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In-Vitro Evaluation of Antiulcer Activity of Selective and Functional Millet

Archana V. Vanjari[1]; Subhash T. Kumbhar[1*]

[1]School of pharmaceutical sciences, Sanjay Ghodawat university, Kolhapur, MS. India 416118

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KEYWORDS
Millets; In-vitro study; Acid neutralizing capacity; Peptic ulcer; Anti-ulcer activity.

ABSTRACT:

Introduction: Peptic ulcer disease (PUD) is a gastric condition which occurs because of an imbalance between defensive and harsh factors, like gastric mucus, secretion of bicarbonate, prostaglandins, and intrinsic resistance of mucosal cell components. There are several medication treatment options available for PUD, but their use is limited due to poor compliance and frequent side effects. Due to this reason, alternative for this treatment was religiously searched and continued and finally found natural phytochemical isolated from plants which possess less side effects, ease of accessibility and affordability and hence considered to be a great traditional medicines in the treatment of PUD

Objective: To design, develop and formulate functional millet formulation and evaluate physicochemical characterization, phytochemical screening and in-vitro study of the prepared powder product for the antiulcer activities.

Method: In present research study, sorghum, finger millet, pearl millet and sesame seeds were used. Millet formulation was used as the novel source of drugs for the antiulcer medications. Its in-vitro H+, K+-ATPase inhibition and Acid neutralizing capacity was analyzed on enzyme extract obtained from fresh goat stomach against standard drug omeprazole and Al(OH)3+Mg(OH)2 respectively

Result: At a concentration of 1000 µg/ml of millet sample and omeprazole found 56.75 % and 84.48 % of inhibition respectively. The millet sample at concentration 2000 mg/ ml was observed to neutralize acid as compared to standard.

Conclusion: From the results that the millet formulation represents a novel drug source for antiulcer treatments. Nevertheless, a comprehensive *in-vivo* study on the pharmacological assessment and Future research will be done to determine the underlying mechanism of action that accounts for its antiulcer efficaciousness.

1. Introduction

Peptic ulcer disease (PUD) is gastric condition which occurs because of an imbalance between defensive and harsh factors, like gastric mucus, bicarbonate secretion, prostaglandins, and intrinsic resistance of mucosal cell components [1]. Peptic ulcers typically occur when aggressive elements overcome defensive ones [2]. The reason behind this include Helicobacter pylori, hyper secretion of pepsin, non-steroidal anti-inflammatory medicines, and occasionally idiopathic. [3,4]. The use of tobacco, mental strain, fast emptying of the stomach, and Zollinger-Ellisson syndrome, which is characterized by excessive and uncontrollably high acid production, all contribute to the development of ulcers [5]. The main pathways involved in the pathophysiology of these illnesses Blood flow, mucus secretion, the formation of hydrochloric acid (HCl),

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non-protein sulfhydryl (NPSH) groups from the stomach and liver, and aggressive stress and gastric defense mechanisms [6, 7]. It is believed that inadequate blood flow to the tissues of gastric linig is the primary factor reducing defense of mucosal system and causing ulcer development. Superoxide anion ($O2^-$), hydrogen peroxide (H2O2), and hydroxyl radical (OH•) are examples of reactive oxygen species that are found in ischemic tissue which are hypothesized to be intermediaries of gastrointestinal injuries with varying aetiologies [8].There are several medication treatment options available for PUD, but their use is limited due to poor compliance and frequent side effects. Due to this reason, alternative for this treatment was religiously searched and continued and finally found natural phytochemical isolated from plants which possess less side effects, ease of accessibility and affordability and hence considered to be a great traditional medicines in the treatment of PUD [7]. One of the first foods that people ate was millets, which may have been the first cereal grain, used in home cookery. For a long time, millets have been the primary food staple for those living in Asia's and Africa's semi-arid tropical regions [9]. The pearl millet mostly grown in India. There are also other important minor millets as Finger millet, Proso millet, and Foxtail millet. Millets are considered to have an evolutionary origin. Micronutrients are commonly found in millets also phytochemicals, proteins, carbohydrates having best amino acid profile etc. also important sources of millets. It also possesses anti-oxidant property. It gives beneficial to the health and increase nutritive value and also prevents the risk of various diseases and disorders [10]. Protein, dietary fiber, vital fatty acids, and minerals including potassium, zinc, magnesium, calcium, iron, and vitamins, especially vitamin B complex, are all rich in millets. Because they maintain constant blood pressure and sugar levels, millets also help prevent a number of illnesses, including diabetes, gastrointestinal disease, heart related disease, blood pressure, thyroid disorders, and celiac disease [11].In present research study, sorghum, finger millet, pearl millet and sesame seeds were used. They were chosen from literature reviews that suggested they had a high nutritional content. In this study, millet formulation was used as the novel source of drugs for the antiulcer medications.

2. Materials and Methods

2.1 Materials

Peral milet (Pennisetum glaucum), Finger millet (Eleusine coracana), Jowar (Sorghum bicolor) and other ingredients like Sesame seeds (Sesamum indicum) were collected from local market. For in-vitro evaluation, reagents like Magnesium hydroxide, aluminum hydroxide, hydrochloric acid, sodium hydroxide were used from laboratory and of analytical grade. The local slaughter was the source of the fresh goat gut.

2.2 Methods

2.2.1 Preparation of millet formulation

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

2.2.2 Organoleptic properties of millet formulation

An organoleptic property of the millet formulation was carried out by visual observation which includes color, smell, appearance, touch and taste. Results were reported.

2.2.3 Physicochemical parameters

For determination of physicochemical parameters of the millet formulation, the conventional protocols described in the literature were followed in order to accomplish the tests. The tests includes, Unsaponifiable matter, Saponification value, Acid value, Specific gravity,

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Weight per ml, Iodine value, pH, and Swelling index. Results were reported [12].

2.2.4 Phytochemical screening

The extract of millets were used for phytochemical screening which involves the detection of steroids, alkaloids, tannins, saponins, phenolic compounds, flavonoids, proteins, amino acids, and carbohydrates. The conventional protocols described in the literature were followed in order to accomplish the phytochemical screening [13].

2.2.5 In-vitro Evaluation of Antiulcer Activity

2.2.5.1 Assessment of H+, K+-ATPase inhibition

Preparation of H+, K+-ATPase enzyme: A local slaughterhouse provided fresh goat stomach, which was used to prepare the H+, K+-ATPase enzyme sample. In order to obtain parietal cells, the stomach was cut open, the mucosa at the gastric fundus was severed, and the innermost layer was removed by scraping out. After homogenising the cells in 16 mM Tris buffer (pH 7.4) with 10% Triton X-100, the mixture was centrifuged for 10 minutes at 6000 g. The H+, K+-ATPase inhibition was measured using the supernatant, or enzyme extract.

Procedure: The reaction mixture was pre-incubated for 60 minutes at 37°C. It contained 0.1 ml of 300 µg enzyme extract and plant extract at various concentrations (200, 400, 600, 800, and 1000 µg/ml). To initiate the reaction, substrate was added together with 2 mM ATP (200 µL), 2 mM MgCl2 (200 µL), and 10 mM KCl (200 µL). The assay mixture containing 4.5% ammonium molybdate and 60% perchloric acid was added to terminate the reaction after 30 minutes of incubation at 37°C. This was followed by centrifugation at 2000 g for 10 minutes. Utilising the Fiske-Subbarow method, the release of inorganic phosphate was measured at 660 nm. Enzyme activity was measured in micromoles of Pi released hourly at different sample VS dosages (0-100 µg). The outcomes were contrasted with omeprazole, a well-known antiulcer PPA inhibitor medication. Using this formula, the percentage of enzyme inhibition was computed; [Activity (control) − Activity (test)/Activity (control)] is the percentage of inhibition. × 100 [14, 15].

2.2.5.2 Acid neutralizing capacity

Aqueous extract in concentrations of 500 mg, 1000 mg, 1500 mg, and 2000 mg has the ability to neutralise acid. For the standard, magnesium hydroxide (500 mg) and aluminium hydroxide have been compared. With the addition of 5ml of the combination, the total volume increased to 70ml. The remaining 70ml was made up of water, and the mixture was then mixed for one minute. After adding 30 milliliters of 1.0 N HCl and stirring for 15 minutes, phenolphthalein was mixed to the preparation of the standard and test samples. The excess HCl was titrated as soon as possible till the pink color was obtained by use of 0.5N NaOH

The moles of acid neutralized is calculated by, Moles of acid neutralized = (vol. of HCl ×Normality of HCl) - (vol.Of NaOH×Normality of NaOH). Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized/ Grams of Antacid/Extracts

3. Results

3.1 Organoleptic properties of millet formulation

Results for organoleptic characteristics of the millet formulation is represented in table 1

Table 1: Organoleptic properties of millet formulation

Sr No.	Parameter	Observation
1	Colour	Off white ,Ceram
2	Smell	Characteristic
3	Appearance	Powder
4	Touch	Characteristic
5	Taste	Sweet

3.2 Physicochemical parameters

Results for all the physicochemical parameters are shown in table 2

Table 2: Physicochemical parameters of millet formulation

Sr No.	Parameter	Observation
1	Unsaponifiable matter	5.10
2	Saponification value	182.7
3	Acid value	3.6
4	Specific gravity	0.512

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5	Weight per ml	0.523
6	Iodine value	4.36
7	pH	6.26
8	Swelling index	11.76 %

3.3 Phytochemical screening

For millet sample, Carbohydrates, glycosides, cardiac glycosides, and protein, and amino acids, alkaloids, reducing sugars, flavonoids and phenolic compounds test found to be positive which is represented in table 3

Table 3: Results of Phytochemical screening

Sr No.	Test	Results
1	Carbohydrates,	+
2	glycosides,	+
3	cardiac glycosides,	+
4	protein,	+
5	amino acids	+
6	Alkaloids	+
7	Reducing sugars	+
8	Flavonoids	+
9	phenolic compounds	+

3.4 Assessment of H+, K+-ATPase inhibition

H+/K+ - ATPase inhibitory activity of the millet sample has been compared to that of omeprazole in different concentrations (200µg, 400µg, 600µg, 800µg, 1000µg) has done comparison with Omeprazole as standard. At 1000 µg/ml of millet sample and omeprazole showed 56.75 % and 84.48 % of inhibition respectively. In contrast to the omeprazole standard medication sample, the millet sample exhibited a moderate level of action. A dose-dependent pattern of activity was observed in the extract. Table 4 and Figure 1 present the findings.

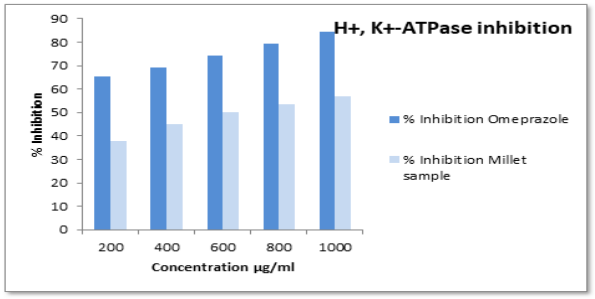

Figure 1: Effect of H+/K+ - ATPase Inhibition

3.5 Acid neutralizing capacity

For four different concentrations (500 mg, 1000 mg, 1500 mg, and 2000 mg) and by using the standard Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2] (500 mg), the neutralizing effect of sample millet was investigated. As per results, the acid-neutralizing capacity (ANC) of the millet sample at different concentrations of 500 mg, 1000 mg, 1500 mg, and 2000 mg have significantly shown the results according to the average value of Al(OH)3+Mg(OH)2 (500 mg). Figure 2 shows the findings.

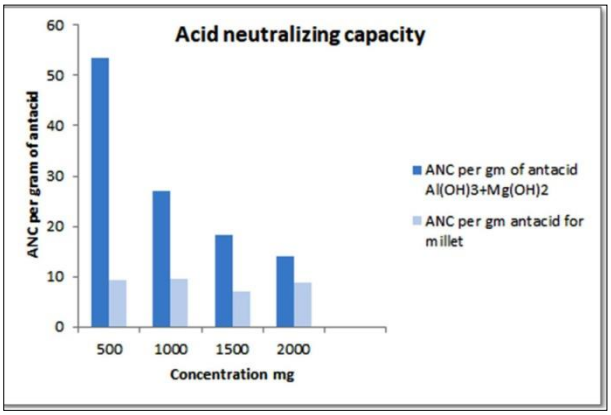

Figure 2: Acid neutralizing capacity for millet

4. Discussion

For millet formulation organoleptic properties, physicochemical properties and phytochemical screening was performed and results are as above. In Phytochemical screening millets samples showed the tests positive for presence of Carbohydrates, glycosides, cardiac glycosides, protein, and amino acids, alkaloids, flavonoids and phenolic compounds. The sample is then ready for further *in-vitro* testing like Assessment of H+, K+-ATPase inhibition and acid neutralizing capacity. As compared to standard drug sample omeprazole the millet sample showed moderate activity and A dose-dependent pattern of activity was observed in the extract. H+/K+ - ATPase is a crucial enzyme for producing acidity, and placed on the parietal cells' apical secretory membrane. The millet sample may contain antacid and antiulcer qualities, which may be due to specific compounds in the mixture, based on the data shown above. To determine the precise mechanism of action and the active ingredients responsible for its antiulcer effectiveness, more investigation is

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nonetheless required. The millet sample showed H+/K+-ATPase inhibitory activity at a dose of 1000 µg, with a maximum percentage inhibition of 56.75% [15]. Compared to standard, the millet sample at a concentration of 2000 mg/ml was observed to neutralise acid. Although the exact etiology of gastric ulcers was not known. It is now widely acknowledged that the illness outcomes from an aggressive imbalance between a factor and the integrity of mucosal lining, which is preserved by the body's defence mechanism. Gastric acid, or excess production of stomach acid, causes ulceration and inflammation of the stomach lining. Acidity is a prevalent gastrointestinal issue linked to a functional condition that can develop for a variety of causes. Antacids function by reducing the pH of the stomach and neutralising stomach acid. A procedure known as back titration has been used to test an antacid's ANC, which is the quantity of acid that it can neutralize. At various doses, the millet sample did not show a discernible decline in ANC. [16, 17].

5. Conclusion

We may conclude from the results that the millet formulation represents a novel drug source for antiulcer treatments. Nevertheless, a comprehensive *in-vivo* study on the pharmacological assessment and Future research suggested to determine the underlying mechanism of action that accounts for its antiulcer activity.

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