

## Investigation of In- Vitro Method of Antiulcer Activity

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### ABSTRACT

It has long been known that medicinal plants include traditional therapeutic ingredients for the treatment and prevention of illnesses and disorders. In order to find out if hydroalcoholic extracts of fenugreek seeds may be used to treat ulcers, this study tested the extracts' capacity to neutralize acids and their ability to block  $H^+/K^+ - ATPase$ . A 1000 mg/ml concentration of the Seeds extract significantly reduced the acidity from 11.90 at the normal 500 mg/ml of Aluminium hydroxide + Magnesium hydroxide to 6.10. The maximum percentage of inhibition of the  $H^+/K^+ - ATPase$  was shown by petals extract at a concentration of 400 g/ml, which is less than the typical dose of esomeprazole (73.82%). The IC 50 value of extract of fenugreek seeds is shown to be 100 g/ml when compared to the average dose of esomeprazole, which is 82.5 g/ml. According to the findings of this study, fenugreek seeds contain compounds that have the ability to neutralize acids and block enzymes, making them a potential alternative treatment for digestive problems.

**Keywords-** Gastrointestinal, Ulcer, In vitro methods, Herbal extract.

### I. INTRODUCTION

Disruption of the gastrointestinal (GI) mucosa due to gastric acid secretion or pepsin causes peptic ulcer disease (PUD). It penetrates the gastric epithelium all the way down to the muscularis propria layer. It typically affects the stomach and the first part of the duodenum. [1] The jejunum, distal duodenum, and lower oesophagus could be affected. In individuals with a stomach ulcer, epigastric pain typically develops within 15-30 minutes after a meal, but in patients with a duodenal ulcer, the pain typically occurs 2-3 hours after a meal. These days, everybody who suspects they may have peptic ulcer illness should get tested for *Helicobacter pylori*. [2] Some patients, especially those with concerning symptoms, may need an endoscopy for diagnosis. Triple-drug therapy based on proton pump inhibitors (PPIs) is now effective in treating the vast majority of patients. When a region of skin is subjected to continuous pressure for an extended period of time, it can cause tissue ischaemia, the stoppage of nutrition and oxygen supply to the tissues, and ultimately tissue

necrosis. The term "distortion or deformation damage" best describes the constant pressure that causes a pressure ulcer. If an external force (shear, compression, or both) is applied to any tissue, the tissue will suffer from localised, acute ischemic damage [3]. Despite the fact that pressure injuries that do not result in open wounds (such as blisters and non-blanching erythema) are not real sores but just "pressure damage" and nevertheless belong to this family of pressure ulcers, the phrase "pressure sores" is often used in the UK. The European Pressure Ulcer Advisory Panel (EPUAP) has adopted the term "pressure ulcers" as the official European term despite its widespread use outside of Europe. Bedsores and decubitus ulcers are two other names for this condition, however these terms are rarely used anymore because it is understood that the ulcers are not brought on by prolonged bed rest. Occipital, trochanteric, sacral, malleolar, and heel skin are the most vulnerable to developing pressure sores. The ulcerogenic process occurs as a result of damage to the protective mucosal lining of the stomach and duodenum. *H.pylori* infections and the use of NSAIDs and low-dose aspirin

are known to damage the mucosal lining. The cost to the mucosal lining in the setting of an H.pylori infection is the result of both bacterial factors and the host's inflammatory response. In the case of NSAID (and aspirin) use, mucosal damage is secondary to inhibition of cyclooxygenase 1 (COX-1) derived prostaglandins which are important in maintaining mucosal integrity.[4-5] Once the mucosal layer is disrupted, the gastric epithelium is exposed to acid, and the ulcerative process ensues. If the process continues, the ulcer deepens reaching the serosal layer. A perforation occurs once the serosal layer is breached at which point the gastric contents are released into the abdominal cavity.[6]

Gastric ulcers are classified into four types based on location.[7]

- **Type 1:** in the antrum, near the lesser curvature
- **Type 2:** combined gastric and duodenal ulcer
- **Type 3:** Prepyloric ulcer
- **Type 4:** ulcer in the proximal stomach or cardia

Gastric ulcers are most commonly found in the lesser curvature (55%), followed by a combination of duodenal and gastric ulcers. Duodenal ulcers are most commonly located in the first part of the duodenum.

Gastric ulcers have malignant potential compared to duodenal ulcers that do not have cancerous risk. A gastric ulcer greater than 3 cm is called a giant gastric ulcer which has a 6%-23% chance to turn into malignancy. In previous literature, the cancer rate in endoscopically diagnosed gastric ulcers ranges from 2.4%–21%.[8][9]

Trigonella, the Latin name for fenugreek, means "little triangle" because of the shape of its yellowish-white blossoms. Methi (in Hindi, Urdu, Punjabi, and Marathi), Hulba (in Arabic), Moshoseitaro (in Greek), Uluva (in Malay), Shoot (in Hebrew), Dari (in Persian), and heyseed (in English) are some of the names it goes by around the world.[10] Fenugreek, or *Trigonella foenum-graecum* L., is a member of the Fabaceae family and has been used medicinally since at least 4000 B.C. Earlier, in 1500 BC in Egypt, the Ebers Papyrus (one of the oldest maintained pharmaceutical record) described it and its benefits. India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, North Africa, the Middle East, and Argentina are among the countries where it is grown commercially. Fibre, phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid, choline, vitamin A, B1, B2, C, nicotinic acid, niacin, and many more useful compounds can be found in fenugreek seeds. [11] There haven't been many studies done to improve its genetics or agronomy for production, but it has outstanding nutritional and therapeutic benefits. In this article, we will cover fenugreek's shape, adaptability, nutritional contents, connected functionality, medicinal relevance, ethnohistorical usage, and pharmacological assumptions. Possible avenues for further study to boost output and versatility are also highlighted. Acid and other unpleasant substances including bile acids, NSAIDs, and

ethanol exacerbate mucosal barrier disruption, H<sup>+</sup> back diffusion, and ulcer vulnerability. [12] The first line of defence in the mucosa is the alkaline buffer layer formed at the epithelial surface by adequate mucosal flow and production of bicarbonate. Healing of an ulcer requires the hormones prostaglandins (PGs). Inducing cyclooxygenase-2 (COX-2) and PGE2 release at the ulcer margin is how growth factors, some gut hormones (such as gastrin and cholecystokinin), and melatonin aid in ulcer healing. Antisecretory dosages of exogenous PG have a similar effect[13]. As well as PGs, several other variables contribute to gastroprotection. These include growth factors, nitric oxide or calcitonin gene-related peptide, and some gut hormones include gastrin and cholecystokinin, leptin, ghrelin, and gastrin-releasing peptide. It has been hypothesised that the release of PG or the activation of sensory nerves is responsible for the protective effect of gut hormones[14-15].

## II. MATERIAL AND METHODS

### 2.1. Collection of Herbal extract

We buy from Seeds of Fenugreek from local vendor for performing an experiment on In vitro model antiulcer Activity. The seed of Plant was washed with tap water 3 times and sterilized by spraying with 70% alcohol. The fine material powder is collected and used for extraction of the crude drug in aqueous solvents by Soxhlet extraction method. This method comprises a constant hot extraction with a controlled amount of water. The plant material is dried, then ground into a powder and stored in an airtight container. After that, water is added and mixed in. In order to speed up the extraction process, heat is applied continuously. The entire procedure takes no more than 15 minutes. The typical dilution factor for a crude medication is between 4:1 and 16:1. Its purpose is to remove plant compounds that are both water- and heat-soluble.

### 2.2. In-Vitro Anti-ulcer Activity

#### 2.2.1 Acid Neutralizing Capacity

The acid neutralizing capacity (ANC) value for hydro-alcoholic extract of Seeds of fenugreek in different concentrations (100 mg/ml, 200mg/ml, 500mg/ml, 1000mg/ml) were compared with the standard antacid AHMH (aluminum hydroxide + magnesium hydroxide -500 mg/ml). To the 5ml quantity of each extract individually, water was added and mixed well to make up the total volume up to 70 ml. Then 30 ml of 1N HCl was added into standard and test preparation and stirred for 15 minutes, 2-3 drops of phenolphthalein solution was added and mixed. The excess HCl was immediately titrated with 0.5N Sodium hydroxide solution drop wise until a pink color is appeared [16].

#### 2.3. Preparation of H<sup>+</sup>/K<sup>+</sup> - ATPase enzyme

Dehradun city market's butcher shop provided the fresh Goat stomach used to prepare the H<sup>+</sup>/ K<sup>+</sup> - ATPase enzyme sample. The stomach of a goat was

dissected open, washed, the mucosa of the gastric fundus was removed, and the inner layer was scraped off to obtain parietal cells. Next, 10% Triton X-100 was added to a homogenate of parietal cells in 16 mM Tris buffer (pH 7.4), and the mixture was centrifuged at 5000xg for 10 minutes. The activity of the enzyme H<sup>+</sup>/K<sup>+</sup>- ATPase inhibitor was measured by isolating the supernatant. Bovine serum albumin was utilised as a reference reagent to calculate the protein concentration in the supernatant. Activity of H<sup>+</sup>/K<sup>+</sup> ATPase was measured in parietal cell extract. [17]

### III. RESULT AND DISCUSSION

#### 3.1. In-vitro Acid Neutralizing Capacity

The in-vitro acid neutralizing effects of hydro-alcoholic extract of Seeds of Fenugreek in different concentrations (100 mg, 200mg, 500mg, and 1000 mg per ml) were compared with the standard antacid AHMH- 500 mg/ml. The results showed concentration dependent reduction in acid neutralizing capacity per gm of antacid was found as 114.9, 41.57, 10.36 and 6.9 respectively. As Similar fashion, AHMH (500 mg) which is found ANC value 11.90 quite similar concentration of test drug. Whereas, test drug concentration 1000 mg was found double to neutralize acid more significantly as compared to standard. The results are tabulated in Table 1.

**Table: 1 Acid Neutralizing Capacity**

Concentration	Volume of NAOH Consumed (ml)	mEq of Acid Consumed	Anc per gram of antacid
HEF -100 mg	35.8	12.25	114.9
HEF -200 mg	28.2	14.63	41.57
HEF -500 mg	43.1	9.65	10.20
HEF -1000 mg	37.2	12.4	6.9
AHMH -500MG	46.5	7.45	11.80

#### 3.2. In-vitro H<sup>+</sup>/K<sup>+</sup> - ATPase Inhibition Activity

Concentrations of the hydro-alcoholic extract of fenugreek seeds (25 g, 50 g, 100 g, 200 g, and 400 g per millilitre) were compared to those of the reference medicine esomoprazole (25 g, 50 g, 100 g, 200 g, and 400 g per millilitre). Inhibition of H<sup>+</sup>/K<sup>+</sup> - ATPase activity by both test and standard drugs was found to be concentration dependent, with results ranging from 45.93 to 46.59 to 72.35% for the test drug and from 55.66 to 63.66 to 74.85% for the standard drug at 100, 200, and 400 micrograms per millilitre. H<sup>+</sup>/K<sup>+</sup> - ATPase activity was observed to be inhibited by the extract at

concentrations higher than 400 g/ml by more than 70%. Table 2 summarises the results.

**Table: 2 In-vitro H<sup>+</sup>/K<sup>+</sup> - ATPase inhibition activity**

Concentration	% Inhibition	
	Standard	HEF
25	-42.56 ± 2.93	-20.41 ± 2.01
50	-29.45 ± 1.56	-13.67 ± 2.21
100	52.45 ± 2.49	47.65 ± 1.45
200	65.87 ± 2.15	56.47 ± 2.17
400	74.34 ± 2.45	70.31 ± 21.58

### IV. CONCLUSION

The majority of individuals don't know what causes peptic ulcers, although it's widely believed that an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence system may be to blame [7]. Due to excessive gastric acid secretion or stomach acid that inflames the mucosa of the stomach and causes ulceration, acidity is a common digestive issue [8]. Antacids work by neutralising stomach acid and lowering the stomach's pH [9].

The amount of acid that an antacid can neutralise per gramme of acid is known as its acid neutralising capacity [5, 10]. Results from ANC demonstrated a concentration-dependent decrease in acid neutralising capacity compared to each gramme of antacid administered. Another digestive issue, hyperchlorhydria, is characterised by excessive hydrochloric acid secretion from the parietal cells of the gastric mucosa via the proton pump. An essential enzyme of parietal cells that causes acidity is H<sup>+</sup>/K<sup>+</sup> - ATPase. The hydroalcoholic extract shown concentration dependent as similar as H<sup>+</sup>/K<sup>+</sup>-ATPase inhibitory activities. It can be said that the hydro-alcoholic extract of Seeds of Fenugreek has strong antiulcer properties.

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