RESEARCH ARTICLE

Evaluation of Antiulcer Activity for Selective and Functional Millet using Pylorus Ligation Induced Ulcer in Rat

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ABSTRACT

Although millet has long been thought to have gastroprotective properties, much research does not assess its efficacy as a treatment for stomach ulcers. Currently, medication therapy is the primary clinical treatment for stomach ulcers. Antacids, cytoprotective medicines, proton pump inhibitors (PPI), H2 histamine receptor antagonists have many side effect and poor patient compliance. Hence, inexpensive, easily accessible, and with less negative effects, millets are an excellent and traditional source in the treatment of a variety of diseases like ulcers. In the present research, pearl millet, finger millet and sorghum millet formulation is used and toxicity and antiulcer activity are determined on wistar rats grouped in six different groups consisting of control, disease, standard, millet formulation, glycerin (gly.) vehicles and millet along with glycerin vehicle. After the test item was administered once at a solitary dosage of 2000 mg/kg, none of the treatment group animals showed several clinical indications of toxicity or mortality. In antiulcer activity, ulcer scoring was found to be less in group treated with millet and glycerin than in the diseased group and standard group and %inhibition was also increased in groups treated with millet with glycerine. Total acidity and pepsin estimation was done and these values for the millet group with glycerine were found within limit compared to disease control. The millet and glycerine treatment significantly inhibited lesions associated with stomach ulcers.

Keywords: Peptic ulcer, Millets, Pylorus ligation, *In-vivo* study, Antiulcer activity.

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INTRODUCTION

One digestive system illness that has a high recurrence rate is gastric ulcers. Between 5 and 10% of people will experience stomach ulcers in their lives, and between 0.1 and 0.3% will experience them annually.[1] The pathological signs include bleeding, erosion, ulcers, and damage to the stomach mucosa. Patients frequently complain of dull discomfort, nausea, vomiting, and regurgitation of acid and epigastric pain.[2] Acute consequences from the disease's long-term progression, such as gastrointestinal bleeding and stomach perforation, can have a major negative impact on patients' health. The pathophysiology of gastric ulcers involves hypersecretion of stomach acid, alcohol misuse, infection of *Helicobacter pylori* infection, smoking and prolonged administration of Non-steroidal anti-inflammatory drugs (NSAIDs). Other significant causes include the dysregulation of ATP-sensitive K+ (KATP) channels and endogenous protective substances like growth hormones, prostaglandins, nitric oxide and mucus.[3]

Currently, medication therapy is the primary clinical treatment for stomach ulcers. Antacids, cytoprotective medicines, PPI, H2 histamine receptor antagonists, and antibacterial medication combinations are among the available treatments for active gastric ulcers. These medications, however, come with a number of negative consequences, such as inadequate healing of ulcers and ulcer returning, which place a significant financial strain on individuals and public health infrastructures.[4-6] Investigating safe and efficient gastro-protective drugs made from natural resources is therefore crucial. There are already a number of edible options that can help treat stomach ulcers[7-9] For ulcer disease, there are a number of pharmacological therapy options, but their use is restricted because of low compliance and frequent side effects. Because of this, every possible substitute for this treatment was looked for and pursued. And finally, natural phytochemicals extracted from plants are inexpensive, easily accessible, and have less negative effects, making them excellent traditional medicines for the treatment of ulcer disease.[10-12]

Asia and Africa are two regions where millets are frequently farmed and is the grain that is most commonly consumed in China. A significant portion of human nutrition comes from millets, whose output has been rising over the past few decades to keep up with the growing global population's

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dietary needs.[13,14] The pearl millet is mostly grown in India. Other significant minor millets comprise foxtail, proso, and finger millet, among others. Millets are considered to have an evolutionary origin. Micronutrients are commonly found in millets, phytochemicals, proteins, carbohydrates having best amino acid profile etc., and are also important sources of millets. It has antioxidant properties as well. It improves nutritional value, is good for health, and lowers the chance of developing a number of illnesses and conditions.[15] Protein, soluble fiber, essential fatty acids, minerals (potassium, zinc, magnesium, calcium, iron), and vitamins (especially vitamin B complex) are all abundant in millet. Millets also help prevent a number of illnesses, such as diabetes, gastrointestinal disorders, heart-related diseases, blood pressure, thyroid disorders, and celiac disease; because they support steady blood pressure maintenance and levels of sugar.[16] Millet can be used as an excellent source of vegetable protein because it contains more of each amino acid than the FAO/WHO advises. According to research by Zhang B *et al*, eating a lot of millet protects against colorectal cancer linked to colitis.[17]

The present study uses finger millet, pearl millet and sorghum as millet formulations to evaluate the antiulcer activity on rat. Singh N *et al* investigated the antibacterial and anticancer effects of extracts from pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*).[18] Known by most as "bajra," pearl millet is a staple diet for the underprivileged. The grains are estimated to contain approximately 12% proteins, 5% ether extractives (including lipids), and 67% carbs. Another name for finger millet is "ragi," which is eaten without being hulled. It is abundant in tannins and polyphenols and is said to be a good supply of antioxidants. Phenolics are the most common secondary metabolic products found in plants. Polyphenols are the most significant phytochemicals found in millet because of their potential as nutraceuticals, including antioxidant activity, anti-inflammatory, anticarcinogenic, antibacterial, antidiarrheal, antiulcer, and anti-cardiovascular activities.[18]

MATERIALS AND METHODS

Materials

Millet formulation includes a mixture of pearl millet, finger millet, jowar and sesame seeds collected from local market. Wistar rats with age of seven weeks and weight variation of ± 20% of the average weight in each sex were obtained from Crystal Biological Solution, Pune and National Institute of Biosciences 69 Dhanagwadi, Pune.

Methods

Formation of millet formulation

The selected millet were cleaned for dust particles, stones, and other dirt, washed under tap water, and moistened for 2 hours. After moistening millets were individually dried under sunlight. Further millets were roasted individually in open pan with direct heat at about 80 to 100°C with stirring continuously by wooden ladle until the shade of millet colour changed slightly compared to original color. Similarly, sesame seeds were roasted in open pan till the colour of seeds slightly changed to light brown. After roasting all ingredients viz., finger millet, pearl millet, sorghum and sesame seeds were cooled to room temperature and then ground. All the powdered ingredients were blended together to get the proper mixture of them. The resulting final product of powder mixture, i.e., formulated millet obtained, was stored in an air tight container at room temperature and further pharmacological investigation to be carried out for the antiulcer study.

Selection of animal and route of administration

One species that is suggested for single dose toxicity study and antiulcer activity was wistar rat. They are widely used in the business to evaluate the safety of goods. A significant quantity of data is available for comparison. Since the oral route is the suggested method of administration for people, it has been selected. The oral route is one of the suggested methods for toxicity testing and antiulcer activity.

Single-dose toxicity study

Toxicity study design

About 2000 mg of millet samples once on the first day of the experiment was given and the total duration was 14 days. The single-dose toxicity study design involves consisting 6 animals. Polycarbonate sterilized cages were used to house the animals. Standard animal husbandry practices were followed in the maintenance of the animal quarters. The animal room was kept at 22 ± 3°C temperature with a 30 to 70% relative humidity level. The 12-hour cycle of light and dark was preserved in the photoperiodicity. The animals were provided standard pelleted feed in terms of the macro- and micronutrient makeup. *Ad libitum* access to purified water gathered using Aqua Guard was granted to animals. Marking was done tails of animals for identification. Individual cage tags identifying the research quantity, animal quantities, dosage, group, route, species, sex, and experiment start and finish dates were attached to each cage.

Observations

Pre-terminal deaths

For 14 days, the animals were monitored twice a day, at intervals of 30 minutes, an hour, two hours, and four hours, after being exposed to the test item, in order towards document whichever signs of toxicity and mortality.

Intake of food and body weight

The food intake profile and each animal's body weight were measured once a week during the trial.

Clinical observations

The animals were observed once after exposure to the test object in the first half-hour and the first four hours. Any clinical symptoms were noted for a period of 14 days.

Antiulcer activity

Adult male wistar albino rats, ages two to three months, weighing 200 to 220 grams, were housed in cages with controlled light (12 hours of light and dark), constant

temperature (24–27°C), and constant relative humidity (50–70%). Standard animal husbandry practices were followed in the maintenance of the animals. The animals were provided standard pelleted feed in terms of the macro- and micronutrient makeup. *Ad libitum* use of Aqua Guard-collected filtered water was granted to the animals. Before the trial, the animals underwent at least two weeks of acclimatization and isolation. This study was carried out in accordance with CCSEA and an experimental protocol authorized by the research ethics committee at Biocyte Institute of Research and Development, Sangli with the serial number IAEC/Sangli/2022-23/07.

Grouping of animals

Six groups of male albino wistar rats weighing 200 to 220 g are created, with six individuals in each group. Rats were kept in cages with grating floors during fasting to prevent coprophagy. Animals were marked to identify them. Individual cage tags identifying the research quantity, animal quantities, dosage, group, route, species, sex, and experiment start and finish dates were attached to each cage. Animal groups and their dose are defined in Table 1.

The pylorus ligation procedure was used to induce ulcers. Animals used in the pylorus ligated ulcer procedure had a 24-hour fast. One hour before pyloric ligation, animals were orally given vehicle, standard, and millet extract. Petroleum ether was used to induce anesthesia in each rats taken for the surgery. The abdomen could be unlocked through a smaller incision made beneath the xiphoid procedure in the midline, and the stomach's pylorus was gently pushed out and sutured. The safety measure was implemented to prevent traction on the pylorus or harm to its blood resources. Carefully positioning the stomach inside the abdomen, interrupted sutures were used to close the incision. After surgery, to recuperate, the animals were housed in different cages and were not given entrée towards water. Six hours later, the stomach was removed after the animals were sacrificed, sliced along the larger curvature, properly cleaned, and ulcer scored. Gastric juice was collected and gastric secretion investigations were executed.[19]

Anti-secretory activity of millets and millets with glycerin

- *Macroscopic examination for ulcer scoring*

Every induction model used the same ulcer score measurement. The ulcer was scored as follows: 0.5 for hemorrhage or redness, 1 for a spot ulcer, 1.5 for streaks of hemorrhage, 2. A severe ulcer 3-Erosions, 4-Perforations. The ulcer index represented the mean ulcer for every animal. This is how the % of ulcer protection will be estimated.

Table 1: Animal groups and their dose induction of ulcer in rats

Groups	*Dose*
Group- I Sham control	10 mL/kg normal saline PO
Group- II – Disease control	10 mL/kg normal saline PO
Group- III – Standard (Omeprazole-20 mg/kg, PO)	0.5 mL of omeprazole (20 mg/kg) PO
Group-IV –Millets	0.5 mL of millets 60 mg/5 mL, PO
Group-V –Glycerine vehicle (50% P.O)	5 mL glycerine (50%), PO
Group-VI –Millets with glycerine	5 mL (1:1) gly.(50%) with millets, PO

%Protection = Mean ulcer index control - Mean ulcer index test/Mean ulcer index control X 100 ------ (1)

Volume of gastric content and pH measurement

The esophagus was used to collect the stomach contents. For ten minutes, the stomach liquid was centrifuged at 3000 rpm and the quantity of gastric juice (mL/kg b.w.) was utilized to express the supernatant's volume that a pH meter was utilized to measure the stomach juice's pH. After that, it was examined using a number of biochemical parameters.[20]

Biochemical evaluation of total acidity in gastric juice

One mL of the supernatant liquid was pipetted out with distilled water and diluted to ten milliliters. Using a few drops of Topfer's reagent as an indicator, the solution was titrated in contrast to 0.01 N NaOH until the solution's red hue disappeared and turned orange. The free acidity was used to calculate the volume of NaOH required. A few drops of phenolphthalein solution were used to continue the titration process until the pink color reappeared. It was determined that the volume of NaOH needed corresponded to the overall acidity.[21]

Acidity = NaOH Volume X Normality of NaOH X 100 mEq/l / 0.1 ---- (2)

Estimation of pepsin activity

Tyrosine was formed due to pepsin's digestion of hemoglobin solution, which was used to measure pepsin activity. The reaction mixture—one mL of gastric juice sample and five mL of substrate (1% BSA in HCl at pH 2.1)—was incubated for fifteen minutes. Ten mL of TCA were added, and this stopped the process. Before substrate addition, the blank (10 mL TCA + 1-mL gastric juice) was incubated 15 minutes before substrate addition. The reaction mixture and blank were filtered after 30 minutes. To filtrate, 10 mL 0.5 M NaOH + 1-mL folin-phenol added, measuring absorbance at 680 nm. Tyrosine concentrations graphed. Pepsin activity expressed as µg tyrosine equivalents/mL gastric juice/min.

Total protein

Total protein was calculated using standard protocol. 0.1 mL gastric juice diluted with 0.9 mL water. About 4.5 mL added alkaline copper reagent was incubated 10 minutes, then 0.5 mL Folin reagent was incubated 20 minutes and measured absorbance at 640 nm. Protein concentration determined from the BSA standard graph, reported as µg/mL.

Histopathological studies

One animal's freshly removed stomach from each group was stored in 10% formaldehyde solution for histological analysis after being cleaned with saline. It was treated using

Table 2: Observations for pre-terminal deaths

Sex	*Animal no*	*Day*													
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Females	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Males	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Where 0= Normal, X- not survive

Table 3: Observations for food intake

Animal ID	*Day*	
	0	*14*
01	109 gm	115 gm
02		
03		
04		
05		
06		

Table 4: Observations for body weight

Animal ID	*Day*		
	0 (gm)	*8 (gm)*	*14 (gm)*
1	195	208	218
2	194	207	215
3	184	189	204
4	187	198	212
5	194	199	207
6	187	195	210

isopropyl alcohol, xylene, and paraffin embedded for a 12-hour investigation under a light microscope (Magnus IV). To confirm the morphological assessment of stomach injury, paraffin-embedded tissue sections cut to a thickness of 5 µm were produced and deparaffinated using hematoxylin and eosin stain (H & E). Photographs under a 40X magnification were taken.[22]

RESULT

Single-Dose Toxicity Study

Pre-terminal deaths

During the study period, no pre-terminal deaths were noted**.** Observations are shown in Table 2.

Food intake and body weight

During the study time, the animals' food consumption was found to be normal, and no discernible effects were observed in them. During the study time, the animals did not exhibit any notable effects, and their body weight gain was normal. Observations are shown in Tables 3 and 4.

Table 5: Observations for clinical signs

General signs	1	2	3
Assessments of posture	0	0	0
Signs of convulsion (Limb paralysis),	0	0	0
Body tone	0	0	0
Lacrimation	Y	Y	Y
Salivation	Y	Y	Y
Tremor	0	0	0
Change in skin color	Y	Y	Y
Piloerection	Y	Y	Y
Defaecation	0	0	0
Sensitivity response	0	0	0
Locomotion	0	0	0
Muscle grips	0	0	0
Rearing	0	0	0
Food intake	0	0	0

Where, 0=Normal, X=Not survived, Y=No any sign

Clinical Observations

The clinical observations, which included a detailed assessment of the skin, fur, eyes, mucous membranes, respiratory, salivation, diarrhea, behavioral pattern, and autonomic and central nervous systems, remained unchanged. A special focus was placed on reports of coma, convulsions, and tremors. Observations for clinical signs are shown in Table 5.

Antiulcer Activity

Macroscopic examination for ulcer scoring

Figure 1 (A to F) shows the macroscopic examination for ulcer scoring. Ulcer index and %inhibition for all the groups are shown in Table 6. In the disease control group (group II) erosion was found where normal saline was given to the animals (Figure 1B). Omeprazole is the antiulcer drug that was given to the standard group (group III) where only spot ulcer was observed (Figure 1C). Group VI of millet formulation with glycerin reduced ulceration at a significant level (Figure 1D). %Inhibition was found to be the maximum in a group where omeprazole standard drug was used. When

Table 6: Ulcer index and %inhibition

Groups	*Dose*	*Ulcer index*	*%inhibition (%)*
Group- I Sham control	10 mL/kg Normal saline PO	-	-
Group- II Disease control	10 mL/kg Normal saline PO	3.0	-
Group- III Standard (Omeprazole-20 mg/kg, PO)	0.5 mL of omeprazole 20 mg/kg PO	1.0	66.00
Group-IV –Millets	5 mL of millets 60 mg/5 mL	2.5	16.66
Group-V –Glycerin vehicle (50% P.O)	5 mL glycerine (50%)	2.0	33.00
Group-VI –Millets with glycerin	5 mL (1:1) gly.(50%) with millets	1.5	50.00

only millet formulation was used the %inhibition was found to be 16.66% only and when it was combined with glycerine, the %inhibition was increased by 3-fold, i.e., 50% inhibition (Figure 2).

Volume of gastric content and pH measurement

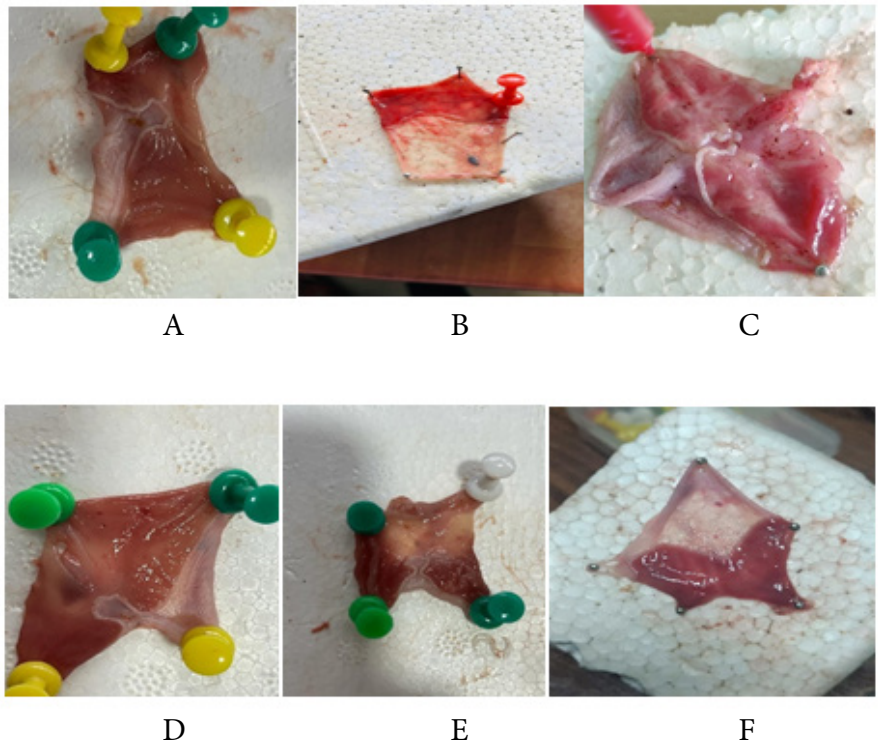
All the observations for a volume of gastric juice and pH of gastric juice are shown in Table 7 and Figures 3 and 4. In a group of VI (Millet+ glycerine) pH for gastric juice was found to be 6.11. For the control group it was found to be 3.8, for the disease control group 4.7; for the standard group, 6.3, and for the millet group it was 6.1.

Biochemical evaluation of total acidity in gastric juice

Values for free acidity and total acidity for all the groups were calculated as per procedure and all the observations are represented in Table 8 and Figure 5. Total acidity in the millet with glycerine group was found to be 80 mEq which is less as compared to the disease control group and standard group.

Estimation of pepsin activity and total protein

The primary ingredient in gastric juice is gastric acid, which is secreted by the stomach's parietal cells. Pepsinogen is transformed into pepsin by gastric acid. Optimal pH for pepsin digestion: 1.5 to 2.5, matching the acidity of normal stomach juice. Because the stomach acids in the gut are neutralized (pH 7), pepsin is no longer functional. Increased pepsin activity indicates that overproduction of stomach acid will damage the protective layer of the gastric mucosa, potentially leading to chronic atrophic gastritis, gastric ulcers, and other disorders.[17] The estimation of pepsin activity and total protein was calculated which are shown in Table 9 and Figure 6. The pepsin values for millet with glycerine group were found to be



(A: Sham Control; B: Disease Control; C: Standard (Omeprazole); D: Millets; E: Glycerine F: Millets+Glycerine)

Figure 1: Macroscopic examination for ulcer scoring

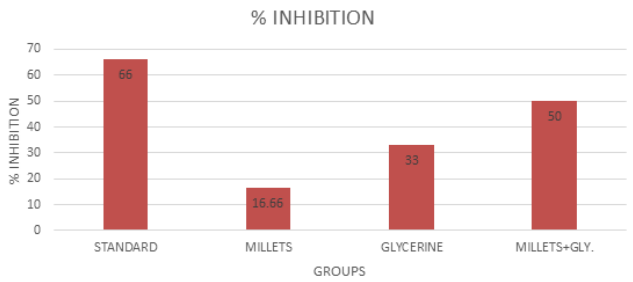


Figure 2: %inhibition for all the groups

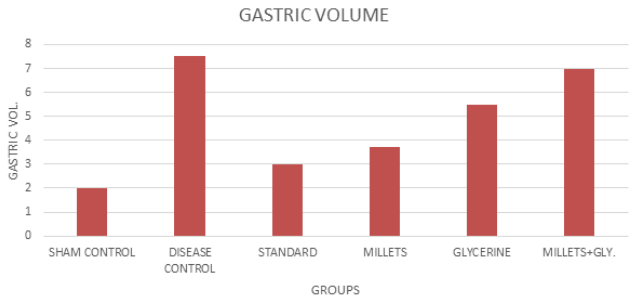


Figure 3: Gastric volume for all groups

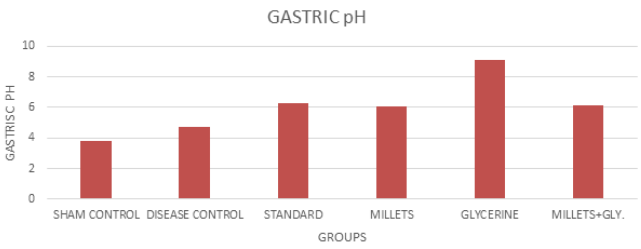


Figure 4: Gastric pH for all groups

15.38 µg/mL which is less compared to disease control, which was 25.49 µg/mL.

Histopathological studies

Hematoxylenol (HE) staining was used to examine gastric tissue sections from rat for histopathological studies. In the control group (Figure 7 A), the submucosa, glandular layer, and mucosal layer were all intact. On the other hand, the diseased group (Figure 7B) showed signs of inflammatory spillover, a markedly disordered glandular layer, a markedly inadequate stomach mucosal epithelium, and prominent edema in the submucosa. When comparison done with diseased rat, the extent of the lesions in the standard treated with omeprazole rat (Figure 7C) decreased. However, the gland's structure was somewhat disorganized, and the epithelial tissue was still noticeably lacking. Lesions associated with stomach ulcers were significantly inhibited by the application of glycerine and millet (Figure 7 D, E and F). The only abnormalities found were a little glandular structural region abnormality and modest deficiencies in the mucosal epithelium of the stomach.

DISCUSSION

Pearl millet, finger millet and jowar millet are rich in polyphenols. Polyphenols are the most important phytochemicals. Although it has long been believed to be helpful for the stomach, there is no scientific proof of its efficacy. Protein is one of millet's main ingredients, which has been found to have a variety of physiologic effects on mice, including bettering lipid metabolism and type 2 diabetes.[23,24] It has been shown that bioactive peptides produced from millet have antioxidant properties. It was previously discovered that the inhibition of intestinal mucin expression underlies the preventative impact of millet protein on colitis. This finding suggests that additional research into this area could be beneficial for the treatment of gastrointestinal problems.[17] These studies were suggesting the antiulcer activity of millet formulation. We have found very limited research on pearl millet, finger millet and sorghum as antiulcer activity. This study reveals the toxicity and antiulcer studies carried out on wistar albino rats.

Single dose toxicity study was based on checking on the pre-terminal death of rat; and during the study period, no pre-terminal deaths were noted. Also, the animals did not exhibit any notable effects during the study time, and their body weight gain was normal. A special focus was placed on reports of coma, convulsions, and tremors in clinical observations and there were no signs was observed.

Pyloric ligation is one of the popular models used to examine stomach ulcer indications. Inflammatory activation, luminal hemorrhage, lipid peroxidative damage and mucosal ulcers can all result from stomach injury.[25] Damage to the stomach mucosa triggers an inflammatory response that upsurges the expression of inflammatory cytokines such TNF-α, IL-1β, and IL-6.[26] It supplementary induces the infiltration of neutrophils and the death of epithelial cells, postponing the curing of

Table 7: Volume of gastric content and pH measurement

Groups	*Dose*	*Gastric juice volume (mL)*	*Gastric juice pH*
Group- I – Sham control	10 mL/kg normal saline PO	2	3.8
Group- II – Disease control	10 mL/kg normal saline PO	7.5	4.7
Group- III – Standard (Omeprazole-20 mg/kg, PO)	0.5 mL of omeprazole 20 mg/kg PO	3.0	6.3
Group-IV –Millets	5 mL of millets 60 mg/5 mL	3.7	6.1
Group-V –Glycerin vehicle (50% P.O)	5 mL glycerine (50%)	5.5	9.1
Group-VI –Millets with glycerin	5 mL (1:1) gly.(50%) with millets	7	6.11

Table 8: Evaluation of total acidity in gastric juice

Groups	*Dose*	*Free acidity (mEq/1/100 gm)*	*Total acidity (mEq/1/100 gm)*
Group- I – Sham control	10 mL/kg normal saline PO	48.00	97.00
Group- II – Disease control	10 mL/kg normal saline PO	94.00	105.00
Group- III – Standard (Omeprazole-20 mg/kg, PO)	0.5 mL of omeprazole 20 mg/kg PO	42.00	92.00
Group-IV –Millets	5 mL of millets 60 mg/5 mL	27.00	67.00
Group-V –Glycerine vehicle (50% P.O)	5 mL glycerine (50%)	30.00	73.00
Group-VI –Millets with glycerine	5 mL (1:1) gly.(50%) with millets	35.00	80.00

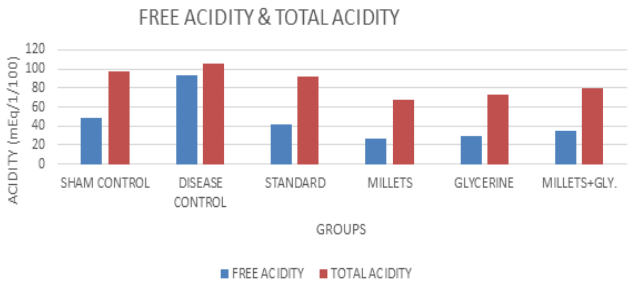


Figure 5: Free acidity and total acidity

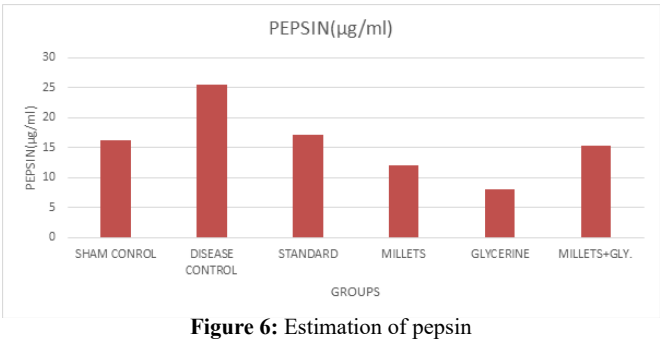


Figure 6: Estimation of pepsin

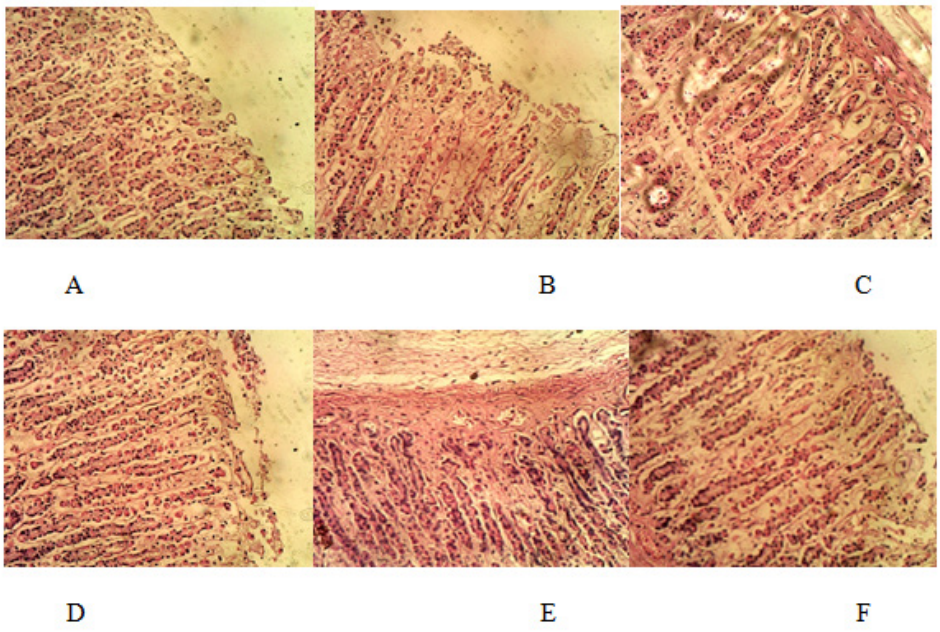


Figure 7: Histopathological studies A) Control group, B) Disease group, C) Standard group treated with omeprazole, D) Group treated with millet, E) group treated with glycerin, F) group treated with millet and glycerin

stomach ulcers.[27-29] By enhancing vagal reflexes and promoting gastric acid output, stomach ligation reveals stomach cells to gastric acid, which might result in gastric ulcers. In this study, pyloric ligation-induced rat model was utilized to assess the effects of millet formulation on the release of digestive enzymes and gastric acid as gastric ulcers advance.[28] The pyloric ligation model was used to test the effects of millet formulation and millet combined with glycerine on gastric acid secretion, total acid production, and pepsin activity in gastric juice. The outcomes recommended the gastro-protective effects of millet formulation along with glycerine. In this study we have used, millet as well millet with glycerine vehicle. In the dose of 60 mg/5 mL of millet formulation %inhibition was found to be 16.66% while for millets with glycerine, it was

Table 9: Results for pepsin and total protein

Groups	*Dose*	*Pepsin (µg/mL)*	*Total Protein (µg/mL)*
Group- I Sham control	10 mL/kg normal saline PO	16.16	0.095
Group- II – Disease control	10 mL/kg normal saline PO	25.49	0.690
Group- III – Standard (Omeprazole-20 mg/kg, PO)	0.5 mL of omeprazole (20mg/kg) PO	17.06	0.376
Group-IV –Millets	5 mL of millets 60 mg/5 mL	12.00	0.560
Group-V –Glycerine vehicle (50% P.O)	5 mL glycerine (50%)	8.07	0.376
Group-VI –Millets with glycerine	5 mL (1:1) gly. (50%) with millets	15.38	0.408

increased to 50% inhibition.[30-33] Total acidity in the millet with glycerine group was found to be 80 mEq which is less as compared to disease control group and standard group. In order to mimic natural digestion, the millet protein in this study was hydrolyzed using pepsin. Therefore, the current findings corroborate the health benefits of a diet rich in millet. Here, we mainly focus on the *in-vivo* toxicity study and in antiulcer activity, total acid estimation, pepsin count, gastric pH, and gastric content measurement and histopathological studies. It will be beneficial in comprehending the function of millet formulation.

CONCLUSION

To sum up, we first presented the results of the toxicity study done on a group of rats; After the test item was administered once at a solitary dosage of 2000 mg/kg, none of the treatment group animals showed some clinical indication of toxicity or mortality. Every animal's food consumption and weight gain were within typical ranges. The animals' physiology, behavior, and physical characteristics did not change much. In antiulcer activity, ulcer scoring was found to be less in group treated with millet and glycerin than in the diseased group and standard group and % inhibition was also increased in groups treated with millet with glycerine. Total acidity in the millet with glycerine group was found to be 80 mEq, less than the disease control and standard groups. The pepsin values for millet with glycerine group were found to be 15.38 µg/mL which is less compared to disease control which was 25.49 µg/mL. The millet and glycerine treatments showed a notable decrease in gastric ulcer lesions. Hence the study can be concluded that, millets are non-toxic and possess antiulcer activity.

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