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IN VITRO EVALUATION OF ANTACID AND ANTI-FLATULENT POTENCY IN AQUEOUS EXTRACT OF FENNEL SEEDS (*FOENICULUM VULGARE*), FENUGREEK SEEDS (*TRIGONELLA FOENUM-GRAECUM*) AND THEIR MIXTURE

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ABSTRACT

Objective: Antacids are popularly used throughout the world to address Gastric ulcer problems. In the present study aqueous extract of fennel seeds (Foeniculum vulgare), fenugreek seeds (Trigonella foenum-graecum) and 1:1 solution of these seeds are estimated of their antacid and antiflatulence activity. **Methods:** The pH of aqueous extracts and their neutralizing properties on artificial gastric acids were determined and compared with water and among themselves. An artificial stomach was used to evaluate the duration of consistent neutralization effect on artificial gastric acids. The anti-flatulent activity was tested by creating foam with sodium laurethsulphate, and volume reduction of foam was calculated post addition of sample solutions. **Results:** The results showed that aqueous extract of both fennel and fenugreek seeds has considerable antacid and anti-flatulent. Compared to the water group, aqueous extract of seeds was found to have significant (P<0.05). Gastric acid neutralizing effects. The duration for consistent neutralization of fennel aqueous extract was significantly longer than the other sample solution. Moreover, these samples exhibited distinct anti-flatulent effects in *in vitro* model scoring marginally higher value for fenugreek. **Conclusion:** Both anti-acidic and anti-flatulence activity recorded by fennel extract in water and also by fenugreek highly corroborate with popular uses of these spices as ethnomedicine and can be recommend in different herbal formulation to cure peptic ulcer problems.

KEYWORDS

Foeniculum vulgare Mill, Trigonella foenum-graecum L, Antacid, Anti-flatulent and Gastric juice.

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INTRODUCTON

Antacids are group of drugs which act as a guard against peptic ulcer disease. Peptic ulcer, a gastrointestinal disorder usually occurs in the stomach and intestine area. The word "peptic" derives from the hormone pepsin that cause mucosal break. The common symptoms of peptic

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ulcer disease are abdominal pain, nausea, vomiting, weight loss, bleeding etc¹.

Major causes of peptic ulcer disease are gastric acid secretion, H. pylori infection, excess alcohol and consumption. non-steroidal tobacco antiinflammatory drugs (NSAIDs)^{2,3}. Treatment of peptic ulcer disease, in modern times involves drug therapy like proton pump inhibitor and H2receptor⁴. The main motive of the treatment is to heal the sore, to relieve the epigastric pain and to prevent acid production. As well as to block the secretion of acid calcium supplement and some antacids like pantoprazole, metronidazole or amoxicillin are used⁵. There's lots of antacid available in market but due to the side effect. especially altered bowel functions, people often try to avoid it. Antacids are a combination of various compounds with various inorganic salts of magnesium, aluminum and calcium. Though aluminum antacids contribute aluminum to the diet, but also cause anemia and constipation. Magnesium antacids are very effective in raising gastric pH, but cause diarrhea due to poor absorption of insoluble magnesium. Calcium containing antacid inhibits peptic activity. The common side effects of this antacid are abdominal pain, milk-alkali syndrome, constipation⁶.

Traditional herbal medicines are often used to prevent the symptoms of peptic ulcer⁷. Some ethnomedicines for treatment of peptic ulcer are cabbage juice⁸, honey⁹, aloe vera¹⁰, turmeric¹¹, chili peppers¹².

Another important side effect of gastritis is flatulence which is a biological process and building up of gas in the digestive system during breaking down of food. The act of swallowing gas while eating and drinking may be a cause of flatulence¹³. Causes of flatulence are also bacterial overgrowth in small intestine, irritable bowel syndrome (IBS). celiac disease. complex carbohydrate, artificial sweeteners (sorbitol/mannitol) in sugar-free sweet foods¹⁴. Some of the symptoms are anorexia, early satiety, nausea. Therapies available for flatulence include lifestyle alteration. modifications. dietarv adsorbents and agents which has surface tension

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reducing property, treatments for gut flora and some drugs that modulate gut transit. Medicines or supplements are also recommended by doctors to reduce gas¹⁵. Some of ethnomedicines are natural substrates like chamomile, mint, basil, cumin, coriander, hyssop, arise, cinnamon, clove, bay, tarragon¹³.

Though some research is done with assorted spices and herbs, this study is focused on two common Indian spice *viz*. fennel, fenugreek seeds and their mixture (1:1 solution). Antiacidity and antiflatulence activity of these spices and their mixture is evaluated in this study.

Foeniculum vulgare Mill. Commonly known as fennel belongs to the Apiaceae family¹⁶. This is used as traditional medicine. Fennel is abundant in carbohydrate and protein and also contains a small amount of fat, sugar, fiber, sodium, potassium, iron, magnesium, calcium, zinc, phosphorus. It contains Vitamin C, Vitamin A, Vitamin B-6, Vitamin E, Vitamin K, thiamin B-1, riboflavin B-2. proline¹⁷. phenylalanine, tryptophan, glycine, Phytoconstituents of fennel are anethole, fenchone, d- α -pinene, Anisic acid, Anisaldehyde¹⁸. This spice is used to control vomiting, diarrhea, dyspepsia, flatulence, stomach distension and gastrointestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves. The pharmacological activities of the seeds reported are antimicrobial, antiaging, antiviral, antioxidant, antidiabetic, antiinflammatory, antitumor, antiallergic, antistress, galactogenic, gastrointestinal effect, antipyretic, chemo preventive, hepatoprotective, hypoglycemic, hypolipidemic. Fennel helps to enhance memory, used as anesthetic, aphrodisiac, and insecticide¹⁹⁻²⁴. Trigonal foenum-graecum L. is known as fenugreek, belongs to Fabaceae family²⁵. This is one of the oldest ethnomedicine. Fenugreek is ironic in fiber, phospholipids, glycolipids, carbohydrate, fat, protein, some aromatic compounds like butanoic acid, 3-isobutyl-2-methoxypyrazine, 1octene-3-one, acetic acid, diacetyl, linoleic acid. Fenugreek seed is an abundant source of vitamin A, B1, B2, C, nicotinic acid, potassium, magnesium, calcium, zinc, manganese, copper, iron²⁶⁻²⁸. It has some bioactive constituents likediosgenin, 4-108 April – June

hydroxyisoleucine (4-HIL), trigonelline, galactomannan (GM), polyphenols²⁹. This traditional medicine has some pharmacological activity such as anti-diabetic agent, anti-bacterial, antioxidant, hypocholesterolemia, anti-fungal, anti-inflammatory, anti-ulcer³⁰⁻³².

In this study, aqueous extract of fennel and fenugreek and their mixture (1:1) has been evaluated in terms Antiacidity and antiflatulence activity to be used as an alternative in treatment of peptic ulcer. To estimate the anti-ulcer and antiflatulence potential of the aqueous extracts on gastric acid, neutralization of the juice was performed *in vitro* and compared to water. Activity of the extract's solution (fennel, fenugreek and the mixture of both seeds) were also compared with each other.

MATERIAL AND METHODS

The Preparation of aqueous solution of fennel and fenugreek

Equal amount of two types of seeds namely fennel (5gm), fenugreek (5gm) and 5gm mixture (2.5gm+2.5gm) were taken separately and 50ml water was added to all. They were soaked in water over night and strained after 24 hours. Each aqueous extracts were made up to 100ml by subsequently adding water.

Chemicals and Reagents

Pepsin was purchased from Apollo Pharmacy (Bhawanipur) and Fennel and fenugreek seeds were purchased from the local market of Howrah (kali babu bazar). And all the other chemicals were purchased from Merck Specialities Private Limited. **Instruments**

The experimental instruments consisted of an electrode stand, a pH meter, a stirrer and hot plate, burette and a graduated pipette.

Preparation of Artificial Gastric Acid

Artificial gastric juice was prepared by adding 3gm of sodium chloride and dissolving 5mg of pepsin enzymes in 500mL water followed by adding 7.0mL hydrochloric acid and adequate water to make a 1000mL solution at pH 1.2.

Determination of the Neutralizing Effects on Artificial Gastric Acids

22.5mL of each test solution was added to 25mL artificial gastric juices at p H 1.2. The pH values were determined to observe the neutralizing effect.

Determination of the Duration of Consistent Neutralization Effect on Artificial Gastric Acids

22.5mL of each test sample was added to 25mL of artificial gastric juice at pH 1.2 in the beaker glass of the artificial stomach at $37\Box$ and continuously stirred (60rpm) with magnetic stirring apparatus. Per minute 3mL of artificial gastric juice at pH 1.2 was pumped into the container of the artificial stomach, and it was pumped out at 3mL/min at the same time. A pH meter was connected to the artificial stomach to check the pH change of the gastric juice. The duration of neutralization effect was determined when the pH value was returned to its initial value (pH 1.2).

Anti-flatulent Test in Removing Foam

50mL of gastric acid was taken a beaker in and 0.938gm sodium laureth sulphate was subsequently added into it. The mixture was heated at $37 \square$ with stirrer speed of 120 rpm to form foam. The volume of foam formed was measured. Then, 10mL of sample solutions were added. The mixtures were observed within 20 minutes and the remaining volume of foams were measured.

Statistical analyses

All the experiments have been done in triplicate. And data from three different experiments were subjected to analysis of variance (ANOVA) (P<0.05).

RESULTS AND DISCUSSION

Anti acid Activity Test

The duration for consistent neutralizing effect of aqueous extract of fennel, fenugreek and the mixture (1:1 solution) of the seeds recorded were 34 minutes, 24 minutes, 33 minutes, respectively and the water was 23minutes.

In this present study we had applied a titration method which mimics the regular physiological functioning of a human stomach, to explore the anti-acidic effects of fennel and fenugreek seeds that were well known for its efficiency to cure

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gastric ulcer. Fennel and fenugreek seeds aqueous extract have antacid and anti-flatulent activities. The anethole and fenchone inside fennel were expected to have antacid and anti-flatulent activity. In the case of fenugreek, it included polyphenol group that has PKa of 10.19, thus it was considered as a week base. Like an antacid the polyphenol group has the same mechanism while reacting with hydrochloric acid and hence neutralizing gastric acid. Here among the four samples (aqueous extract of fennel, aqueous extract of fenugreek, and 1:1 solution of two extracts and water), fennel showed the best result on anti-acidic test. When the fennel extract added, the pH of gastric juice had reached 1.79 and took 34 minutes to come back the initial pH (1.2). In case of fenugreek pH reached 1.51 and took 24 minutes. PH reached 1.62 for the 1:1 solution and took 33 minutes and water took 23minutes to come back the initial pH from pH 14.8. Thus, fennel and fenugreek seeds exhibited gastric acid neutralizing potential of the most efficient is fennel.

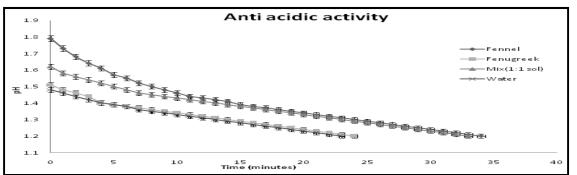
Anti-flatulent test was also performed by checking the ability to reduce foam forming tendency. The reduction of volume of foam was observed. Fennel extract reduced 20.00019114% foam whereas fenugreek extract reduced 20.00020206% foam. 1:1 solution reduced foam by 18.51926704% for and water by 15.9993534%. Fennel and fenugreek both showed almost similar result in the test of antiflatulent.

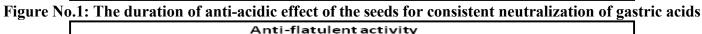
Thus, it can be concluded that fennel and formulations of fennel can be used as herbal drugs to prevent peptic ulcer and flatulent.

S.No	Test solution	pH after adding the solution	duration of neutralizing (minutes)	
1	Fennel extract	1.79	34	
2	Fenugreek extract	1.51	24	
3	Mix (1:1 sol)	1.62	33	
4	Water	1.48	23	

S.No	Test solution	Initial vol of foam (cm ³)	Final vol of foam (cm ³)	Reduced vol(cm ³)	% of reduction
1	Fennel extract	104.634	83.707	20.927 ± 0.201	20.00019114 ± 0.201
2	Fenugreek extract	98.979	79.183	19.796 ± 0.193	20.00020206 ± 0.193
3	Mix (1:1 sol)	103.908	84.665	19.243 ± 0.197	18.51926704 ± 0.197
4	Water	98.979	83.143	15.836 ± 0.155	15.9993534 ± 0.155

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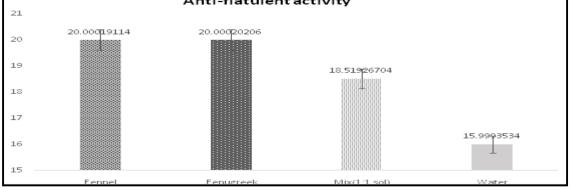


Figure No.2: % of remaining volume foam within 20minutes

CONCLUSION

This present study revealed significant anti-acidic and anti-flatulent activities in aqueous extract of fennel seeds and fenugreek seeds and also in their 1:1 mixture in the in vitro models tested. Results clearly indicate higher antacid potential of fennel compared to fenugreek. Anti-flatulent activity of both fennel and fenugreek was found to be similar (marginally higher for fenugreek) though the 1:1 recorded less. These observations claim further probe into the exact determination of the constituents responsible for these activities in particular. More research can help to find out and exact proportion of effective dosage formulation and other also with other extracts to cure gastric ulcer problems.

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CONFLICT OF INTEREST

We declare that we have no conflict of Interest.

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