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"Evaluation of Phytochemicals and Study of Phoebe Lanceolata for Antiulcer Effect"

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ABSTRACT
This study focuses on the phytochemical evaluation of Phoebe lanceolata for their antiulcer effect. The methanolic extract of Phoebe lanceolata yielded 8.96% w/w, indicating good extractive value. Phytochemical screening confirmed the presence of flavonoids, phenols, proteins, carbohydrates, and selected alkaloids, while glycosides, saponins, diterpenes, and tannins were absent. Quantitative estimation revealed that the extract contains a high concentration of total flavonoids (0.956 mg/100 mg) and phenols (0.857 mg/100 mg), suggesting strong pharmacological potential and supporting its traditional medicinal use. Acute toxicity study revealed that extracts at the dose of 2000 mg/kg didn't exhibit any sign of toxicity or mortality up to 14 days. This finding suggested that the experimental plant had a wider safety margin and an LD50 value greater than 2000 mg/kg in rats, since any test substance that is not toxic at 2000 mg/kg is considered relatively safe. The percentage of ulcer inhibition for Phoebe lanceolata extract exhibited a significant reduction compared to the indomethacin group. These effects were seen in cases of indomethacin-induced gastric mucosal injury and were similar to those produced by the reference standard drug.

Keywords: Phoebe lanceolata, antiulcer activity, phytochemical screening, flavonoids, phenolics,

I. INTRODUCTION

Various medicinal plants are used traditionally in the treatment of peptic ulcer. Plants and phytomedicines exhibit their action by various mechanisms like antioxidant, cytoprotective, antisecretory action. The need to replace certain drugs due to their adverse effects exemplifies the universal role of plants in the treatment of disease and the maintenance of health **(Sharifi-Rad et al., 2018).** The utilization of medicinal plants for the treatment of various ailments has been practiced since ancient times and is commonly referred to as phytotherapy. The healing effects of medicinal plants come from the fact that they accommodate a wide range of secondary metabolites, called phytochemicals, which can be synthesized again and again in nature. The richest sources of active metabolites are contained in seeds, bark, roots, leaves, and fruits of plants. Therefore, a multitude of plants have employed these phytochemicals as a defence mechanism against pathogens **(Alamgir, 2018).**

Phoebe lanceolata (Lauraceae) and Stephania glabra (Menispermaceae) are common plants of central Himalaya. These plants have been used in the traditional system of medicine in India for treatment of various diseases. Aqueous extract of berries from P. lanceolata is important remedy for wounds and sore and S. glabra tubers has been used in psycho-disorders, tuberculosis, asthma, diabetes, dysentery and fever **(Omar, 2015)** various alkaloids from these plant species [6-10]. n-hexane, acetone and ethanol extracts of these plants were evaluated for antimicrobial activities against the test microorganisms to determine the MIC and IZD. n-hexane and acetone extracts showed poor activity against most of the test microorganisms **(Doughari and Saa-Aondo 2021).**

Ulcers represent a chronic and recurrent condition distinguished by the intermittent occurrence of sores within the mucosal membranes of the stomach. Peptic ulcer is one of the most common, chronic gastrointestinal disorder in modern era. Now it has become a common global health problem affecting a large number of people worldwide and also still a major cause of morbidity and mortality **(Bereda, 2022).** Peptic ulcer disease can be characterized by inflamed lesions or excavations of the mucosa and tissue that protect the gastrointestinal tract. Damage of mucus membrane which normally protects the oesophagus, stomach and duodenum from gastric acid and pepsin causes peptic ulcer. The pathophysiology of this gastro-intestinal disorder is viewed as an imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric

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oxide, peptides, growth factors and injurious factors like acid, pepsin **(El-Deeb and Al-Madboly 2021).** Though different classes of drugs are used in the treatment of peptic ulcer but most of the drugs are exhibits serious side effects like arrhythmias, impotence, gynaecomastia, arthralgia, hypergastrinemia and haemopoeitic changes. The use of phyto-constituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer **(Bharathajothi and Bhaaskaran, 2017).**

This review aims to offer the phytochemical composition of Phoebe lanceolate and explore its potential as a therapeutic agent foe managing ulcers.

II. METHODS AND MATERIALS

2.1 Chemicals

Iodine, Potassium Iodide, Sodium nitroprusside, Sodium hydroxide, Lead acetate and Folin-Ciocalteu reagent was produced from Loba chemie Pvt. Ltd., Mumbai. Potassium Mercuric Iodide, Picric acid and Ferric chloride, was received from Thomas Baker, Mumbai. Potassium Bismuth Iodide, Pyridine, Gelatin, Nitric acid, Copper acetate and Sodium Chloride was acquired from S. D. Fine Chem. Ltd., Mumbai. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Qualigens Fine Chemicals, Mumbai and Himedia.

2.2 Collection of plant materials

The plants have been selected on the basis of its availability and folk use of the plant. Roots of Phoebe lanceolata were collected from local area of Bhopal in the month of February, 2025. Drying of fresh plant parts was carried out in sun but under the shade. Dried roots of Phoebe lanceolata were preserved in plastic bags, closed tightly and powdered as per the requirements.

The plant was authenticated by the taxonomist Dr. Jashwinder Mehta, Department of Botany, Career College, Bhopal (M.P.). Herbarium of plant was submitted in the Department of Botany.

2.2.1 Extraction by maceration process

Extraction from plant materials is an important step in phytochemical processing for discovering bioactive secondary metabolite. Selection of a suitable extraction technique is also important for the standardization of herbal products. Extraction is used to extract suitable soluble constituents, with the aid of the chosen solvents except those not necessary. The products obtained from the plant were thoroughly washed in tap water and rinsed in purified water. The cool, stable samples obtained from the plants were cut into small pieces and dried under shade for 3 to 4 weeks. 50 gram shade dried plant materials were coarsely powdered. Powdered of Phoebe lanceolata has been extracted with methanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C **(Mukherjee, 2002).**

2.3 Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:\

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

2.4 Phytochemical Screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods **(Kokate, 1994).**

2.5 Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method**.** 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 2 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 2 ml of 2% AlCl3 solution was added to 2 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm **(Garg et al., 2019).**

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2.6 Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer **(Mishra et al., 2018).**

2.7 In vivo antiulcer activity of Phoebe lanceolata extract

2.7.1 Animals

Wistar rats (180±20g) were group housed (n=6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise- free room between 08.00 to 15.00 h. A separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted to control and supervise experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India.

2.7.2 Toxicity study

Healthy adult male Wistar rats were fasted overnight before the experiment. Different doses (50-2000 mg/kg, P.O) of Phoebe lanceolata extract were administered to each group of six rats. The rats were observed continuously for 1 hour, then at half-hourly intervals for 4 hours, and further monitored up to 72 hours for any gross behavioral changes. Additionally, observations continued for 14 days to check for mortality, in accordance with OECD Guideline 425. The Phoebe lanceolata extract was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. The doses selected for antiulcer evaluation were 100 and 200 mg/kg, respectively **(Deshpande et al., 2015).**

Table 1:- Toxicity study

Observation	Acute toxicity
Skin and Fur	Normal
Eyes	Normal
Respiration	Normal
Sleep	Normal
Coma	Not seen
Mortality	Not seen

2.7.3 Experimental designs

Indomethacin-induced gastric ulcer

The rats were divided into five groups (n=6) randomly, and six animals were placed in each group.

Group I: The normal group was treated with distilled water

Group II: The control group was induced with indomethacin (40 mg/kg) administered to rats after fasting for 36 h

Group III: The standard drug group was treated with Cimetidine 100 mg/kg/p.o

Group IV: Ulcerated rats pretreated with Phoebe lanceolata extract 100 mg/kg

Group V: Ulcerated rats pretreated with Phoebe lanceolata extract 200 mg/kg

Animals were treated with either vehicle, extracts or Cimetidine orally 30 min prior to induction of gastric lesions. The animals were sacrificed 5 h after treatment with the ulcerogenic agent to assess the ulcer score and mucous-producing activity. The stomach was cut open along the greater curvature, and ulcer scoring was done by using a magnifying lens, and the ulcer was scored according to its severity in comparison with that of the standard. The ulcer index was determined using the formula:

Ulcer index = 10/X

Where X = Total mucosal area/Total ulcerated area. Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis,

3 = perforated or penetrated ulcer.

2.7.4 Determination of volume and pH

The volume of gastric juice of each rat was measured after centrifugation at 1000 rpm for 10 min and analyzed. An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water, and the pH of the solution was measured using a pH meter **(El-Ashmawy et al., 2016).**

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2.7.5 Statistical analysis

The experimental data are presented as the mean ± SD for each experimental group. One- way analysis of variance (ANOVA) was employed to assess statistical differences between groups, followed by Tukey's post hoc test for multiple comparisons. A statistically significant effect was observed ($p < 0.05$) **(Kim, 2014).**

III. RESLUT AND DISCUSSION

3.1 Results of percentage yield

Table 2: % Yield of extract of Phoebe lanceolate

Extract	% Yield (w/w)
Methanolic	8.96

The percentage yield of the methanolic extract of Phoebe lanceolata was found to be 8.96% (w/w), indicating a moderate extraction efficiency.

3.2 Results of phytochemical screening of extract

Small portion of the dried extracts was subjected to the phytochemical tests using standard methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml.

Table 3: Phytochemical screening of extract of Phoebe lanceolate

Constituents	Methanolic extract
Alkaloids	
Mayer's Test	-ve
Wagner's Test	+ve
Dragendroff's Test	-ve
Hager's Test	+ve
Glycosides	
Legal's Test	-ve
Flavonoids	
Lead acetate	+ve
Alkaline test	+ve
Phenol	
Ferric chloride test	+ve
Proteins	
Xanthoproteic test	+ve
Carbohydrates	
Molisch's Test	-ve
Benedict's Test	+ve
Fehling's Test	+ve
Saponins	
Froth Test	-ve
Diterpenes	
Copper acetate test	-ve
Tannins	
Gelatin Test	-ve

3.3 Results of estimation of total flavonoids and phenol content

3.3.1 Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.030X - 0.008$, $R^2=0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance.

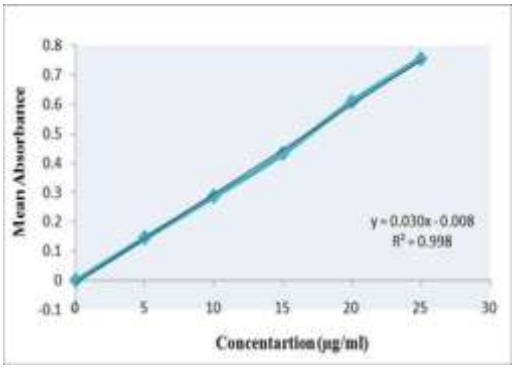

Figure 1: Graph of calibration curve of Quercetin

3.3.2 Total phenol content estimation (TPC)

The content of total phenol compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.0309X + 0.014$, $R^2= 0.9989$, where X is the gallic acid equivalent (GAE)

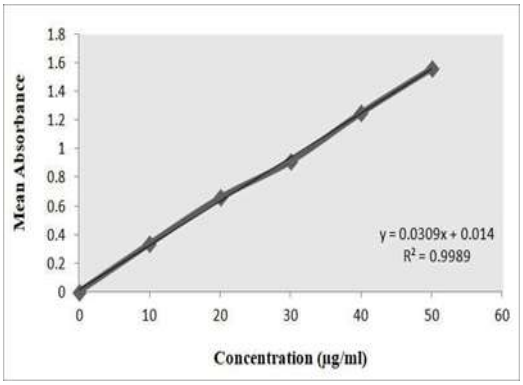

Figure 2: Graph of calibration curve of Gallic acid

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Table 4: Estimation of total flavonoids and phenol content of extract of Phoebe lanceolata

Extract	Total flavonoids content	Total phenol content
	(mg/ 100 mg of dried extract)	
Methanolic	0.956	0.857

The methanolic extract of Phoebe lanceolata was found to contain 0.956 mg/100 mg of total flavonoids and 0.857 mg/100 mg of total phenolic content, indicating a high concentration of polyphenolic compounds in the extract.

3.4 Results of antiulcer activity of Phoebe lanceolata extract

The anti-ulcerogenic effect of Phoebe lanceolata extract was evaluated against indomethacin-induced gastric ulcers in rats. The normal control group treated with distilled water showed no ulcers (0.00 ± 0.00), confirming the integrity of the gastric mucosa. In contrast, the indomethacin-treated group exhibited a significant increase in the number of ulcers (14.8 ± 0.37), indicating severe gastric damage. Cimetidine, a standard anti-ulcer drug, reduced the ulcer count to 4.2 ± 0.29 when administered at 100 mg/kg along with indomethacin. Treatment with Phoebe lanceolata extract at 100 mg/kg significantly decreased the number of ulcers to 7.9 ± 0.33 compared to the indomethacin-only group. Furthermore, the higher dose of 200 mg/kg showed enhanced protection, reducing the ulcer count to 5.1 ± 0.31.

Table 5: Anti-ulcerogenic effect of Phoebe lanceolata extract against ulcerogenic agents in rats (Number of Ulcers)

Group	Treatment	Number of Ulcers
Group-I	Distilled water	0.00 ± 0.00
Group-II	Indomethacin (40 mg/kg)	14.8 ± 0.37#
Group-III	Cimetidine 100 mg/kg + Indomethacin (40 mg/kg)	4.2 ± 0.29***
Group-IV	Phoebe lanceolata extract 100 mg/kg + Indomethacin (40 mg/kg)	7.9 ± 0.33*
Group-V	Phoebe lanceolata extract 200 mg/kg + Indomethacin (40 mg/kg)	5.1 ± 0.31***

Values are expressed as mean±S.E.M. (n = 6). #P<0.001 vs Group I; ***P < 0.001, ** P < 0.01, * P < 0.05 vs Group II (One-way ANOVA followed by Tukey's post hoc test).

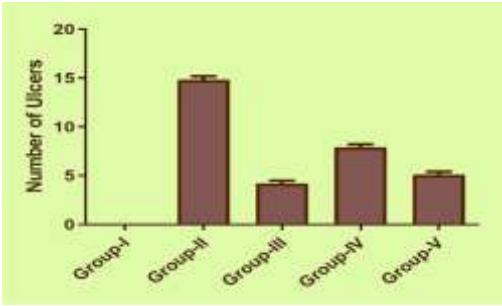

Figure 3: Anti-ulcerogenic effect of Phoebe lanceolata extract against ulcerogenic agents in rats (Number of Ulcers)

The results of the study demonstrated that indomethacin administration (Group II) significantly increased the ulcer index to 17.4 ± 0.42, indicating substantial gastric mucosal damage compared to the normal control group (Group I), which exhibited no ulcers (0.00 ± 0.00). Pretreatment with cimetidine (Group III) effectively reduced the ulcer index to 4.8 ± 0.26, serving as a positive control. Notably, treatment with Phoebe lanceolata extract at 100 mg/kg (Group IV) resulted in a marked reduction in ulcer index to 8.9 ± 0.33, while a higher dose of 200 mg/kg (Group V) showed even greater protection, lowering the ulcer index to 5.3 ± 0.28.

Table 6: Anti-ulcerogenic effect of Phoebe lanceolata extract against ulcerogenic agents in rats (Ulcer index)

Group	Treatment	Ulcer Index
Group-I	Distilled water	0.00±0.00
Group-II	Indomethacin (40 mg/kg)	17.4±0.42#
Group-III	Cimetidine 100 mg/kg + Indomethacin (40 mg/kg)	4.8±0.26***
Group-IV	Phoebe lanceolata extract 100 mg/kg + Indomethacin (40 mg/kg)	8.9±0.33*
Group-V	Phoebe lanceolata extract 200 mg/kg + Indomethacin (40 mg/kg)	5.3±0.28***

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Values are expressed as mean±S.E.M. (n = 6). #P<0.001 vs Group I; ***P < 0.001, ** P < 0.01, * P < 0.05 vs Group II (One-way ANOVA followed by Tukey's post hoc test).

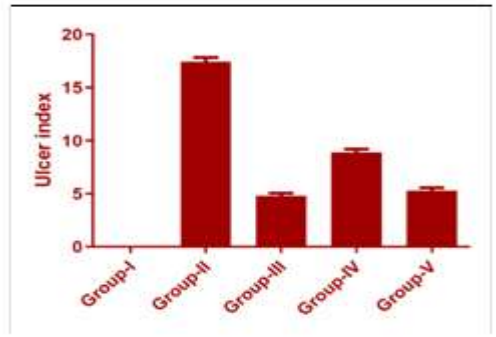

Figure 5: Anti-ulcerogenic effects of Phoebe lanceolata extract against ulcerogenic agents in rats (Ulcer index)

In the evaluation of anti-ulcerogenic activity, the pH of gastric contents in the control group (distilled water) was 6.45 ± 0.12, indicating normal gastric acidity, whereas administration of indomethacin (40 mg/kg) significantly reduced the pH to 3.21 ± 0.10, suggesting marked gastric acidification. Treatment with cimetidine (100 mg/kg) restored the pH to 5.95 ± 0.15, demonstrating effective acid suppression. Similarly, Phoebe lanceolata extract at 100 mg/kg and 200 mg/kg increased the pH to 5.28 ± 0.11 and 5.90 ± 0.13, respectively, showing a dose-dependent improvement in gastric acidity.

Table 8: Anti-ulcerogenic effect of Phoebe lanceolata extract on gastric pH in rats

Group	Treatment	pH
Group-I	Distilled water	6.45 ± 0.12
Group-II	Indomethacin (40 mg/kg)	3.21 ± 0.10
Group-III	Cimetidine 100 mg/kg + Indomethacin (40 mg/kg)	5.95 ± 0.15
Group-IV	Phoebe lanceolata extract 100 mg/kg + Indomethacin (40 mg/kg)	5.28 ± 0.11
Group-V	Phoebe lanceolata extract 200 mg/kg + Indomethacin (40 mg/kg)	5.90 ± 0.13

Values are expressed as mean±S.E.M. (n = 6). #P<0.001 vs Group I; ***P < 0.001, ** P < 0.01, * P < 0.05 vs Group II (One-way ANOVA followed by Tukey's post hoc test).

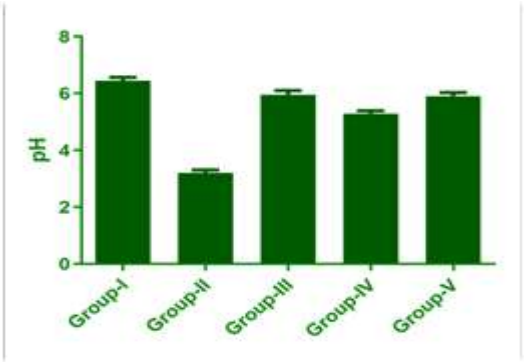

Figure 7.6: Anti-ulcerogenic effect of Phoebe lanceolata extract on gastric pH against ulcerogenic agents in rats

Regarding gastric volume, indomethacin treatment significantly increased the volume to 4.75 ± 0.13 ml compared to the control value of 2.10 ± 0.09 ml, indicating excessive gastric secretion. Cimetidine treatment reduced this to 2.42 ± 0.08 ml, while Phoebe lanceolata extract at 100 mg/kg and 200 mg/kg decreased the volume to 2.89 ± 0.10 ml and 2.33 ± 0.07 ml, respectively.

Table 9: Anti-ulcerogenic effect of Phoebe lanceolata extract on volume in rats

Group	Treatment	Volume (ml)
Group-I	Distilled water	2.10 ± 0.09
Group-II	Indomethacin (40 mg/kg)	4.75 ± 0.13
Group-III	Cimetidine 100 mg/kg + Indomethacin (40 mg/kg)	2.42 ± 0.08
Group-IV	Phoebe lanceolata extract 100 mg/kg + Indomethacin (40 mg/kg)	2.89 ± 0.10
Group-V	Phoebe lanceolata extract 200 mg/kg + Indomethacin (40 mg/kg)	2.33 ± 0.07

Values are expressed as mean±S.E.M. (n = 6).

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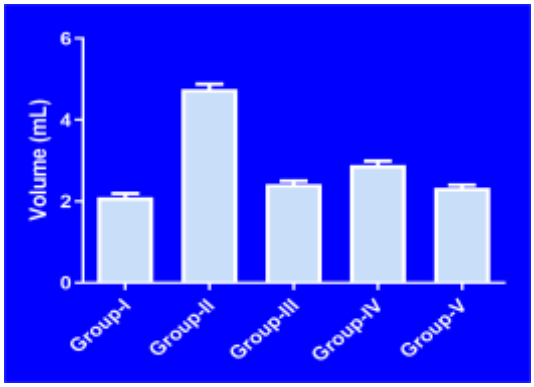

Figure 7.7: Anti-ulcerogenic effect of Phoebe lanceolata extract on volume against ulcerogenic agents in rats

IV. CONCLUSION

This study has shown the significant gastroprotective effects of Phoebe lanceolata extract. These effects were seen in cases of indomethacin-induced gastric mucosal injury and were similar to those produced by the reference standard drug.

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