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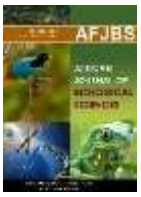

Antiulcer effect of Sorghum Bicolor leaves extract on Ethanol induced mucosal damage in Wistar rats

Garima Vishwakarma[1], Raj.K. Prasad[1], Kuldeep Singh[1]#
[1]Department of Pharmacology and Toxicology, Shambhunath Institute of Pharmacy (SIP), Jhalwa, Prayagraj, Uttar Pradesh, Pin Code:211015

[#] Corresponding author

Kuldeep Singh, M. Pharm.
Department of Pharmacology and Toxicology
Shambhunath Institute of Pharmacy (SIP),
Jhalwa, Prayagraj, Uttar Pradesh,
Pin Code:211015

Abstract:
Peptic ulcer disease (PUD) presents a common challenge for both primary care providers and gastroenterologists, characterized by varied symptoms such as abdominal pain, nausea, vomiting, weight loss, and potentially severe complications like bleeding or perforation. Understanding the underlying risk factors and mechanisms driving PUD is crucial for effective diagnostic and treatment approaches.
This study investigates the protective effects of the ethanolic extract of Sorghum bicolor leaves (EESBL) against ethanol-induced mucosal damage in Wistar rats. Nitrite levels, glutathione (GSH) levels, catalase concentration, and lipid peroxidation (MDA content) were evaluated to elucidate EESBL's mechanisms of action. In the vehicle-treated ethanol group, nitrite levels were significantly elevated, and GSH levels were reduced compared to sham controls. Treatment with EESBL effectively mitigated these changes, lowering nitrite levels and boosting GSH concentrations. Catalase levels were decreased in the ethanol-treated group but remained unchanged with EESBL treatment. Moreover, ethanol-induced lipid peroxidation, indicated by increased MDA content, was markedly reduced by EESBL.
Morphological examination revealed that EESBL-treated rats exhibited fewer gastric lesions and preserved mucosal integrity compared to ethanol-only controls. These findings underscore the antiulcer properties of EESBL, suggesting its potential as a therapeutic agent for attenuating ethanol-induced gastric mucosal damage in clinical settings.
Keywords: Peptic ulcer, Sorghum bicolor, Stomach, Antioxidant,

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1. Introduction

Peptic ulcer disease is one of the most common ailments of the gastrointestinal tract observed in various clinical settings. This results from an imbalance of aggressive factors like acid, pepsin, Helicobacter pylori; and defensive mechanisms (gastric mucus and bicarbonate secretion, prostaglandins, and inherent resistance of mucosal cells) (1). The gastric mucosa is often subject to various agents potentially harmful for it including bile acids, food components, bacterial products and different drugs (2). Excessive stress, smoking, poor diet, and NSAID use are other risk factors that may contribute to the development of duodenal ulcers. Others, like prolonged anxiety, emotion stress, hemorrhagic surgical shock, burns and trauma are also recognized as being acute gastric irritants (3).

Various antiulcer medications with potentially harmful side effects such as arrhythmias, impotence, and hematopoietic changes are available including H2 receptor antagonists, proton pump inhibitors, and cytoprotective agents (4). The therapeutic premise of peptic ulcer disease is three-fold: neutralization of aggressive factors (acid, pepsin, active oxidants and a variety of exogenous substances including NSAIDs) or enhancement of mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins and nitric oxide) (5).

Even though many strategies have been and are being employed to combat ulceration including H2 receptor antagonists, proton pump inhibitors, cytoprotective agents etc., these drugs continue to offer a number of serious side effects like arrhythmias, impotency and hematopoietic changes. So the search for the new, safe and effective antiulcer compounds is at a premium (6). Treatment rationale in peptic ulcers is based on stopping the aggressive agents (such as acid, pepsin, active oxidants and various exogenous substances such as NSAIDs) or on reinforcing protection of the mucosa (e. BeginLineg. mucus, bicarbonate, normal blood flow, prostaglandins e.c and nitric oxide) (7).

Interestingly, a range of agents that have shown beneficial antiulcer effects with few of the problems described above (as adverse effects) are available in alternative medicine. For example, in Ayurvedic medicine, many plants and herbs are suggested to manage gastrointestinal problems, including gastric ulcers. The gastro-protective activity of most medicinal plants is

largely due to their effectiveness in enhancing the mucosal defensive factors rather than a decrease in aggressive elements (8).

Sorghum bicolor, widely known as sorghum is a multipurpose and widely grown plant source used in agriculture, for feeding and nutritional purposes, and medicine (9). Sorghum includes nearly all the essential amino acids and is a rich source of B group vitamins, vitamin E, phosphorous, potassium, magnesium, and iron. Earlier, different components of sorghum plants were employed in folk medicine to manage numerous ailments such as skin disorders, inflammation, pain, and other GIT-related problems. Sorghum has various pharmacological effects because of its abundance of phytochemicals such as antioxidant activity, anti-inflammatory activity, and antimicrobial activity (10). Thus far, no research has assessed the antiulcer efficacy of *S. bicolor* by employing particular gastric ulceration models, and this can be proposed as one of the directions for further investigation.

2. Methods and materials

2.1. Collection and Preparation of Plant Material

Fresh leaves of Sorghum bicolor were collected from a village in Phoolpur, U.P., state of India. Identification of plant material was done in coordination with Dr.Vinay Rajan, Scientist-E H. O D, and Mr. Lakshman, Senior preservative in Herbarium, Botanical Survey of India, Allahabad, U.P., India. The isolation of ethanol extract was done using the Soxhlet-based extraction method. Ethanol Solvent Fractions Phytochemical screening: The Qualitative Phytochemical Evaluation for the ethanol extracts were tested for the presence of the below phytochemical using standard methods: Tannins, Alkaloids, Glycosides, Terpenoids, Flavonoids, Amino Acids and Proteins (11).

2.2. Extraction of *Sorghum bicolor*

Specimens of Sorghum bicolor were acquired, washed, and then air-dried before being ground into a fine powder using a domestic food processor. The powdered material was then continuously heated by percolation using Soxhlet's technique. Petroleum ether was used as non-polar solvent and soxhlation was done at 60°C till the extraction was thought to be through. The extracts were then evaporated using a rotary vacuum evaporator at 40°C to completion. Based on the observation, the final prepared extract was reddish brown and had no specific odor, after

which the extract was transferred to an airtight container ready for use in subsequent analyses. On this extraction process, it was possible to separate the active compounds of the plant material derived from Sorghum bicolor plant (12).

2.3. Experimental Animals

The ethical clearance for this experiential study was sought from the Institutional Animal Ethical Committee of Shambhunath Institute of Pharmacy, Prayagraj, having Registration No. 1217/PO/Re/S/08/CPCSEA. Albino Wistar rats of both sexes were used in this study after considering their average body weight of 150-250 g. They were purchased from S. S. Chakorborty Laboratory, the address of which is Kolkata. The rats were kept in polypropylene cages and were bred under the standard best environment (illumination for 12 hours' light and dark period at 25 ±3 degree centigrade and relative humidity of 35-60%). Standard pelletized feeds and tap water were offered to the rats on an as-required basis or as an ad-libitum basis.

2.4. Acute toxicity studies

Using the modified method of acute toxicity, the current study aimed at testing the following parameters (13). The method determines the concentration of the extract that would be lethal to 50% of the animals (LD50). The estimation of LD50 values for the crude extract was conducted on Swiss albino mice and Healthy adult Wistar rats of both sexes. The test routes were both intraperitoneal, and only a small dose increase from the original study was used in the experiments and oral. The administration of the extract in both rats and mice was done in phases. The first phase comprised the distribution of various concentrations of the extract (100, 1000, 1500, and 2000 mg/kg i.p. and p.o.) to find the range in which toxicity was located. The second phase was based on the observations that were made in the first and was part of the second phase and included more specific dosages (800, (of 1000, and 1200 mg/kg i.p.) to new sets of experimental animals. The animals treated i. p. and p. o. were observed for 24 and 72 h, respectively. Respectively, for behavioral effects such as nervousness, ataxia, enthusiasm, wakefulness, lethargy, and death. The LD50 was calculated as the geometric mean of the dose that proved lethal in 100 percent of cases and that which gave no lethality at all.

2.5. Experimental design

Male Wistar albino rats were used, and these rats were randomly divided into five groups, each consisting of five rats. The animals were starved for 24 hrs prior to the study, but water was available at will. Group I, which was used as vehicle control, was given only distilled water; Group II, which was used as diseased control, was administered with aspirin 200mg/kg/p.o; Group III, which was used as standard control, was administered with esomeprazole 20 mg/kg (P. O); Group IV and Group V treated as treatment groups administrated with the graded dose of ethanol extract of *S. bicolor* at 200 and 400 mg/kg respectively, once daily for seven days.

2.6. Ethanol-induced mucosal damage in rats

Before the experiment, the rats were put on a fasting regimen for 24 hours. Sub-groups 4 and 5 were administered EESB, Esomeprazole, and vehicle control treatment for 1 hour, respectively, after which 1ml of absolute ethanol 0.5ml/100g each was orally administered to each rat in every sub-group. After 1 hour, the animals were euthanized with an excess of anaesthetic ether, and the stomachs were longitudinally incised along the greater curvature with the help of scissors, and the content was washed slowly using running tap water. For ulcers, subjects were placed on a glass slide and examined at 10X power of the lens. The ulcers were scored. The mean ulcer score in each group was calculated and was designated as the ulcer index, and the percentage was calculated using the following formula: formula:

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

Where C = ulcer index in the control group.

T = ulcer index in the treated group.

Pylorus ligation-induced gastric ulceration

In this method, overnight-fasted albino rats were put into separate cages; EESB, reference drug, and control vehicle were given one hour before pyloric ligation. Then the pre-treated animals were made unconscious by anaesthetic ether; the peritoneum was then incised by a small centrally placed ventral midline incision below the xiphoid process. The forbidden tie of the pyloric portion of the stomach also did not affect the blood vessels of that area. Consecutive sutures were taken to close the abdominal wall after proper incision of the stomach. Specifically, postoperative care of animals involves withholding water during recovery time. After 4 hours of

ligation, the stomach was removed, and content was induced into clean test tubes. The quantity of gastric juice that was secreted, the pH of the secretion, and the total acid strength were measured. The contents were spun in a centrifuge was done, and later filtered; titration to estimate the total acidity was made. From the supernatant, 1-ml aliquots were used to determine the pH several or free total/ acidity activity.

Each stomach was then scored for gross lesions in the fore stomach portion and was indexed in accordance with the severity identified. The numbers of ulcers were counted, and the scoring of ulcers was made as follows: Normal colored stomach (0), Red coloration (0. 5), Single pinpoint ulceration (1), Haemorrhagic changes (1. 5), Multiple ulcers (2) and Perforation (3). Ulcero of each animal was quantified by averaging the number of ulcers of that animal and the termed it ulcer index. Ulcer index (UI) was measured by using the following formula: UI = (UN + US + UP) x 10 −1' where. UI = Ulcer index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers. The ulcers' size inhibition percent and also the control had been estimated and compared with the control. Percentage protection against ulcer= [(UI control – UI treated) / UI control] x 1 (14).

2.7. Biochemical analysis:

After sacrifice, the stomach tissue from each group was isolated and immediately stored at -80ºC for further analysis. A 100 mg frozen sample from stomach tissue was homogenized using phosphate buffer saline (PBS) at pH 7.4. Following centrifugation of the homogenates at 12,000 rpm for 10 minutes, the supernatants were collected. The concentration of each marker was normalized to the protein content of each stomach sample, which was determined using the Bicinchoninic acid (BCA) assay. The methods for estimating biochemical levels are briefly discussed below.

2.7.1. Evaluation of nitrite levels: The Griess reagent was prepared by dissolving 150 mg of sulphanilamide, 50 mg of N-(1-naphthyl) ethylenediamine (NED), and 900 µl of phosphoric acid in 14.1 ml of distilled water. For plating, 100 µl of each standard or sample was mixed with 100 µl of Griess reagent and incubated at 37°C for 30 minutes. The absorbance of the resulting purple color was measured at 545 nm using a multimode reader (15).

2.7.2. Measurement of glutathione level: The working reagent was prepared by dissolving 1.98 mg of DTNB in 50 ml of PBS. According to protocol, 750 µl of the working reagent was mixed

with 200 µl of the standard or supernatant, vortexed for 1 minute, and incubated at 37ºC for 10 minutes. The absorbance of the resulting yellow product was measured at 412 nm (16).

2.7.3. Evaluation of catalase levels: Blank, control blank, and sample tubes were prepared according to protocol, each with a final volume of 220 µl. In the control blank (C), 20 µl of phosphate buffer and 100 µl of H2O2 were added, followed by 100 µl of phosphate buffer. In the sample (S) wells, 20 µl of the sample supernatant was added instead of the buffer. For the blank (B), only 220 µl of phosphate buffer was added. The catalase concentration in the stomach was quantitatively measured colorimetrically at 610 nm (17).

2.7.4. Evaluation of Lipid peroxidation: The MDA level was quantified using 1,1,3,3-tetramethoxypropane (TMP) as a standard, which generates MDA through acid hydrolysis. In the test tube, 750 µl of TBA was mixed with 100 µl of the sample, followed by 100 µl of SDS solution and 750 µl of acetic acid. Then, 300 µl of distilled water was added. The test tubes were heated in a water bath at 95°C for 60 minutes. The optical density was measured at 532 nm (18).

2.8. Statistical analysis

The average and standard error of the mean (SEM) (n = 6) were used to present all of the experimental data. One-way analysis of variance (ANOVA) was performed to statistically process the data in order to identify differences between several groups (Graph pad prism 4.0 software). It was thought that a p-Value of 0.001 was quite significant.

3.**Result & discussion**

3.1. Yield Percentage

The leaves of *Sorghum bicolor* have been extracted using a Soxhlet apparatus with solvents such as ethanol and distilled water. Table 1 shows the percentage yield in solvents: the highest percentage value was found in ethanol.

S.No.	Extract	% Yield
1.	Ethanolic	9.07
2.	Water	6.45

Table 1- % Yield of *Sorghum bicolor* leaves extract

3.2. Physico chemical parameter

S.No.	Parameter	Result
1.	Total ash	13.63
2.	Water soluble ash	3.49

3.	Acid insoluble ash	1.28
4.	Moisture content	7.94

Table 2- Physicochemical parameter of EESBL

3.3. Preliminary phytochemical analysis of *Sorghum bicolor* leaves

S.No.	Phytoconstituents	Result
1.	**Carbohydrates**	
	Molish's test	+ive
2.	**Tannins**	
	Lead acetate solution	+ive
	Acetic acid solution	+ive
3.	**Glycoside**	
	Legal's test	-ive
4.	**Flavonoids**	+ive
5.	**Proteins**	
	Biuret test	+ive
6.	**Alkaloids**	
	Wagner's test	+ive
	Hager's test	+ive
7.	**Steroids**	+ive
8.	**Saponins**	+ive

Table 3- Phytochemical screening of EESBL.

3.4. Thin layer chromatographic analysis

Extract Name	Solvent system	Light			
		Visible		UV	
		Spot	Rf	Spot	Rf
Sorghum bicolor	Ethyl acetate: n-hexane 5:2	2	4.2/8.9=0.47	3	3.5/6.7=0.52
			7.6/8.9=0.85		6.7/8.9=0.75
					4.6/8.9=0.516

Table 4- Qualitative results of TLC of EESBL.

(a)  (b) 

Fig.1 : TLC of ethanolic extract of *Sorghum bicolor* (Ethyl acetate : n-hexane(5:3))
(a)UV Light (b)Visible Light

3.5. Acute Toxicity Study

The acute oral toxic test in rats, as defined by the Organization for Economic Co- operation and Development (OECD) as per 423 guidelines, was used to evaluate a safe oral dose of ethanolic extract of *Sorghum bicolor* leaves (EESBL) (OECD Guidelines Chemical 2010). The EESBL was made by dissolve extract on ethanol then changing a concentration so It's not exceed 1 ml/100g in the rat at various doses up to 200 for test of was then given to the animal (p.o.) and they were monitored for behavioral changes, mortality and toxicity, and for the next 24 hours. Based on the mg/kg. For the extract acute toxicity testing, two separate doses of EESBL (400 and 200 mg/kg. p.o) were chosen in this study.

3.6. EESBL reduces ethanol-induced Oxidative Stress markers

3.6.1. Nitrite levels: In the vehicle-treated ethanol group, nitrite levels were significantly elevated compared to the sham group. However, treatment with EESBL markedly reduced nitrite levels compared to the ethanol group, as illustrated in Figure 2.

3.6.2. Glutathione levels: GSH levels were significantly reduced in the ethanol + vehicle group compared to the sham group. However, treatment with EESBL increased the antioxidant marker GSH in the ethanol + EESBL group compared to the ethanol + vehicle group, as illustrated in Figure 2.

3.6.3. Catalase levels: The vehicle-treated ethanol group showed a significant decrease in catalase concentration compared to the sham group. Treatment with EESBL did not alter catalase levels in brain tissue following ethanol-induced mucosal damage.

3.6.4. Lipid peroxidation levels: The vehicle-treated ethanol group exhibited a significant increase in MDA content compared to the sham group, indicating enhanced lipid peroxidation. In contrast, EESBL treatment significantly reduced the ethanol-induced rise in MDA content, demonstrating its potential protective effect against lipid peroxidation in mucosal damage, as illustrated in Figure 2.

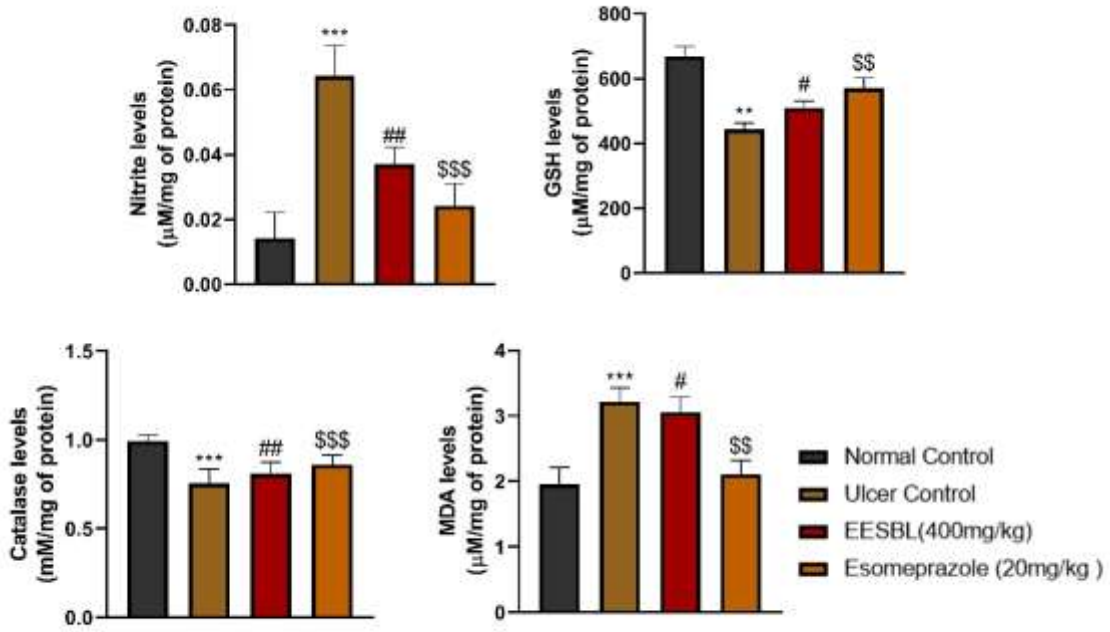

Fig. 2: Representative Bar diagram of antioxidant assay. The data were expressed as the mean ± SEM (n=5), and statistical analysis was conducted using one-way ANOVA followed by Dunnett's multiple comparison test. Where significant difference was observed between *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs control group, #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 vs ethanol group, and $p<0.05, $$p<0.01, $$$p<0.001, $$$$p<0.0001 vs ethanol group.

3.7. Effect of EESBL on mucosal damage

The ethanolic extract of Sorghum bicolor leaves (EESBL) significantly attenuated ethanol-induced mucosal damage in Wistar rats. The group treated with EESBL exhibited markedly fewer gastric lesions compared to the control group that received ethanol alone. Morphological examination showed that EESBL treatment preserved gastric mucosal, substantially reducing erosion. These findings suggest that EESBL has protective properties that counteract the harmful effects of ethanol on gastric mucosal tissue.

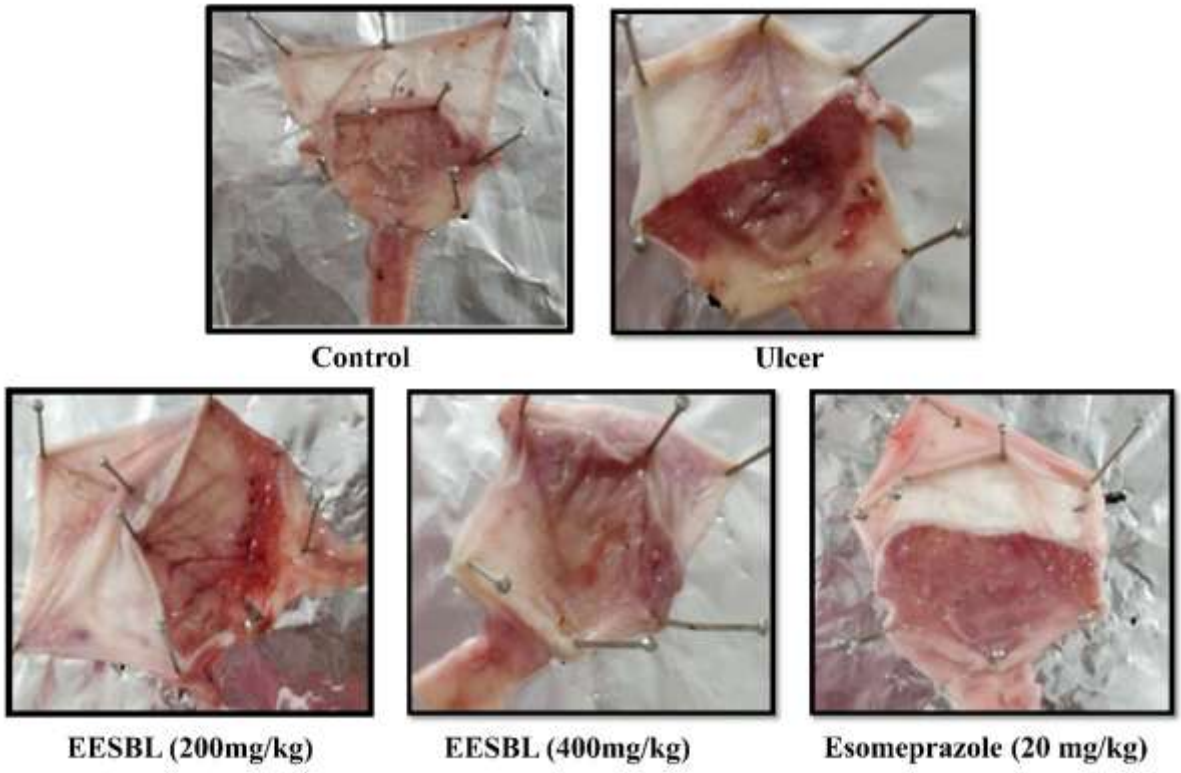

Fig. 2: Representative microscopic images of Stomach of different groups

3.8. Effect of EESBL on antiulcer activity:

The ethanolic extract of Sorghum bicolor leaves (EESBL) demonstrated significant attenuation of ethanol-induced mucosal damage in Wistar rats. In the experimental group treated with ethanol, there was a marked increase in ulcer index, a decrease in the percentage of protection, and increases in gastric volume, free acidity, and total acidity. However, treatment with different doses of EESBL significantly reduced the ulcer index, increased the percentage of protection,

and decreased gastric volume, free acidity, and total acidity. These findings indicate that EESBL effectively mitigates the harmful effects of ethanol on the gastric mucosa, suggesting its potential as an antiulcer agent.

Group	**Treatment**	**Dose**	**Ulcer index ± SEM**	**% protection**	**Gastric pH ± SEM**	**Gastric volume ± SEM**	**Free acidity mEq/l ± SEM**	**Total acidity mEq/l ± SEM**
Group I Normal Control	Inducer(Ethanol)	(5 mL/ kg b. wt., p.o.	-	-	3.18±0.55	3.04± 0.56	34.23± 3.72	62.93± 5.23
Group II Ulcer Control		2ml/ kg, b.w, p.o	4.58± 0.34	-	2.35± 0.05	5.23± 0.32	80.01±14.21	88.33± 13.10
Group III Standard	Esomeprazole	20mg/kg b.w., p.o.	2.19± 0.25	52.18	5.14± 0.76	3.56± 0.60	38.26± 5.09	44.20± 8.65
Group IV Test 1	EESBL(200mg/kg)	200 mg/ kg b.w., p.o.	3.07± 0.38	32.96	4.76± 1.11	4.99± 0.9	50.17± 8.66	48.18± 5.90
Group V Test 2	EESBL(400mg/kg)	400 mg/ kg b.w., p.o.	2.10± 0.69	54.04	4.83± 0.81	4.30± 0.80	37.65± 0.44	48.18± 4.20

Table 7.5- Effect of EESBL on various parameters in pyloric ligation model

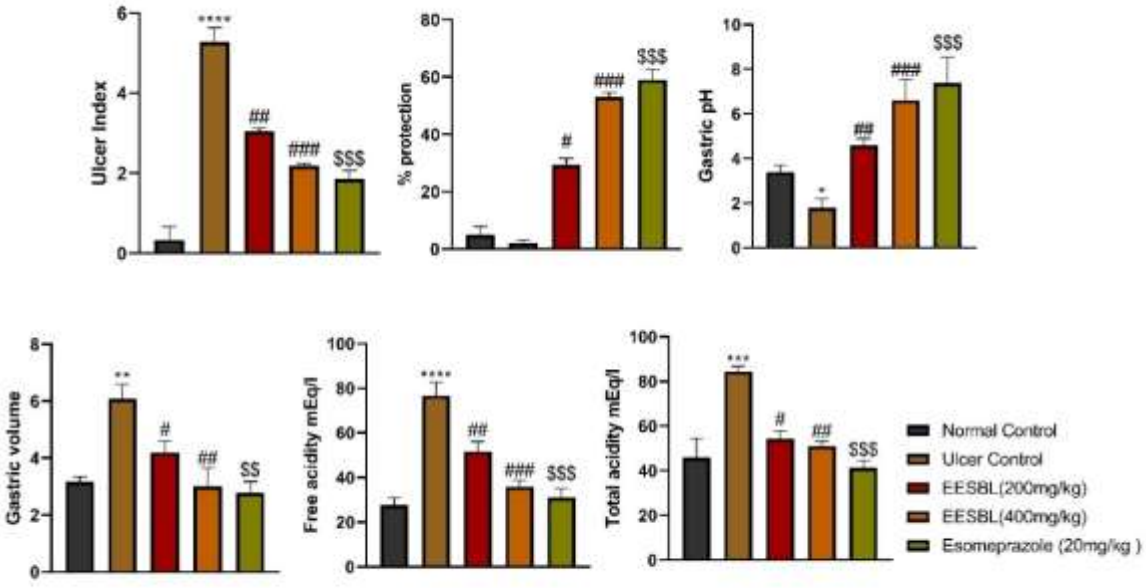

Fig.3: Representative bardiagram of various parameters in pyloric ligation model. The data were expressed as the mean ± SEM (n=5), and statistical analysis was conducted using one-way ANOVA followed by Dunnett's multiple comparison test. Where significant difference was observed between *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs control group, #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 vs ethanol group, and $p<0.05, $$p<0.01, $$$p<0.001, $$$$p<0.0001 vs ethanol group.

3.9. Effect of EESBL on ethanol-induced mucosal damages in Wister rats

The ethanolic extract of Sorghum bicolor leaves (EESBL) demonstrated a significant attenuation of ethanol-induced mucosal damage in Wistar rats. The experimental group treated with EESBL showed markedly reduced gastric lesions compared to the control group that received ethanol alone. Histopathological examination revealed that EESBL treatment preserved the integrity of the gastric mucosa, with a substantial reduction in the extent of erosion. These findings indicate that EESBL possesses protective properties that mitigate the deleterious effects of ethanol on gastric mucosal tissue.

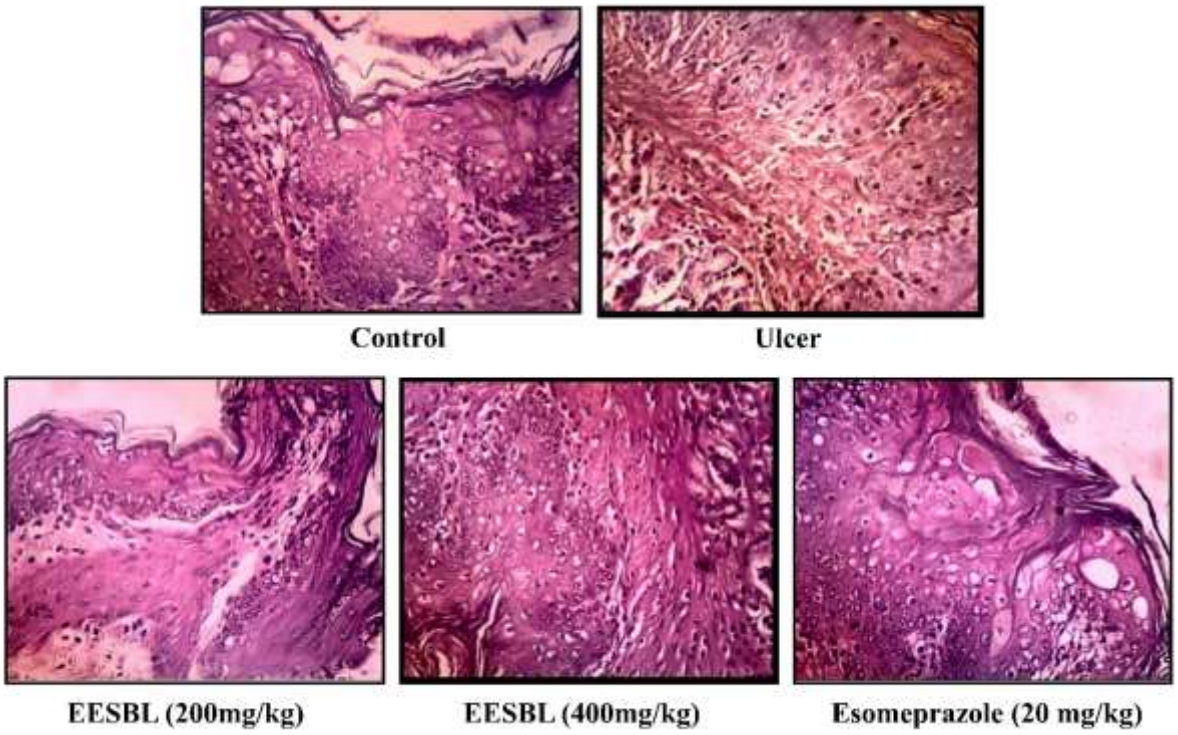

Fig. 4: Representative microphotographs of H & E staining in different group

4. Conclusion

In conclusion, there is a growing preference for herbal products due to their perceived safety compared to synthetic alternatives. Peptic ulcers affect 10% of the global population and are influenced by factors such as alcohol consumption, smoking, stress, NSAIDs, and H. pylori infection (10). Phytochemical screening reveals that the leaves of Sorghum bicolor contain valuable secondary metabolites, including carbohydrates, proteins, flavonoids, alkaloids, steroids, tannins, saponins, and glycosides (19). Our findings suggest that the ethanolic extract of Sorghum bicolor leaves demonstrates significant antiulcer effects in a pyloric ligation-induced gastric ulceration model in rats, showing efficacy comparable to the reference drug esomeprazole. Further research using additional experimental models is warranted to elucidate the specific mechanisms of action of the active compounds responsible for the observed antiulcer activity.

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Declaration of competing interest:

NONE

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