RESEARCH ARTICLE

Formulation and Development of Solid Oral Dosage Form of Millet and Evaluation of Antiulcer Activity using Ethanol Induced Ulcer in Rat

Archana Vilasrao Vanjari, Subhash T. Kumbhar*

School of Pharmaceutical sciences, Sanjay Ghodawat university, Kolhapur, Maharashtra S. 416118, India

Received: 13th Dec, 2024; Revised:6th Jan, 2025; Accepted: 17th Feb, 2025; Available Online: 25t Mar, 2025

ABSTRACT

An unbalanced mix of harsh and defensive components, such as mucus from stomach, bicarbonate secretion, prostaglandins, and inherent resistance of the components of mucosal cells, leads to the stomach condition known as Peptic Ulcer Disease (PUD). PUD can be treated with a variety of medications, but their usage is restricted because of frequent side effects and low compliance. This led to a thorough search and ongoing investigation for alternatives to this treatment, which ultimately resulted in the discovery of a natural phytochemical, derived from plants that is thought to be an excellent conventional medication to treat PUD because it has less adverse effects and is more easily accessible and affordable. The objective of this in vivo antiulcer activity by using ethanol induced rat and to formulate and develop solid oral dosage form of millet. Millet formulation of tablets and capsules developed and physicochemical evaluation done. Five groups of albino wistar rats weighing 200–220g were created, with six individuals in each group. The ethanol induced ulcer procedure was used to induce ulcers.Animals were given Vehicle, Standard, and millet test orally. Observations for gastric content volume, pH, ulcer scoring, total acidity, pepsin activity and Histopathological studies were performed. The total acidity in the millet group at 300 mg and 600 mg was determined to be 93 and 68 mEq, respectively. Pepsin values for test 1 and test 2 were found to be 24.76 and 21.65 µg/ml. The millet treatment for the concentration of 300mg and 600mg treatment revealed a significant decline of gastric ulcer lesions. The millet with concentration of higher dose as 600mg shows the higher significant activity than the millet with concentration 300mg

Keywords: Antiulcer activity, millets, functional millets, in vivo study.

How to cite this article: Archana Vilasrao Vanjari, Subhash T Kumbhar. Formulation and development of solid oral dosage form of millet and evaluation of Antiulcer Activity using ethanol Induced Ulcer in Rat. International Journal of Drug Delivery Technology. 2025;15(1):244-53. doi: 10.25258/ijddt.15.1.34

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Gastric ulcer is the digestive disease which is having high recurrence rate. Between 0.1 and 0.3% of persons will get ulcers in stomach each year, and between 5 and 10% will get them in their lifetime. Frequent complaints from patients include nausea, vomiting, acid regurgitation, dull discomfort, and epigastric pain.[1] The health of patients may suffer significantly from acute effects of The long-term course of the disease, such as stomach perforation and gastrointestinal haemorrhage.[2] Alcohol abuse, Helicobacter pylori infection, smoking, excessive stomach acid production, and prolonged use of NSAID's are all factors in the pathogenesis of gastric ulcers. ATP-sensitive K+ (KATP) channel dysfunction and endogenous defensive chemicals such as growth hormones, prostaglandins, nitric oxide, and mucus are additional important factors.[3]

The primary clinical intervention for gastric ulcers at the moment is pharmaceutical therapeutic treatment. Different treatments for active stomach ulcers contains proton pump inhibitors, antacids, cytoprotective drugs, H2 histamine receptor antagonists, and a mix of antimicrobial drugs.[2] But there are many side effects of these drugs, such as poor ulcer recovery and recurrence, come at a high financial cost to patients and public health systems. [4]Hence its a essential to research safe and efficient gastroprotective medications made derived from natural resources. Many edible choices are currently available to help treat stomach ulcers.[5-7]

This led to a thorough search and ongoing investigation for alternatives to this treatment, which ultimately resulted in the discovery of a natural phytochemical derived from plants that is thought to be an excellent conventional therapy of PUD because it has very less adverse effects and easy to use and affordable.[8] Millets, maybe the first cereal grain employed in home cooking, were among the first meals consumed by humans. Millets have long been the main nutritional source for people residing in semi-arid tropical regions of Asia and Africa. [9]India is the main location for pearl millet cultivation. The protein content of millet is between 11 and 18 percent,[10] with albumin having the highest proportion and globulin, and gluten following. Millet protein is composed of 10.2% proline, 10.2% aromatic amino acids, and 19.89% branched-chain amino acid. [11]Protein from millets has unique physiological properties, as indicated by the component analysis.Numerous vital amino acids are abundant in millet. Millet has high concentrations of the other seven amino acids, especially tryptophan and methionine, with the exception of lysine, which is in little supply. Millets

**Author for Correspondence : director.pharmacy@sanjayghodawatuniversity.ac.in*

Figure 1: Tablet manufacturing

Figure 2 : Millet capsules

Table 1: Millet tablet

Sr. No.	Ingredients	Role of ingredient
1.	Mixed Millets	Active compound
2.	Excipients	Binder
3.	Sodium Benzoate	Preservative

Table 2: Millet capsule

S. No.	Ingredients	Weight of ingredient sample
1.	Mixed Millets	Active compound
2.	Sodium Benzoate	Preservative

are a good source of protein, dietary fibre, essential fatty acids, minerals, and vitamins.Millets also help prevent many diseases like diabetes, gastrointestinal disorders, because they keep blood pressure and sugar levels steady.[12] Sesame seeds, finger millet, pearl millet, and sorghum were employed in this study. They were selected based on evaluations of the literature that indicated they were high in nutrients. The main aim of current study was to assess the gastroprotective effect of certain functional and selective millets on mice's stomach ulcers caused by ethanol and ligation, as well as to look into the mechanism of it.

MATERIALS AND METHODS

Materials

Pearl millet, Finger millet, Jowar and other ingredients like Sesame seeds and sugar were procured from market. For assessment in vivo, Wistar rats from Crystal Biological Solution in Pune were seven-week-old and had weight variations of ± 20% of the average weight for each sex. Every other chemical utilised was of analytical grade.

Methods

Millet formulation preparation

After being cleared of dust and debris, all millet samples were rinsed with tap water and kept moist for two hours. Millets were individually dried in the sun after being moistened. Other millets were roasted independently in an open pan over direct heat at 80 to 100° C while being constantly stirred with a wooden ladle until the millet's colour somewhat altered from its initial tint.In a similar manner, sesame seeds were toasted in an open skillet until they began to become a light brown. Following roasting, the millets samples were allowed to cool to room temperature before being ground. To get the right mixture, all of the powdered ingredients were combined. The finished powder mixture product, or formulated millet, was kept at room temperature in an airtight container while additional pharmacological research was conducted for the antiulcer study.

Organoleptic properties or sensory evaluation of prepared millet powder

Table 3: Animal groups and their dose

S. No	Groups	Dose	No. of animals
1.	Normal Control	NA	6
2.	Disease Control	Ethanol (1mL/200 g)	6
3.	Standard (Ranitidine)	50 mg/Kg P.O.	6
4.	TEST 1 (300 mg/Kg)- Millets	Millets 300mg/Kg, P.O.	6
5.	TEST 2 (600 mg/Kg- Millets	Millets, 600 mg/Kg, P.O.	6

Table 4: Sensory features of millet formulation

Sr No.	Parameter	Observation
1	Color	Off white
2	odour	Characteristic
3	Visual characteristics	Powder
4	texture	Characteristic
5	Taste	Not specific

Visual inspection of a millet formulation's colour, smell, look, touch, and taste was used to assess its organoleptic property.

Physico-chemical characterization

In this evaluation, BD, TD, CI, Hasner's ratio, moisture content and swelling index was determined by using the official procedure. Bulk density was accomplished by determining the volume that a 5 gm weight of powder occupied in a dry measuring cylinder. Tapped Density was accomplished by determining the volume that a 5 gm weight of powder occupied in a dry measuring cylinder. Additionally, the measuring cylinder was tapped 100 times, and the volume of each tap was noted.Carr's index was computed using the bulk and tapped density data.The tapped and bulk densities' data were used to calculate Hausner's Ratio. Hausner's ratio = Tapped density / Bulk density, this is formula for it.

Table 5: Physicochemical properties

Parameters	Pearl millet	Finger millet	Sorghum	Sesame seeds
BD	0.65	0.56	0.4	0.46
TD	0.86	0.75	0.56	0.56
HR	1.32	1.33	1.4	1.21
CI	24.41	25.33	28.57	17.85
Evaluation (CI /HR)	Passable	Passable	Poor	Fair

Table 6: Physicochemical characteristics of the millet mixture

Sr No.	Parameter	Observation
1	Bulk Density	0.520
2	Tapped density	0.595
3	Hausner's ratio	1.125
4	Carr's index	11.11
5	Angle of repose	33.42
6	pH	6.26
7	Swelling index	11.76 %
8	Dispersibility	65.36%
9	Moisture content	5.3%
10	Total ash	2.3%

Table 7. Weight variation of tablet

Sr No	Weight of tablet mg	Sr no	Weight of tablet mg
1	300	11	310
2	310	12	315
3	315	13	305
4	310	14	305
5	300	15	300
6	310	16	310
7	310	17	300
8	300	18	310
9	295	19	300
10	310	20	310

Moisture Content

Using a hot air oven, the prepared millet powder's moisture content was ascertained.

The powdered ingredients were initially weighed and the wet weight of powder was taken as A. The powder was dried in the oven at 100°C for 30 minutes and then its dry wet was taken as B. It was determined using the formula below:

%W = [(A – B/A)] x 100

Where, %W = % of moisture**,** A = Wet weight of the powder**,** B = Dry weight of the powder

Swelling index

About one gram of the produced millet powder sample was taken in a graduated centrifuge tube, along with 10 mL of water. The tube was then centrifuged after 30 minutes of shaking in a water bath that was held at 30 °C. After being moved to a pre-weighed Petri plate, the supernatant in a hot air oven to dry it set at 105 °C for three hours. After that, it dried out on a water bath by evaporating.To determine the swelling index, the dried Petri plates and the residue in the centrifuge tube were weighed

Dispersibility

One hundred millilitres of water were mixed with about five grams of millet powder. The dispersion was taken in an aliquot (10 mL) and filtered through filter paper that had been pre-weighed. After drying, the filtrate was weighed individually. The percent of dispersed solids was determined by the solids still on the filter paper, and the % of soluble solids was evaluated by the dried filtrates.

Total ash

In a crucible that had already been dried and weighed, three grams of the millet sample were precisely measured. The dish was heated to 550ºC in a muffle furnace, first gradually over a flame and then vigorously till it turned grey. The crucible underwent cooling in a desiccator before being weighed. Heating in muffle furnace for 30 min, cooling and weighing were repeated till concordant weights were obtained.

Table 8. Pre-formulation study

Sr. No.	Contents	Observations
1.	Appearance	Powder
2.	Powder colour	Cream colour
3.	Powder taste	Not specific
4.	Powder smell	Mild

$$Total\ ash\ \% = \frac{wt.with\ ash - wt.empty}{total\ wt} \times 100$$

Where, wt. = weight of crucible

Design and development of millet formulation

Millet Tablets

The prepared powder of millet was further designed for tablets and evaluated their pre-formulation studies.

Method for Tablet Preparation

Millet tables were prepared by standard procedure of wet granulation method. This method is possibly the most frequently used means of producing a compression mix for tablets, ingredients mentioned in below table 1.

Pre-formulation study of prepared millet tablet

Friability Test

After being carefully weighed, twenty tablets were added to the drum. The were removed after the drum was turned 100 times for four minutes at 25 RPM. The extracted tablets were carefully weighed, and their friability was assessed using the procedure. Calculate the friability (loss of weight in %).Friability = (A – B) x 100 / A

Where,A = starting tablet's weight in gm, B = weight of intact tablets in gram following the completion of the rotation.Acceptance criteria for friability of uncoated tablet are not more than 1.0%.

Hardness Test

The tablet is positioned in between top and lower anvils. While the top anvil presses down on tablet, Support is provided by the lower anvil.Until the tablet cracks or breaks, the force is progressively increased. The force is

Table 9. Weight Variation Test

Sr. No.	Weight of capsule (mg)	Sr. No.	Weight of capsule (mg)
1.	450	11.	450
2.	440	12.	450
3.	450	13.	445
4.	455	14.	455
5.	450	15.	450
6.	450	16.	450
7.	445	17.	450
8.	450	18.	450
9.	450	19.	445
10.	455	20.	450

continually measured during the test, and the highest force needed to shatter the tablet is noted.

Disintegration Test

Six three-inch glass tubes with an open top were used in the USP device to evaluate disintegration. The tubes were placed up against a screen with 10 meshes located at the basket rack assembly's base.One pill is put in each tube to measure the disintegration time. Select six pills to measure the disintegration time for in a batch.Fill the beaker with immersion fluid until it reaches 900 mL. Put one tablet into each tube after the HMI displays the desired temperature, for a total of six tablets in six tubes.The discs are employed in accordance with the particular monograph's description. Attached to the rotating shaft is the basket.

Weight Variation Test

Table 10. Gastric content volume and pH

Sr. No.	Group Name	Gastric Content Volume	Gastric Content pH
1.	Normal Control	2.36	3.90
2.	Disease Control	2.04	2.61
3.	Std (RANITIDINE)	3.13	8.13
4.	TEST 1 (300 mg/Kg)	1.15	7.15
5.	TEST 2 (600 mg/Kg)	2.15	7.62

After taking twenty tablets, weigh each one individually. Ascertain the mean weight and contrast it with the mass of every tablet.The tablet passes the U.S.P. test if no tablet deviates from the limit by more than twice the percentage restriction and There is a limit of no more than two tablets.

Millet Capsules

The prepared powder of millet was further designed for second dosage form as capsules and evaluated their pre-formulation studies. The prepared millet capsule composition as given in table 2

Method for millet capsule preparation

The loading tray was loaded with empty capsules, and the tray was then placed into the machine. Verify that the front knob is turned to the right.Lever locks were facing front. Pull down the lengthy handle to remove all of the body's caps. Put the tray with all of the caps aside.Pull the locking lever back to for the capsule bodies to descend and level off against the filling surface.To prevent powder spills,

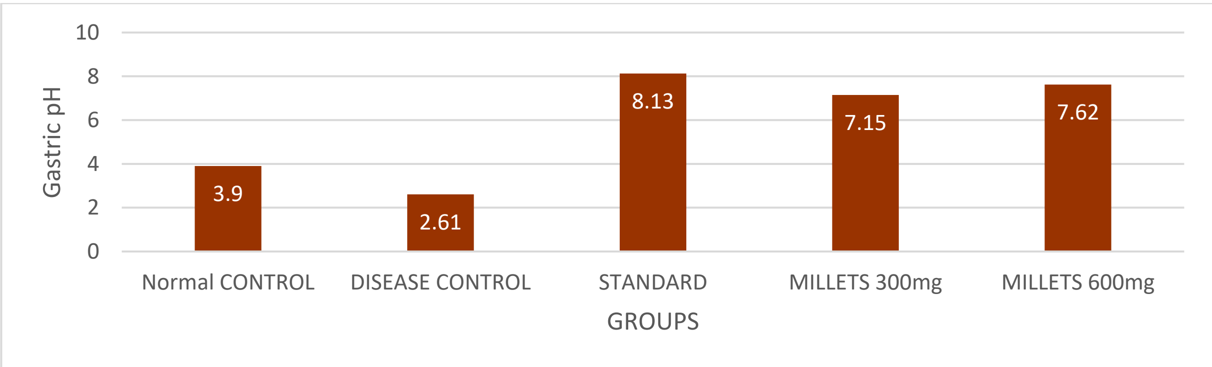


Figure 3: Gastric content pH. *Every value is shown as Mean ± SEM (n = 6), and after comparing them using a one-way ANOVA pair and the findings were statistically significant according to Dunnet's "t" test., *p < 0.05 vehicle against ethanol-induced control; #p < 0.05 treatment groups versus control.*

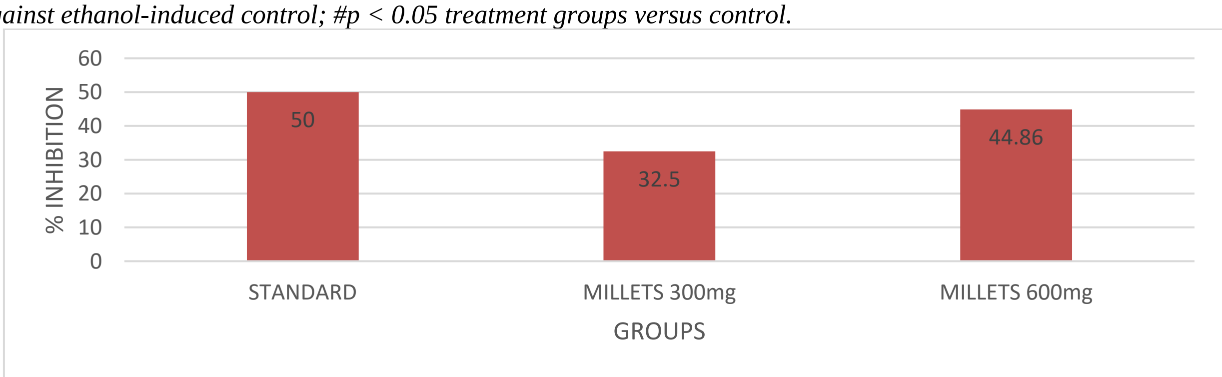


Figure 4: Percentage inhibition. *Every value is shown as Mean ± SEM (n = 6), and after comparing them using a one-way ANOVA pair and the findings were statistically significant according to Dunnet's "t" test, *p < 0.05 vehicle against ethanol-induced control; #p < 0.05 treatment groups versus control.*

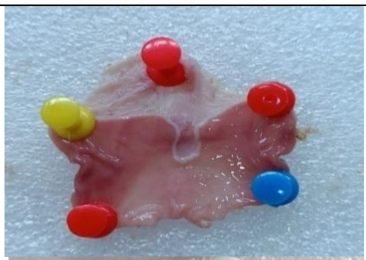
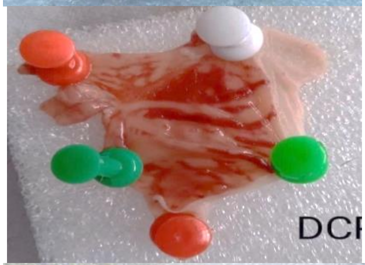
Sr. No.	Group Name	Images Of Stomach
A	Normal Control	
B	Disease Control	
C	Standard	
D	300 mg/kg	
E	600 mg/kg	

Figure 4: Macroscopic examination for Ulcer scoring (A: Normal Control; B: Disease Control; C: Std(Ranitidine); D: Millets 300mg/kg; E: Millets 600mg/kg;

place the millet powder tray on top of the filler.After measuring, pour and sprinkle the millet powder. Place excess powder on the shelf of the powder tray. Reduce tamper and secure.To compress powder, turn the handle.This lets you pack each capsule with additional powder.To guarantee uniform fill weights, raise the tamper and spread more powder from the shelf into the capsules.Place the tray with the caps back into the filler. Slide the front knob to the left to reveal the locking plate. To lock the plate, engage the lock. Press down on the long handle while holding the tamper handle. The bodies are forced up into the caps, locking each capsule in place at once.Take the lock off of the locking plate. Turn the front knob to the right and lift the locking plate.

Lower the extended grip and take out the finished capsule tray. The capsules are now full. The capsules will all fall out of the tray as you turn it.

Pre-formulation study of prepared millet capsule

Disintegration Test

Table 11: Ulcer Index and % Protection

Sr. No.	Group Name	Ulcer Index	% Protection
1	Normal Control	0	-
2	Disease Control	60.00	-
3	Std (RANITIDINE)	30.00	50.0
4	TEST 1 300 mg/Kg	40.50	32.50
5	TEST 2 600 mg/Kg	33.08	44.86

Table 12: Assessment of the stomach juice's overall acidity

Sr. No.	Group Name	Free Acidity	Total Acidity
		(mEq/1/100 gm)	*(mEq/1/100 gm)*
1	Normal Control	25.6	67.66
2	Disease Control	69.6	109
3	Std (RANITIDINE)	34	73
4	TEST 1 (300 mg/Kg)	64.3	93
5	TEST 2 (600 mg/Kg)	35	68

Six three-inch glass tubes with an open top were used in the USP device to evaluate disintegration. The tubes were placed up against a screen with 10 meshes located at the basket rack assembly's base. One capsule is put in each tube to measure the disintegration time. Fill each of the six basket tubes with one dosage unit, and if directed, add a disc.

Weight Variation Test

Around 20 capsules were taken, which are individually weighed. Ascertain the mean weight and contrast it with the mass of every capsule.If no capsule deviates from the limit by more than twice the percentage restriction, the capsule passes the U.S.P. test. and the maximum amount of two capsule is exceeded.

Methods for in-vivo antiulcer activity

Selection of animals

One species that is suggested for antiulcer activity was Wistar rat. These are frequently employed in the industry to assess the safety of products.There is a substantial amount of information that can be compared. The oral route has been chosen since it is the recommended administration strategy for humans. Two to three-month-old male Wistar albino rats weighing 200 to 220 grammes were kept in cages with uniform temperatures (24–27 °C), controlled light and consistent relative humidity (50–70%).The animals were maintained according to standard animal husbandry procedures. The pelleted feed given to the animals had a conventional composition of macro- and micronutrients. The animals were given unlimited access to filtered water collected by Aqua Guard.

The animals were isolated and acclimated for at least two weeks before the study. This research was conducted in compliance with CCSEA and an experimental protocol with serial number IAEC/Sangli/2024-25/08 that has been approved by the research ethics committee at the Biocyte Institute of Research and Development, Sangli

Grouping of animals

There are five sets of six albino Wistar rats, each weighing between 200 and 220 grams. During the fasting phase, Rats were kept in cages with floors made of gratings to avoid coprophagy. Rats were identified by their markings. Each cage had a unique tag attached to it with the study, animal numbers, dose, group, route, species, sex, and dates of the experiment's beginning and end. Table 3 provides an overview of animal categories and their dosage

Ethanol Induced Ulcer Method

Induction of ulcers in rats

After 45 minutes after oral millet sample administration to all animal groups, gastric ulcers were caused by administering 100% alcohol orally at a dose of 1 mL / 200 g of body weight. An hour after 100% ethanol was administered, the animals were slaughtered.After removing the stomach and cutting it along its greater curvature, the ulcer index was scored and the stomach was cleaned with regular saline (0.9%).

Gastric content volume and measurement of pH

The stomach contents were collected via the oesophagus. The stomach fluids undergone centrifugation at 3000 rpm for ten minutes, and the amount of gastric juice was used to indicate the volume of the supernatant. A pH metre was used to calculate the acidity of the stomach liquid. Following that, it was investigated utilising a number of biochemical characteristics. [13]

Macroscopic examination for Ulcer scoring

The ulcer score was measured in the same way for each induction model. By using a magnifying glass to view ulcers, ulcer scoring was accomplished. The incidence and severity of the lesions were graded using the following arbitrary scoring system.

Scoring system for ulcer

Shedding of epithelium =10, One or two ulcers = 30, Perforated ulcers = 50, Frank hemorrhages = 20, More than two ulcers =40

Ulcer index was computed using the following scoring

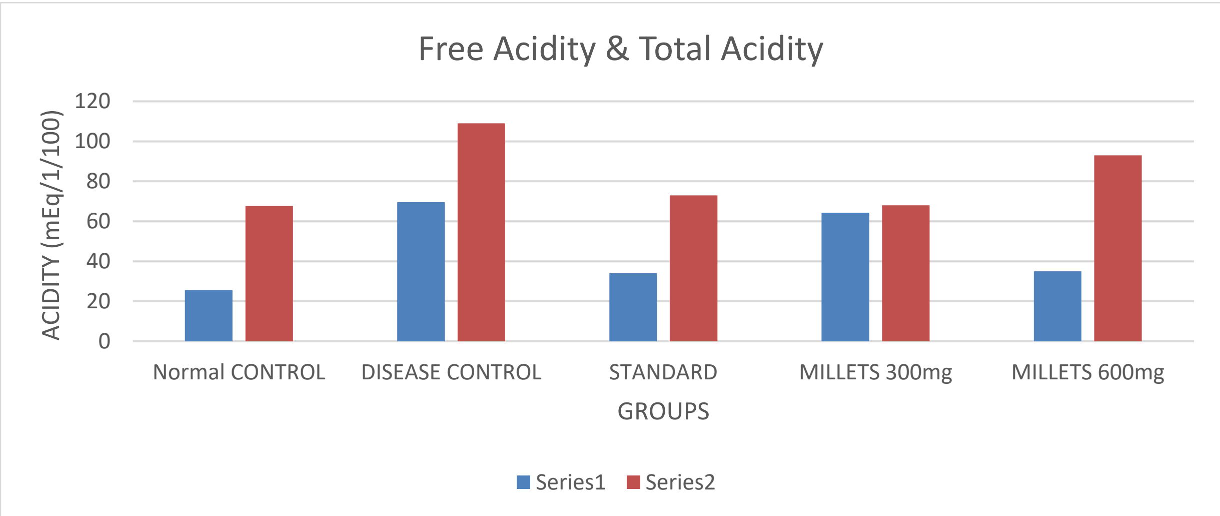


Figure 6 :Free Acidity and Total acidity

*Each value is given as Mean ± SEM (n = 6), and ANOVA pair with Dunnet's "t" test was used to perform statistical significance for comparison.*p < 0.05 vehicle against ethanol-induced control; #p < 0.05 treatment groups versus control.*

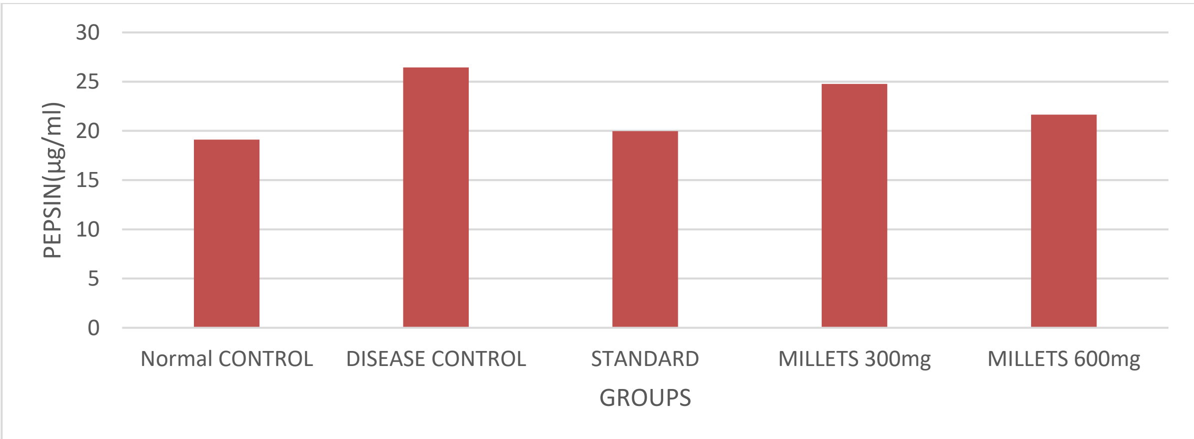


Figure 7: Pepsin Estimation. Every value is shown as Mean ± SEM (n = 6), and after comparing them using a one-way ANOVA pair and The findings were statistically significant according to Dunnet's "t" test., *p < 0.05 vehicle against ethanol-induced control; #p < 0.05 treatment groups versus control.

method:
UI = Us + Up × 10-1
Where,
Us = Average ulcer severity score
Up = proportion of animals that get ulcers
The formula for the percentage protection index was,
C - T/C × 100
Where, C = ulcer index in the group under control;
T = the treated group's ulcer index

Biochemical evaluation of total acidity in gastric juice

Distilled water was used to dilute one millilitre of the supernatant liquid to ten millilitres. Titration was carried out against 0.01 N NaOH until its red colour vanished and got orange, where Topfer's reagent was used as an indicator. The necessary volume of NaOH was determined using the free acidity.The titration procedure was repeated with a few drops of phenolphthalein solution till the pink colour returned. It was discovered that the required volume of NaOH matched the total acidity. [14]

Acidity = NaOH Volume X Normality of NaOH X 100mEq/l / 0.1

Estimation of Pepsin activity

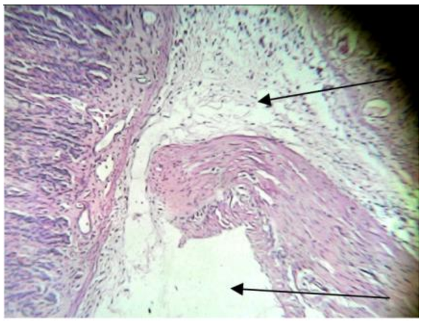
The mechanism by which pepsin broke down the haemoglobin solution produced tyrosine, which was used to gauge the activity of the enzyme. Fifteen minutes were spent incubating the sample of mixture. Together with one millilitre of Sample of gastric juice and five millilitres of the substrate (1% BSA in HCl at pH 2.1). After adding ten millilitres of TCA, the procedure was halted. The blank, which included a mixture of 10 mL TCA and 1 mL gastric juice sample, incubation was done for 15 minutes prior to mixing of 5 mL of the substrate. After half an hour, Separate filters were used for the reaction mixture and the blank.The absorbance was measured at 680 nm following the addition of 1 mL of folin-phenol reagent and 10 mL of 0.5 M NaOH to the filtrate. Different tyrosine concentrations were shown on a graph.The microgrammes of tyrosine equivalents released per millilitre of gastric juice per minute were used to measure the pepsin activity.

Estimation of Total protein

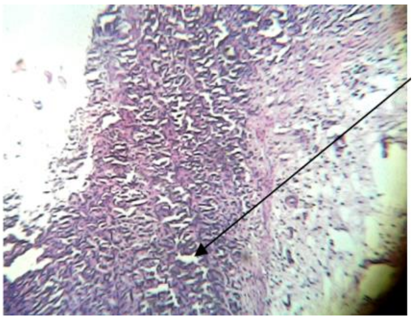
It was determined by using industry standard procedure. 0.9 mL of water was used to appropriately dilute 0.1 mL of gastric juice. It was then let to sit at room temperature for ten minutes following the addition of 4.5 mL of alkaline copper reagent. After that, Folin reagent (0.5 mL) was added, and after 20 minutes, the blue color's absorbance at 640 nm was measured. The protein concentration was expressed as µg/mL and was computed by utilising a typical graph made of albumin from cows.

Histopathological studies

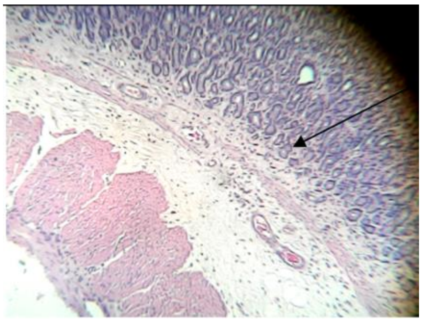
After being cleansed with saline, the just removed stomach from a single animal from every group was preserved in a 10% formaldehyde solution for histological examination Isopropyl alcohol, xylene, and paraffin embedding were used to prepare it for a 12-hour analysis using a light microscope (Magnus IV). Hematoxylin and eosin stain (H & E) was used to make tissue encased in paraffin sections that were sliced to 5 µm thickness. This process confirmed the morphological assessment of stomach injury.Images at a 40x magnification were captured. [15]



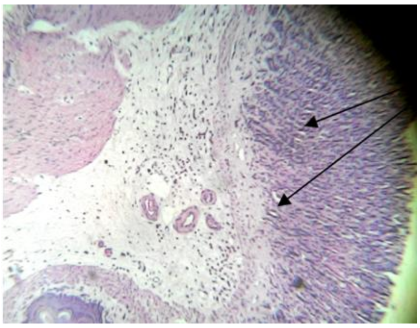
A) The cell on the mucus surface was showed in good architecture manner.



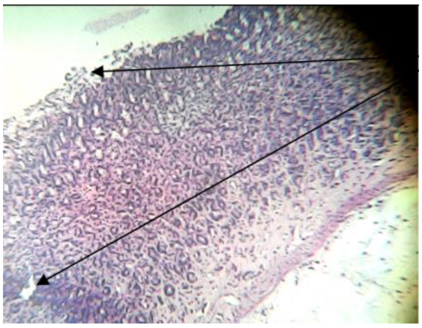
B) The cell on the mucus surface was showed in disrupted manner



C) The cell on the mucus surface was showed restored manner



D) The cell on the mucus surface was showed restored manner



E) The cell on the mucus surface was showed restored moderate manner

Figure 8: Histopathological studies A) Control group, B) Disease group, C) Standard group treatment withwith Ranitidine, D) Group with millet 300mg/kg treatment, E) Group with millet 600mg/kg treatment

Table 13. Pepsin content and Total Protein

Sr. No.	Group Name	Pepsin µg/mL	Total Protein µg/mL
1	Normal Control	19.12	107.03
2	Disease Control	26.45	177.66
3	Std (RANITIDINE)	19.98	123.53
4	300 mg/Kg	24.76	145.87
5	600 mg/Kg	21.65	104.95

RESULTS

Organoleptic properties of millet formulation

Table 4 lists the findings of a visual inspection conducted to assess an organoleptic quality of the millet formulation.

Physicochemical parameters

Tables 5 and 6 show the results of the physicochemical parameters of the millet formulation that were determined as described below.

Design and development of millet formulation

Dosage Form: Millet Tablets

Physical evaluation of tablets: General Appearance of tablets was cream white , Hardness range was found to be 2-3 kg/cm² and disintegration time range was found to be 1 min. Weight variation was observed which isshown in table. As the calculated friability was found to be 0.9% and it is within the limit. weight variation of tablet given in table 7.

Dosage form: Millet Capsule

Preformulation study parameters are studied and observations are shown in table 8 Physical evaluation was done and weight variation results shown in tabl3 9. Disintegration time range was found in the range of 2-3 mins

Gastric content volume and pH

Table 10 and Figures 3 display all of the findings for the volume of stomach content and pH of gastric juice. Stomach juice pH in the millet sample group in Tests 1 and 2 (300 and 600 mg/kg, respectively) was determined to be 7.15 and 7.62.It was discovered to be 3.90 for the normal control group, 2.61 for the illness control group, and 8.13 for the standard group. Similarly gastric content was also found to be 1.15 and 2.15 for Test 1 and Test 2 millet samples respectively. For standard, it was found to be 3.13.

Macroscopic examination for Ulcer scoring

Table 11;figure 4 and figure 5shows the ulcer index and percentage inhibition for each group. The antiulcer medication administered to the standard group (group III)in which ulcers was seen was ranitidine.Comparing group V (600 mg/kg) of millet formulation to groups III (Standard) & IV (300 mg/kg), a substantial reduction in ulceration was seen.Maximum inhibition was observed in the group administered with the conventional medication ranitidine.Upon utilizing solely the millet formulation at dosages of 300 and 600 mg/kg, the percentage of inhibition was determined to be 32.50% and 44.86%, respectively

Assessment of the overall acidity of stomach juice biochemically:

For each group it was determined by following the methodology and table 12 and figure 6 show all of the observations.In contrast to the group under disease control, which had 109 ± 1.45 mEq and resp., the total acidity in the millet test groups 1 and 2 was determined to be 93 and 68 mEq, respectively

Estimation of Pepsin activity and total protein

Using a technique that takes into account pepsin's digestion of haemoglobin solution, which produces tyrosine, pepsin activity was measured.Gastric acid is the primary component of gastric juice, It is released by stomach parietal cells stomach. Gastric acid converts pepsinogen into pepsin. Atrophic gastritis, stomach ulcers, and other conditions may result from the protective layer of the gastric mucosa being harmed by excessive stomach acid production, which is indicated by elevated pepsin activity.Pepsin activity and total protein estimates were computed and are displayed in Table 13 and Figure 7.The millet 300 mg/kg and millet 600 mg/kg groups' respective pepsin levels were determined to be 24.76 and 21.65 µg/mL, which is lower than the disease control group's value of 26.45 µg/mL.

Histopathological studies

Rat stomach tissue sections were stained with hematoxylenol (HE) for histopathological analysis. The sick group, on the other hand, displayed evidence of spread of inflammation, a notably disorganised glandular layer, a notably insufficient epithelial layer of stomach, and a significant edoema in the submucosa. The conventional ranitidine-treated rat's lesions were less severe when compared to the diseased rat; still, the gland's structure remained disorganized, and the epithelium tissue was still conspicuously absent. Application of millet formulation at 300 mg/kg and 600 mg/kg substantially prevented lesions related to stomach ulcers.The only anomalies discovered were minor deficits in the stomach's mucosal epithelium and a little aberration in a glandular structural region (Figure 8)

DISCUSSION

Millet formulation of tablet dosage forms and capsule dosage forms were developed and the physicochemical parameters were evaluatedMillets have been employed as an innovative pharmacological source with antiulcer properties. Naturally occurring phytochemicals were isolated from the millets and were subjected to *in-vivo* evaluation. Wistar rats were used in an in-vivo investigation that included an antiulcer activity.In this study, the ethanol-induced ulcer method was used to determine antiulcer activity where, five distinct groups comprising control, disease control, standard, and millet formulation (TEST 1) 300 mg & (TEST 2) 600 mg was used. Polyphenols are abundant in millet varieties such as Jowar, Finger, and Pearl. Among phytochemicals, polyphenols are the most significant. There is no scientific evidence to support the long-held belief that it is beneficial for stomach issues. One of the primary components of millet is protein, which has been shown to improve lipid metabolism and Type 2 diabetes in mice among other physiologic effects.[16,17] It was previously found that millet protein's ability to prevent colitis is due to its reduction of intestinal mucin expression. This result implies that further investigation in this field may prove advantageous in the management of gastrointestinal issues.[18] These findings imply that millet formulation has antiulcer properties. The antiulcer studies that were conducted on Wistar albino rats are disclosed in this paper. In vivo model was used as ethanol induced ulcer model. In this case, 1 mL/200 g of body weight of 100% ethanol was used to cause the stomach ulcers. This model is useful for assessing the efficacy of anti-ulcer medications since it properly depicts a number of characteristics of human gastrointestinal ulcers.[19] One of the main causes of stomach ulcers is ethanol, which damages the stomach mucosa through a variety of mechanisms, such as cytotoxicity and dehydration-induced collapse of mucosal cell barriers. Because of ethanol's cytotoxic properties, leukocytes are drawn to the area and produce inflammatory cytokines and reactive oxygen species (ROS), which can all lead to cell death.[20] Using an ethanol-induced rat model, this study assessed how millet formulation affects the generation of stomach acid and the release of digestive enzymes when gastric ulcers worsen. Using this paradigm, the effects of millet alone on pepsin activity in the gastric juice, total acid production, and gastric acid secretion were investigated. The findings indicated that the millet formulation, at concentrations of 300 mg and 600 mg/kg, had a gastroprotective effect. Percentage inhibition with millet formulation at the dose of 300mg/kg and 600mg/kg was found to be 32.50% and 44.86% resp. as compared to standard it shows the significant level of percentage inhibition for the Test 1 (300mg) and Test 2 (600mg). Although, as compare to the millets having dose 600mg/kg shows higher % inhibition than millet 300mg/kg. When comparing the total acidity of the millets group to the disease control group and the standard group, it was discovered that the millets group had 93 and 68 mEq for 300 and 600 mg, respectively. Therefore, the findings of this study provide credence to the benefits of millet in reducing the incidence of ulcers. Additionally, the millet formulation with a dose of 600 mg/kg proved to be more effective than one with a concentration of 300 mg/kg. Although, the in-vivo toxicity study, ethanol induced ulcer model, Gastric pH and gastric content measurement, estimation of total acid, pepsin count, and histopathological studies are helpful in understanding the activity of millet formulation.

CONCLUSION

Treatments with millet greatly decreased the lesions from stomach ulcers and % inhibition was likewise higher in the millet 300 mg and 600 mg group. In contrast to the standard group and the disease control group, the total acidity in the millet group at 300 mg and 600 mg was determined to be 93 and 68 mEq, respectively. Compared to the disease control group, which had pepsin values of 26.45 µg/ml, the millet group had lower pepsin values, 24.76 and 21.65 µg/ml. The millet treatment for the concentration of 300mg and 600mg treatment revealed a significant drop in stomach ulcer lesions. Although, as the millet with concentration of higher dose as 600mg shows the higher significant activity than the millet with concentration 300mg. As 600mg dose for millet is more effective than the smaller dose 300mg.As a result, the study's findings support the notion that millets are safe and have antiulcer properties.

REFERENCES

1. Zhang B, Rao X, Zhang Y, Dai W, Xu Y, Zhao C, Chen Z, Wang J, Fan D, Wang X, Wang S. Protective effect of foxtail millet protein hydrolysate on ethanol and pyloric ligation-induced gastric ulcers in mice. Antioxidants. 2022;14;11(12):2459. doi: 10.3390/antiox11122459
2. Kavitt RT, Lipowska AM, Anyane-Yeboa A, Gralnek IM. Diagnosis and treatment of peptic ulcer disease. The American journal of medicine. 2019;1;132(4):447-456. doi: 10.1016/j.amjmed.2018.12.009.
3. Ribeiro AR, do Nascimento Valença JD, da Silva Santos J, Boeing T, da Silva LM, de Andrade SF, Albuquerque-Júnior RL, Thomazzi SM. The effects of baicalein on gastric mucosal ulcerations in mice: Protective pathways and anti-secretory mechanisms. Chemico-Biological Interactions. 2016;25;260:33-41. doi: 10.1016/j.cbi.2016.10.016.
4. Byrne BE, Bassett M, Rogers CA, Anderson ID, Beckingham I, Blazeby JM. Short-term outcomes after emergency surgery for complicated peptic ulcer disease from the UK National Emergency Laparotomy Audit: a cohort study. BMJ open. 2018; 1;8(8):e023721. doi: 10.1136/bmjopen-2018-023721.
5. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li S, Gan RY, Li HB. Natural products for the prevention and management of Helicobacter pylori infection. Comprehensive reviews in food science and food safety. 2018;17(4):937-952.
6. Sousa C, Ferreira R, Azevedo NF, Oleastro M, Azeredo J, Figueiredo C, Melo LD. Helicobacter pylori infection: from standard to alternative treatment strategies. Critical reviews in microbiology. 2022;4;48(3):376-396. doi: 10.1080/1040841X.2021.1975643
7. Abd El-Ghffar EA, Al-Sayed E, Shehata SM, Eldahshan OA, Efferth T. The protective role of Ocimum basilicum L.(Basil) against aspirin-induced gastric ulcer in mice: Impact on oxidative stress, inflammation, motor deficits and anxiety-like behavior. Food & function. 2018;9(8):4457-4468.
8. Lin HC, Sheu SY, Sheen LY, Sheu PW, Chiang W, Kuo TF. The gastroprotective effect of the foxtail millet and adlay processing product against stress-induced gastric mucosal lesions in rats. Journal of Traditional and Complementary Medicine. 2020;1;10(4):336-344
9. Kamdi S, Bhure S, Gupta SK, Kankal G, Dadas N. genetic improvement of soybean cultivar for yield and its related traits by induced mutation. souvenir &. 2020; 28 8(11):523-529. DOI:10.33545/26174693.2024.v8.i11g.2891

10. Ji Z, Mao J, Chen S, Mao J. Antioxidant and anti-inflammatory activity of peptides from foxtail millet (Setaria italica) prolamins in HaCaT cells and RAW264. 7 murine macrophages. Food Bioscience. 2020;1;36:100636.
11. Kumar KK, Parameswaran KP. Characterisation of storage protein from selected varieties of foxtail millet (Setaria italica (L) Beauv). Journal of the Science of Food and Agriculture. 1998;77(4):535-542. https://doi.org/10.1002/(SICI)1097-0010(199808)77:4<535::AID-JSFA77>3.0.CO;2-G
12. Ambati K, Sucharitha KV. Millets-review on nutritional profiles and health benefits. International Journal of Recent Scientific Research. 2019;10(7):33943-33948.
13. Danai P, Patel S, Pandey V, Singh P, Yadav G, Kumar A, Agarwal T. Antiulcerogenic activity of Anogeissus pendula hydroalcoholic extract on pylorus ligated induced gastric ulcers in Albino Wistar rats. Phytomedicine Plus. 2021;1;1(4):100127.
14. Srivastava V, Verma SK, Mathur N, Goel KK. Ameliorative effect of Albizzia procera leaves extract against experimentally induced gastric ulcer models in Wistar albino rats. Journal of Applied and Natural Science. 2021;13;13(1):308-315. DOI: https://doi.org/10.31018/jans.v13i1.2513
15. Dahariya N, Sathapathy S, Patra R, Mishra UK, Sahu SK, Samal L, Jena B, Joshi SK. Age wise histological studies on the major lymphoid organs in various chicken genotypes. Journal of Animal Research. 2020;10(5):811-819.
16. Fu Y, Yin R, Guo E, Cheng R, Diao X, Xue Y, Shen Q. Protein Isolates from Raw and Cooked Foxtail Millet Attenuate Development of Type 2 Diabetes in Streptozotocin Induced Diabetic Mice. Molecular nutrition & food research. 2021;65(6):2000365. doi: 10.1002/mnfr.202000365.
17. Choi YY, Osada K, Ito Y, Nagasawa T, Choi MR, Nishizawa N. Effects of dietary protein of Korean foxtail millet on plasma adiponectin, HDL-cholesterol, and insulin levels in genetically type 2 diabetic mice. Bioscience, biotechnology, and biochemistry. 2005;1;69(1):31-37
18. Zhang B, Xu Y, Liu S, Lv H, Hu Y, Wang Y, Li Z, Wang J, Ji X, Ma H, Wang X. Dietary supplementation of foxtail millet ameliorates colitis-associated colorectal cancer in mice via activation of gut receptors and suppression of the STAT3 pathway. Nutrients. 2020; 7;12(8):2367

La XQ, Liu YZ, Zhang LC, Li HQ, Li ZY. Protective effect of foxtail millet bran-derived polyphenols on alcohol-induced gastric mucosal injury. Nutrition & Hygien. 2022; 43 (13): 64-71.doi: 10.7506/spkx1002-6630-20210719-221

19. Lin HC, Sheu SY, Sheen LY, Sheu PW, Chiang W, Kuo TF. The gastroprotective effect of the foxtail millet and adlay processing product against stress-induced gastric mucosal lesions in rats. Journal of Traditional and Complementary Medicine. 2020;1;10(4):336-344. doi: 10.1016/j.jtcme.2020.01.003.