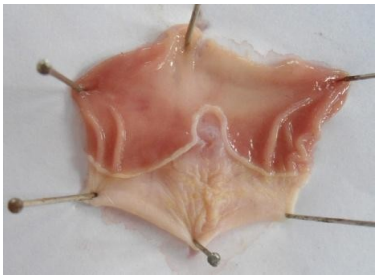
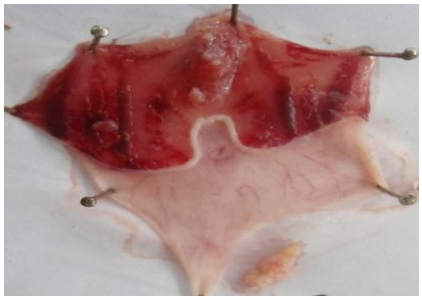
GROUP	ULCER INDEX (UI)	PERCENTAGE INHIBITION (%)
Normal Control	00.00 ± 0.00	-
Ulcer Control	11.7 ± 2.13***	-
Pergularia extensa Linn., (100mg/kg)	8.52 ± 1.61*	27.18
Pergularia extensa Linn.,(200mg/kg)	6.38 ± 1.07	45.47
Pergularia extensa Linn., (400 mg/kg)	4.53 ± 1.63	61.28
Pantaprazole (20 mg/kg)	2.51+ 0.83	78.54

Table-2: Effect of Pergularia extensa Linn., on Ulcer Index in Aspirin induced gastric ulcer

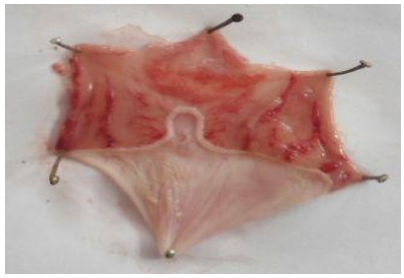
All values are expressed as mean ± S.E.M.; (n=6) animals in each group. **P<0.001, *P<0.01, Ulcer control group was compared with Normal control group. Pantoprazole and Extract treated groups were compared with ulcer control group.



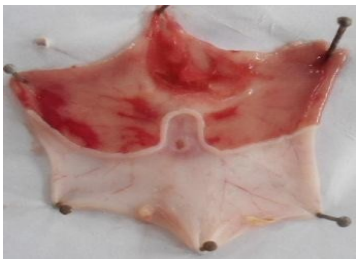
1. NormalControl



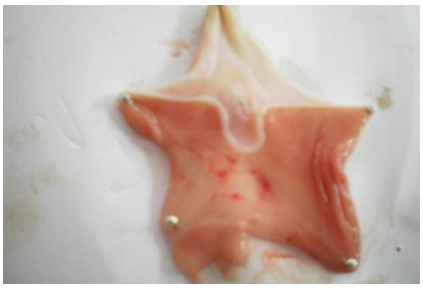
2. Ulcer control



3. MEPE (100mg/kg)



4. MEPE (200mg/kg)



5. MEPE (400mg/kg)

6. Pantoprazole (20mg/kg)

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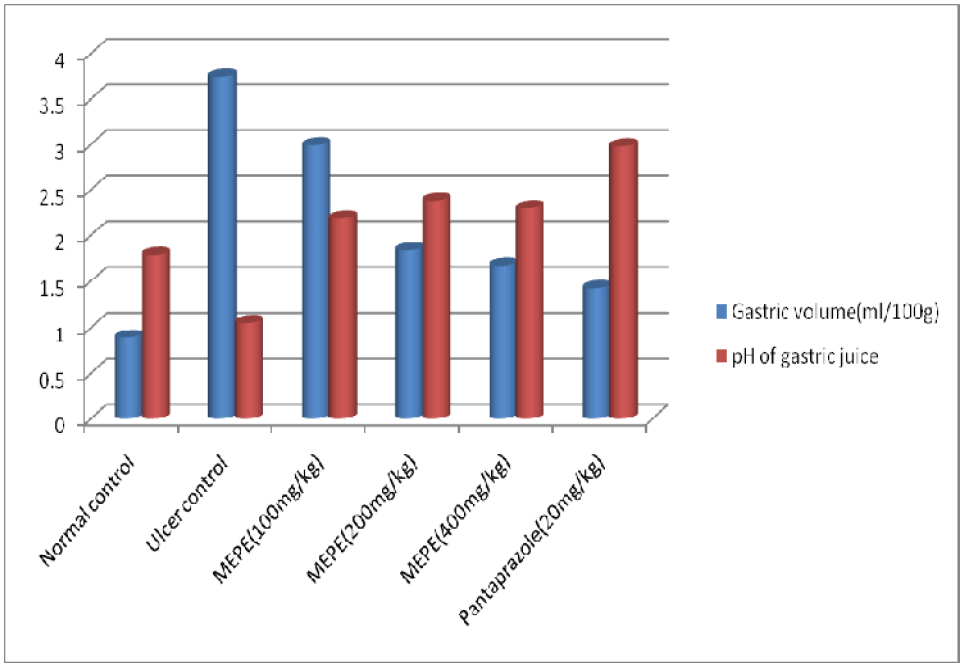
Figure-1: Effect of Pergularia extensa Linn., on Ulcer Index in Aspirin induced gastric ulcer



Ulcer index (UI) and acid parameters

The effects of Methanolic extract of Pergularia extensa Linn.,on acid parameters showed significant effect at 100mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of the gastric juice was increased compared to ulcer control group. But, in this gastric environment also able to induce ulcer, so it can be thought that the anti-secretory activity might not be the main mechanism of action of these extracts.

Group	Gastric volume (ml/100g)	pH of gastric juice
Normal Control	0.89 ± 0.08	1.79 ± 0.06
Ulcer control	3.74 ± 0.71***	1.05 ± 0.39
Pergularia extensa Linn., (100mg/kg)	2.99 ± 0.45	2.19 ± 0.61
Pergularia extensa Linn., (200mg/kg)	1.84 ± 0.30	2.38 ± 0.23
Pergularia extensa Linn.,(400mg/kg)	1.67 ± 0.35	2.30 ± 0.54
Pantaprazole(20mg/kg)	1.42+0.22	2.98+0.68

Table-3: Effect of Pergularia extensa Linn., on Gastric secretion, pH using Aspirin induced ulcer

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. ***P<0.001, **P<0.01, Ulcer control group was compared with Normal control group. Pantoprazole and Extract treated groups were compared with ulcer controlgroup.

Conclusion

The present study was undertaken to determine the antiulcer activity of the methanolic extract from the barks of Pergularia extensa Linn. The preliminary phytochemical investigation showed the presence of alkaloids, saponins, flavonoids, terpenoids, tannins, cardiac glycosides, gums andphytosteroids. The pharmacological and acute toxicity studies of Methanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed (3 days) up to 2000mg/kg of body weight. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritant. Similarly, the Methanolic extract of Pergularia extensa Linn.,, showed the presence flavonoids and their glycosides, tannins, triterpenoids and saponins. These

phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by aspirin. Pergularia extensa Linn., showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 27.18, 45.47 and 61.28 at doses of 100, 200 and 400mg/kg doses respectively. The ulcer protective action of extracts at 400mg/kg was good to that of standard drug, pantoprazole, which exhibited an inhibition percentage of 78.54.

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