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EVALUATION OF ANTIULCER ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF SEEDS OF *PASPALUM SCROBICULATUM* LINN. (POACEAE) IN PYLORUS LIGATED WISTAR ALBINO RATS

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ABSTRACT

Objective: The purpose of this research work is to determine the ulcer protective activity of hydroalcoholic extraction of seeds of *Paspalum scrobiculatum* Linn in pylorus ligated Wistar albino rats. **Materials and Methods:** The ulcer protective effects of *Paspalum scrobiculatum* was studied by using pylorus ligation modal in Wistar albino rats. Wistar albino rats of either sex were divided into four groups. The control group received 0.1 mg/ml of CMC, the reference group receive omeprazole (20 mg/kg) and the test group received 200 mg/kg and 400 mg/kg of hydroalcoholic extract of *Paspalum scrobiculatum* by oral route. The parameters measured for antiulcer activity were gastric volume, pH, free acidity, total acidity, ulcer index and percentage protection. **Results:** In pyloric ligation model; the gastric volume, total and free acidity was significantly decreased at $p<0.0001$ and and pH of the gastric juice was significantly increased at $p<0.01$ and $p<0.0001$ in *Paspalum scrobiculatum* treated groups as compared to control group. All the doses of *Paspalum scrobiculatum* showed dose dependent antiulcer effect as well as significant ($p<0.01$ and $p<0.0001$) reduction in the ulcer index as compared to control group. **Conclusion:** The present study concluded that the hydroalcoholic extract of seeds of *Paspalum scrobiculatum* has a significant anti-ulcer effect.

KEYWORDS: *Paspalum scrobiculatum*; antiulcer; pylorus ligation.

INTRODUCTION

Peptic ulcer is a serious gastrointestinal disorder that affects four million people each year. An estimated 15,000 deaths occur each year as a consequence of peptic ulcer disease.[1] Peptic ulcer are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue.[2] There are many types of ulcers such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer of this peptic ulcer is seen among many people. The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer." Gastric ulcers are located in the stomach, characterized by pain; ulcers are common in the older age group. Eating may increase pain rather than relieve pain. Other symptoms may include nausea, vomiting, and weight loss.[3] Duodenal ulcers are located at the beginning of the small intestine and are characterized by severe pain with a burning sensation in the upper abdomen that awakens patients from sleep.[4] The pathophysiology of Peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and *H. pylori)* and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors).[5]

The risk factors responsible to produce peptic ulcer disease are alcohol consumption, cocaine, tobacco, and amphetamine use, chronic administration of nonsteroidal anti-inflammatory drugs (NSAIDs), fasting, Zollinger–Ellison syndrome, and cancer treatment with angiogenesis inhibitors.[6],[7] The existing drugs cause several adverse effects; conversely, indigenous natural drugs are without side effects which would possibly better deal with peptic ulcers. Medicinal plants possess numerous active phytoconstituents that are responsible for several biological activities. So the drug of natural origin used for the management of gastric ulcers is a better alternative to synthetic drugs.[8]

Paspalum scrobiculatum Linn. belonging to the family Poaceae, is identified as the true botanical source of Kodrava. It is commonly known as 'Kodo millet'. It is a minor grain crop in India, and an important crop in the Deccan plateau. Its cultivation in India is generally confined to Karnataka, Gujarat, Madhya Pradesh Chhattisgarh, Eastern, and Tamil Nadu. Several medicinal properties such as antidiabetic, tranquilising, antirheumatic and wound-healing are attributed to this grain. Traditionally it is used in diabetes, hypertension, depression, wound healing, cancer, hyperlipidaemia, microbial infections, inflammation, haemorrhages, and general debility. According to the phytochemical investigation, the seeds of *Paspalum scrobiculatum*, contain tannins, phenolic compounds, terpenoids, glycosides, amino acids, fixed oil and fat, saponins, flavonoids, protein, and carbohydrates. Accordingly,

this systematic study was carried out to evaluate the antiulcer activity of hydro-alcoholic extract of seeds of *Paspalum scrobiculatum* Linn. in Wistar albino rats.

MATERIALS AND METHODS

Collection and authentication of plants

The seeds of *Paspalum scrobiculatum* Linn. were collected from the Mana velugu Vintage farmers, Andhra Pradesh. Plants were cultivated and harvested in Oruvathilkotta, Trivandrum. The plant materials used for the study were authenticated by Dr T.S. Swapna, Professor and Head of the department of botany, University of Kerala, Kariavattom, Thiruvananthapuram. The herbarium of plant materials was prepared and submitted to the University of Kerala, Kariavattom (voucher specimen number: KUBH 10477).

Preparation of extracts

Seeds were washed with water and dried in shade to remove the traces of moisture. The seeds were powdered for the easiness of extraction and stored in an airtight container. The dry powdered plant material was sieved through sieve No: 60. Then the coarse powder was collected. First, the powdered seeds were subjected to extraction with petroleum ether (60-80^{0}C) by using the Soxhlet apparatus for the removal of fatty materials. Then defatted plant material was dried at room temperature and finally subjected for Soxhlation with 70% ethanol. A total of 250 g of plant material was used for hydro-alcoholic extraction. After successive soxhlation for 24 hours, the extract was distilled to remove the solvent completely and dried in a desiccator. Finally, the extract was weighed and calculated the percentage yield.[3]

Preliminary phytochemical analysis

The hydro alcoholic extract of *Paspalum scrobiculatum* Linn. Seed was screened for the presence of various phytochemical compounds.[9]

Experimental animals

Wistar albino rats (150 – 350 g) of both sexes were used for this study. The animals were housed under the standard conditions of temperature (25 ± 2 ^{0}C) and relative humidity (33 – 70%) with a 12:12 light-dark cycle. The animals were fed with a standard rodent pellet diet and water ad libitum except during experimentation. Experiments were conducted as per the CPCSEA guidelines. The Institutional Animal Ethics Committee approval number was 02/05/2020/IAEC/MCT.

Chemicals and Reagents

Omeprazole, Ethanol, Ketamine, CMC, Haematoxylin and eosin.

Anti-ulcer activity

Albino Wistar rats of either sex was weighed (150-350 g) and divided into four groups of six animals each. In this method albino rats were fasted in individual cages for 24 hours. Care was taken to avoid coprology. Test drug or standard drug or control vehicle was administered 30 minutes prior to pyloric ligation. After providing light anaesthesia, (ketamine 75mg/kg i.p) an incision of 1 cm long was made in the abdomen just below the sternum. Stomach was exposed. Then a tight knot was tied around the pyloric sphincter. While putting the knot care was taken so that no blood vessels is tied along the knot. After that the abdomen was sutured. Skin was cleaned from any blood spots and bleeding. Collodion was applied over the wound. The animal was kept in separate cages after the procedure. At the end of 4 hours after ligation the animals was sacrificed with excess of ketamine, and the stomach was dissected out. Gastric juice was collected and drained into tubes and centrifuged at 1000 rpm for 10 minutes and the volume was noted. The pH of gastric juice was recorded by pH meter. Then the contents were subjected to analysis for free and total acidity. The stomachs were then washed with running water to see for ulcers in the glandular portion of the stomach. Put it on the slide glass and observe under 10 X magnification for ulcers. Score the ulcers as below.[10],[11]

0 = Normal coloured stomach

0.5 = Red colouration

1 = Spot ulcers

1.5 = Haemorrhagic streaks

2 = Ulcers > 3 but < 5

3 = Ulcers > 5

$$Acidity = \frac{Vol.\,of\ NaOH\ \times Normality\ \times 100}{0.1}$$

Histopathological Evaluation

The ulcerated gastric tissue was stained by using Haematoxylin and eosin stain and examined under the microscope for histopathological changes such as infiltration, inflammation and erosion.

Statistical analysis

The results were expressed as mean ± SEM, data were determined using one way ANOVA followed by Dunnett's multiple comparison.

RESULTS

Preliminary phytochemical screening revealed the presence of carbohydrate, protein, amino acid, glycosides, tannins, phenols, flavonoids and steroids or triterpenoids.

Table 1: Effect of different concentration of plant extract on volume, P^H and acidity.

Group	Dose (mg/kg)	Volume of gastric juice (mL)	P^H	Acidity (mEq/L/100 g)	
				Free	Total
Group I (Control)	CMC (0.1mg/mL)	7.8 ± 0.0966	2.04 ± 0.0200	96.666 ± 1.9264	122.166 ± 2.1512
Group II (Low dose of PS)	PS (200mg/kg)	6.25 ± 0.15 ****	2.67 ± 0.1264 *	79.5 ± 1.6072 ****	80.333 ± 1.9607 ****
Group III (High dose of PS)	PS (400mg/kg)	4.43 ± 0.1542 ****	4.60 ± 0.1657 ****	58.333 ± 1.8559 ****	65.33 ± 1.7826 ****
Group IV (Standard)	Omeprazole (20mg/kg)	3.71 ± 0.0980 ****	5.26 ± 0.1475 ****	43.5 ± 2.8838 ****	50.833 ± 1.4925 ****

All values are Mean ± SEM, N = 6, Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test, *p<0.05 and ****p<0.0001 when compared with the control.

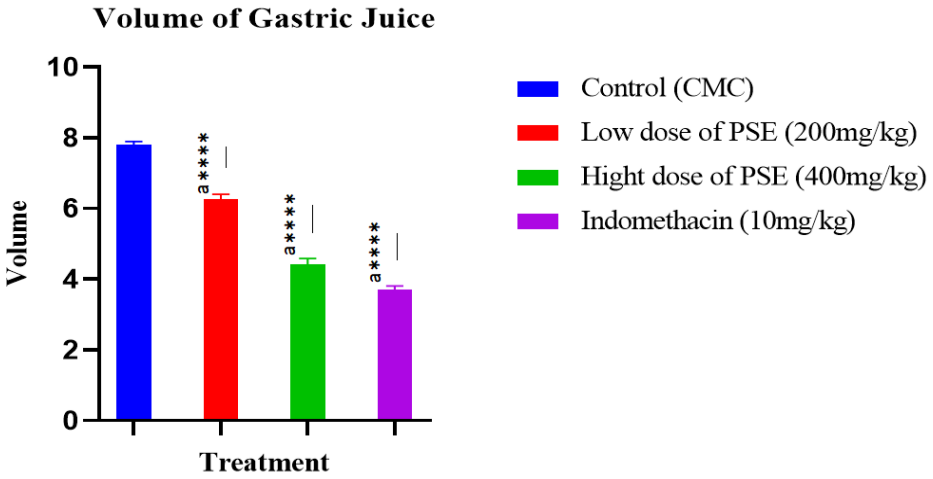


Fig. 1. Comparison of volume of gastric juice. N = 6 Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Post-hoc Tukey's. A** indicates P<0.0001 when compared to control group.**

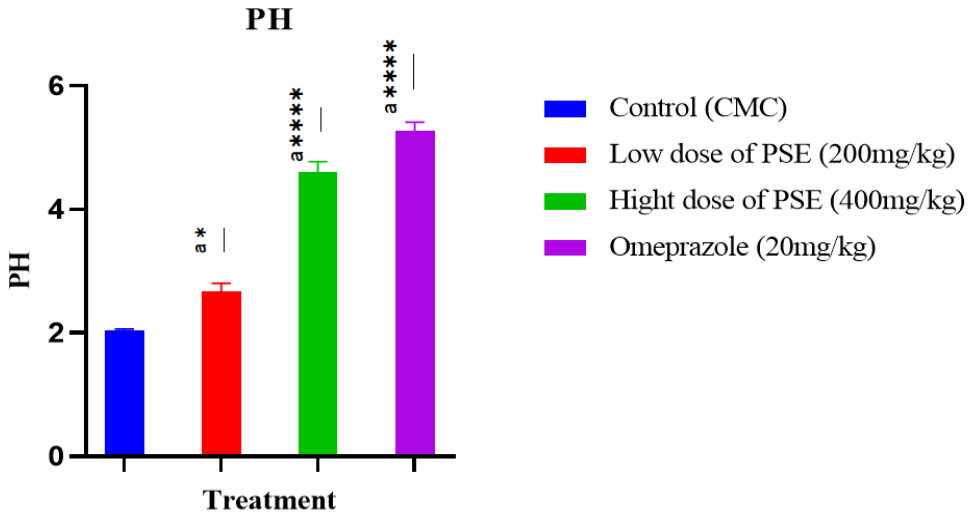


Fig. 2. Comparison of P^H. N = 6 Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Post-hoc Tukey's. a* indicates P<0.05 and a** indicates P<0.0001 when compared to control group.**

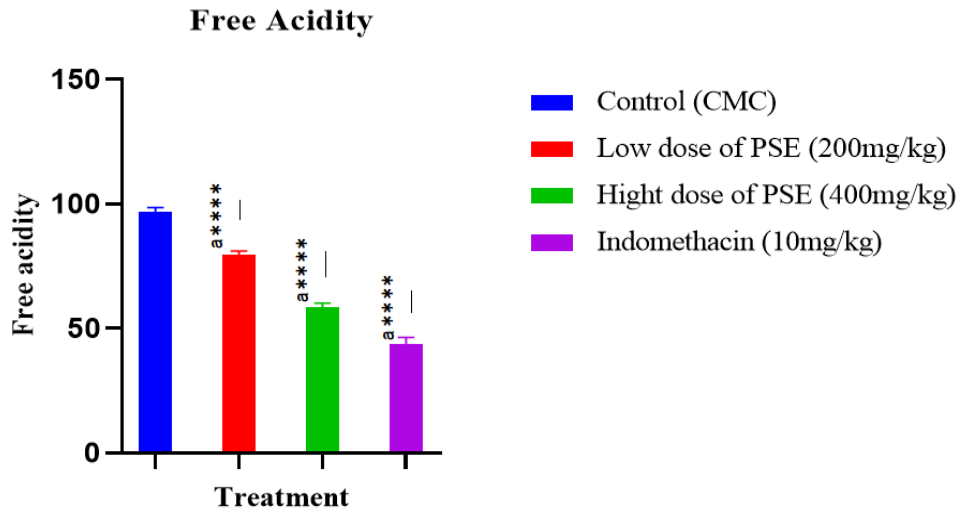


Fig. 3. Comparison of free acidity. N = 6 Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Post-hoc Tukey's. a** indicates P<0.0001 when compared to control group.**

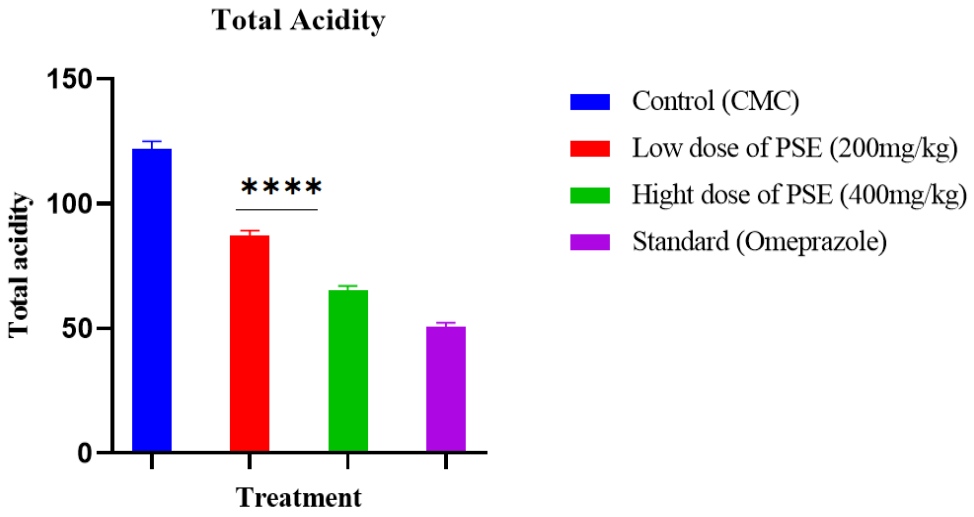


Fig. 4. Comparison of total acidity. N = 6 Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Post-hoc Tukey's. ** indicates P<0.0001 when compared to control group.**

In the pylorus ligation induced ulcer method, The volume of gastric juice of 200 mg/kg and 400 mg/kg of the plant extract treated group were 6.25 ± 0.15 ($p<0.0001$) and 4.43 ± 0.1542 ($p<0.0001$) respectively. This study showed significant decrease in the gastric volume of plant extract treated group as compared with control group which showed the gastric volume 7.8 ± 0.0966. The gastric volume of plant extract treated group indicates a positive effect when compared with control group (Figure 1). The 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.0001$) plant extract showed significant increase in the P^H compared with control group (Figure 2). The effect of plant extract in acid parameter showed significant ($p<0.0001$) effect at the 200 mg/kg and 400 mg/kg dose compared to control group (Figure 3), (Figure 4) and (Table 1).

Table 2: Effect of different concentration of plant extract on ulcer index.

Group	Dose (mg/kg)	Ulcer index	Percentage protection
Group I (Control)	CMC (0.1mg/mL)	16.66 ± 0.0506	-
Group II (Low dose of PS)	PS (200mg/kg)	8.24 ± 2.3291 **	50.50
Group III (High dose of PS)	PS (400mg/kg)	3.33 ± 1.4922 ****	79.97
Group IV (Standard)	Omeprazole (20mg/kg)	2.98 1.3342 ****	82.09

All values are Mean ± SEM, N = 6, Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Dunnett's multiple comparison test, **p<0.01 and ****p<0.0001 when compared with the control.

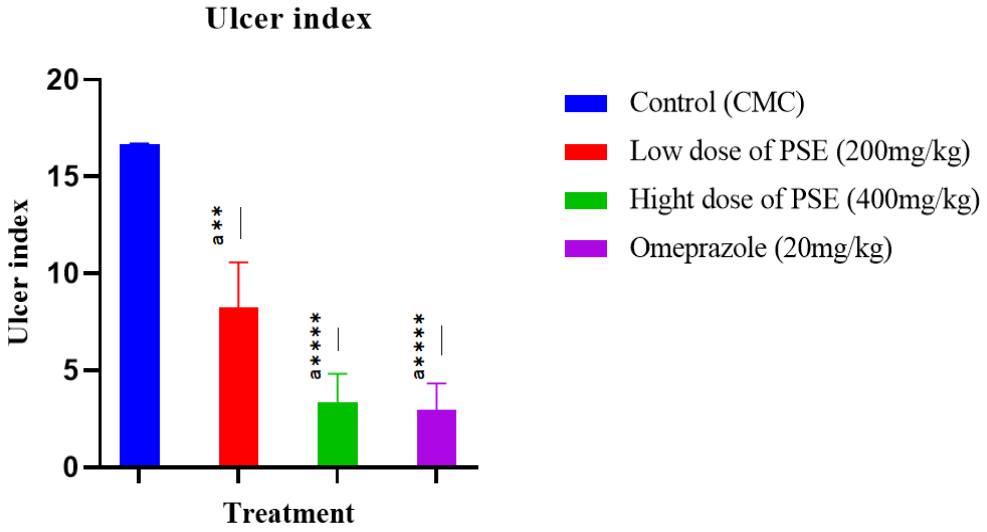
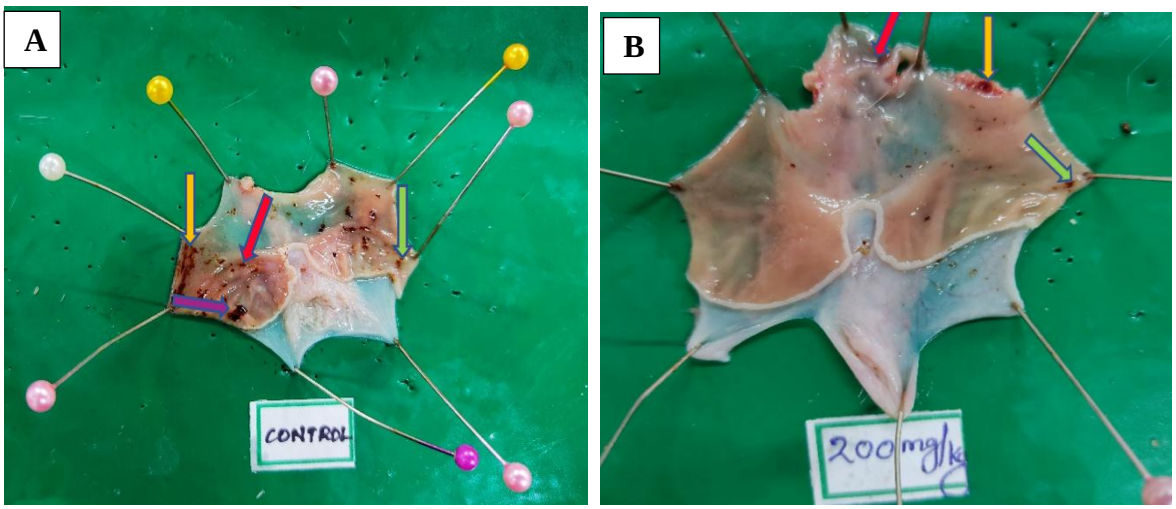


Fig. 5. Comparison of ulcer index. N = 6 Values are expressed as mean ± SEM. Data were analysed by one way ANOVA followed by Post-hoc Tukey's. a indicate P<0.01 and a**** indicates P<0.0001 when compared to control group.**



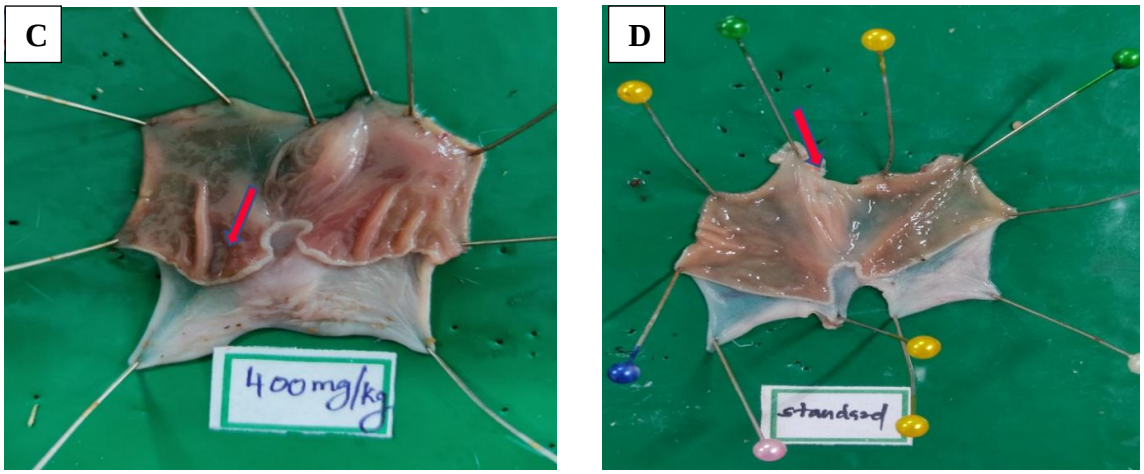


Fig. 6. Rat stomach of test and standard drug treatment group.

A: Control (0.1 % CMC)

B: Low dose (200 mg/kg) *Paspalum scrobiculatum*

C: High dose (400 mg/kg) *Paspalum scrobiculatum*

D: Standard (Omeprazole 20 mg/kg)

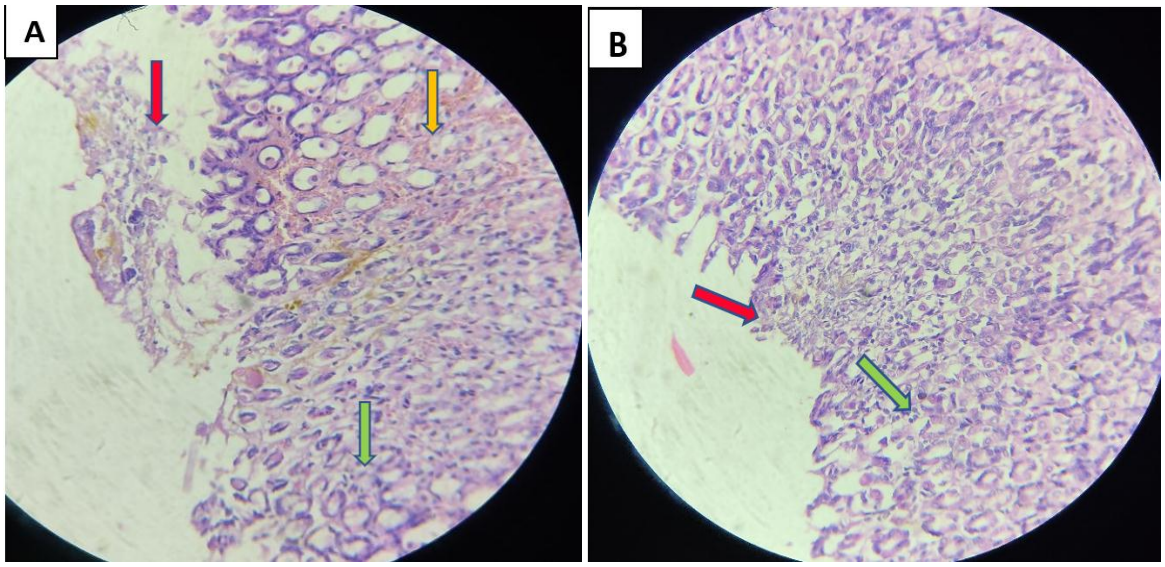
Redness

Spot ulcer

Haemorrhagic streak

Deep ulcer

The ulcer index of 200 mg/kg ($p<0.01$) and 400 mg/kg ($p<0.0001$) of the plant extract treated group showed significant decrease in the ulcer index as compared with control group. It has also been observed that plant extract treated group shows significantly and dose dependently reduces the gastric ulceration (Table 2), (Figure 5).



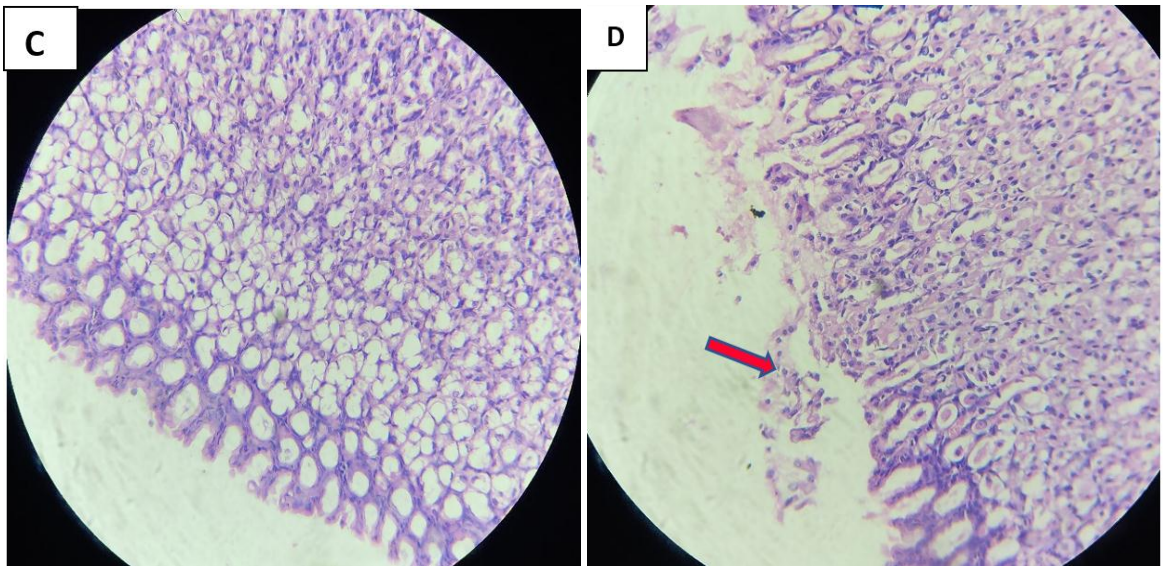


Fig. 7. Histopathological evaluation of rat stomach.

A: Control (0.1 % CMC)

B: Low dose (200 mg/kg)

C: High dose (400 mg/kg)

D: Standard (Omeprazole 20 mg/kg)

⇒ Haemorrhage

⇒ Inflammatory cells

⇒ Mucosal erosion

Histopathological evaluation of pylorus induced group showed a diffused mucosal ulceration, Haemorrhage, inflammatory cells infiltration in mucosa, secretions and mucosal erosion. Plant extract at 200 mg/kg showed partial healing of ulcer with diffused mucosal ulceration, No Haemorrhage, inflammatory cells infiltration in mucosa, less secretions and mucosal erosion. In 400 mg/kg treatment group showed the healed ulcer with intact epithelium, No Haemorrhage, No inflammatory cells infiltration in mucosa and no secretions.

DISCUSSION

In preliminary phytochemical studies, the seeds extract of *Paspalum scrobiculatum* contain carbohydrate, protein, amino acid, glycosides, tannins, phenols, flavonoids and steroids or triterpenoids. Some phytoconstituents extracted from medicinal plants possess antiulcerogenic activity and act by various mechanisms. Phenolic compounds and flavonoids possess antiulcer effect due to their antisecretory, cytoprotective, antioxidant, anti-inflammatory, and anti-*H. pylori*. Phenolic compounds and flavonoids also promote prostaglandin synthesis, stress defense, and antioxidant enzymes synthesis, and wound

healing properties.[12] Tannins directly protect the outermost layer of mucosa and change the mucosa structure that can resist to chemicals and mechanical injury.

Pyloric ligation-induced ulcer model is an important method for the measurement of mean ulcer index and Percentage protection in ulcerogenesis. The ligation of the pyloric end of the stomach leads to stress induced secretion of excess HCl from the parietal cells and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid.[13] Pylorus ligation induced ulcers are shown by auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier which resulted as upper gastrointestinal damage including lesions, ulcers and life-threatening perforation and haemorrhage.[14] The agents who decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers induced by this method.

Paspalum scrobiculatum caused a significant decrease in the gastric volume, free acidity, and total acidity compared to the control group, indicating an antisecretory mechanism. The antisecretory activity could be due to the inhibition of the H + -K + -ATPase enzyme. The ulcer index and percentage inhibition are parameters commonly used to determine the gastroprotective effect of investigational agents.[15] The low dose (200mg/kg) and high dose (400 mg/kg) of *Paspalum scrobiculatum* caused a significant reduction in the ulcer index and an improvement in the percentage inhibition compared to the control group, indicating a gastroprotective effect.

CONCLUSION

The present study concluded that the hydroalcoholic extract of seeds of *Paspalum scrobiculatum* has a significant anti-ulcer effect may be related to antisecretory as well as cytoprotective activities of one or more of the identified phytochemicals.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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