ISSN 2063-5346

IN-VITRO ANTIULCER ACTIVITY OF AQUEOUS EXTRACT OF FENUGREEK SEED

Neha Tamta[1*], Dr. Amandeep Singh[2], Neelam Painuly[3*]

Article History: Received: 10.05.2023 Revised: 29.05.2023 Accepted: 09.06.2023

Abstract

In India, numerous traditional practitioners employ the evergreen tree *Trigonella foenum-graecum* to heal gastric ulcers. The goal of the current study was to assess the in-vitro antiulcer activity of *Trigonella foenum-graecum*seed aqueous extract (AE). In this study, we performed anti-ulcer activities using aqueous extract concentrations of 100 mg, 500 mg, 1000 mg, and 1500 mg in an in-vitro method for the H+/K+ - ATPase inhibition activity method. In the acid-neutralizing capacity (ANC), the extract significantly decreased ANC as compared with standard Aluminium hydroxide + Magnesium hydroxide (500 mg). Significant antiulcer action has been found in *Trigonella foenum-graecum*seed powder, and this species' active ingredients have been thoroughly studied.

[1]Research Scholar, Department of Pharmacology, School of Pharmacy and Research,

Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India.

[2]Professor, Department of Pharmacology, School of Pharmacy and Research,

Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India.

[3*] **Correspondence to Author:** Assistant Professor, Research Scholar, Department of Pharmacology, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India. E –mail: nehatamta14@gmail.com

DOI:10.48047/ecb/2023.12.9.05

INTRODUCTION

Gastric and duodenal ulcers, often known as peptic ulcer disorders, have been posing a serious threat to the world's population for the past 200 years because of their high penitence and transience. Numerous causes, including stress, smoking, drunkenness, and *Helicobacter pylori*, can cause these ulcers. Around the world, at least 40% of men and 20% of women are known to be affected by infection [1]. Another disorder that can lead to the development of ulcers is achalasia, which is brought on by autoimmune reactions, oesophageal infections, and hereditary factors. Production of saliva and bicarbonate, which counteract refluxed acid, is a protective mechanism for gastro-oesophageal reflux. Stress from the modern way of life has been severely exacerbated. Smoking, drinking, and spicy food habits have also disrupted the reflux condition, which results in the development of ulcers [2]. Over use of nonsteroidal anti-inflammatory drugs (NSAIDs), hyper secretion of gastric acids, decreased mucous secretions, inhibition of Arachadonic acid pathway, mucosal blood flow, surface active phospholipids, prostaglandins (PG), nitric oxide (NO), as well as enzymatic and non enzymatic antioxidant performance has also contributed to this condition. H. pylori infection has go undetected for long as it is asymptomatic. Approximately 70% of the total population is colonized by H. Pylori, and has 10%-30% susceptibility of developing into peptic ulcer [3].

Trigonella foenum-graecum is a plant that grows best in saline-sodic soil conditions and has a high folk medicinal value. Australia, China, India, and Sri Lanka are drug's native countries. In Java, it naturally grows in grassy fields, wasteland, and thickets, as well as on ridges and by the sides of roads. *Trigonella foenum-graecum* is a 40–80 (–150 cm) tall, upright, annual or living perennial legume shrub. It has several thin branches that are erect or decumbent at the base, together with a long, strong taproot. The cylindrical stems have stiff, coarse hairs at the base and are typically reddish in colour. Seeds show good therapeutic activity like antioxidant, anti-inflammatory and anticancer. But there are less activities proved like antiulcer, anticancer, anthelmintic, wound healing for stem part[4]. This study was aimed at investigating the occurrence of constituents in *Trigonellafoenum-graecum.* Theseed extract prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and by considerable decrease in superoxide dismutase, H+K+ATPase and increase in catalase activity. The H+K+ATPaseare the dimeric enzyme responsible for H+ secretion by the gastric parietal cells. H+K+ATPase are selectively blocked by the action of *Trigonellafoenum-graecum* seed extract.

MATERIALSANDMETHODS:

Processing of Plant Material: The collected plant material has been identified by Dr. S. Prasad Rao, botanist, Sir C R Reddy College of Autonomous sciences, Eluru. A voucher specimen(Voucher No.ATC32/10/2022) has been deposited at the herbarium unit of the Department of Botany, Sir C R Reddy Degree College, Eluru, west Godavari (District), Andhra Pradesh, India.

The plant was washed with tap water 3 times and sterilized by sprinkling with 70% alcohol. The plant is then dried in shade at room temperature and checked regularly for fungal contamination (if any). Once the plant is dried completely, it is then grinded into fine powder with pestle and motor. The fine powder is collected and used for extraction of the crude drug in aqueous solvent by Soxhlet extraction method.

Chemicals used: Aluminium hydroxide, sodium hydroxide, hydrochloric acid, sodium CMC, Tween80, Sodium benzoate,

Orange oil and magnesium hydroxide and other chemicals used were of analytical grade [5].

Extraction by Soxhlet Apparatus: This extraction procedure has been practiced for a long time for crude drugs. The mode of extraction process depends on the plant materials content to be extracted. Usually, the crude extract is taken from the Soxhlet apparatus with the aqueous solvent. This apparatus consists of three parts; a round bottom flask to take the solvent, the main jar is loaded with material from which the compounds have to be extracted anda condenser in which condensation of solvent vapour takes place. 100 g of the powder of plant material is taken into Soxhlet main jar. The solvent is poured into the round bottom flask and extract condensation under reduced pressure, and in a temperature of 60-80 °C set to boil through the regulated heating mantle.

The vapour of the solvent pass-through drive tubes enters the condenser through the main jar and gets condensed where there is a continuous flow of water in the condenser [6].

The condensed solvent falls back on the packed material in the main jar before collecting in a jar itself. The collection and extraction of material take place simultaneously in the main jar, as seen by the coloring of the solvent as a compound of material gets dissolved in the solvent. Thus, the crude plant material extract has been obtained, and it usually takes7-8htocomplete an extraction. The solvent was evaporated, and finally, it yields brown extract; this has been stored in the refrigerator for further studies.

Test for Flavonoids:

- The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

Test for phenol:

- **Bromine Water:** Take 5ml of bromine add 100ml of distilled water and shake well. Decant off the clear liquid.

Test for Alkaloids [7]**:**

- **Dragendorff's test**: To the 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution).An orange-red precipitate indicates the presence of alkaloids.

Test for Saponins:

- Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. No layer of foam indicates the absence of saponins.

Test for Glycosides:

- **Legal'stest:** Dissolve the extract in pyridine and add sodium nitroprusside solution make it alkaline. No formation of pink to red color shows absence of glycosides.

Test for Carbohydrates:

- **Fehling's test:** To 1ml of the extract, add equal quantities of Fehlings solution and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

Test for Tannins:

- Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

Test for Proteins:

- **Biuret test:** Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% $CuSO_4$ solution till a blue color is produced, and then add to the 1ml of the extract. Formation

of pinkish or purple violet color indicates the presence of proteins[12].

Preparation of herbal suspension dosage form

The composition of formulation for preparing 100 ml of suspension of *Trigonella foenum-graecum* powder was as shown in Table 1. The 60-mesh size fine particles of the drugs are properly mixed by triturating the drug mixed in solvents which are different additive such asTween-80, sodium carboxymethyl cellulose (CMC), as sweetening agent, flavoring agent like orange oil, and stabilizing agent sodium benzoate during shelf life of formulation. The study of antiulcer suspension formulation in vitro antiulcer study is to be conducted after In-vitro Evaluation of Antiulcer Activity of aqueous extract of stem of *Trigonella foenum-graecum* [8].

Table No. 1: Composition of aq. extract of stem of *Trigonella foenum-graecum* herbal suspension

S.No	Ingredients list	Quantities in suspension			
		F1	F2	F3	F4
1.	T.F. Extract	100mg	0.5gm	1gm	1.5gm
2.	Sodium CMC	0.6%	0.6%	0.6%	0.6%
3.	Tween80	0.1w/v	0.1w/v	0.1w/v	0.1w/v
4.	Sodium benzoate	1.5gm	1.5gm	1.5gm	1.5gm
5.	Orange oil	1ml	1ml	1ml	1ml
6.	Purified water q.s	100ml	100ml	100ml	100ml

***In-vitro* Evaluation of Antiulcer Activity:**

Acid Neutralizing Capacity: The aqueous extract of acid-neutralizing capacity values areF1, F2, F3 and F4. The aluminium hydroxide and magnesium hydroxide (500mg) are taken as the standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1.0 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed. With 0.5N Sodium hydroxide, the excess HCl was immediately titrated until the pink colour is attained [9-10].

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 divided by Grams of Antacid/Extract.

H+/K+ - ATPase Inhibition Activity: Preparation of H+/K+ - ATPase Enzyme: To prepare H+/K+ - ATPase enzyme sample the fresh goat stomach has purchased from the local slaughterhouse, the gastric mucosa of the fundus was cut-off and opened, the inner layer of the stomach was scrapped out for the parietal cell. The parietal cell obtained from the stomach was homogenized in 16mM Tris buffer with PH of 7.4, which has 10% Triton X-100 and centrifuged at 6000 rpm for 10 mins after centrifuge the supernatant solution was used for the H+/K+- ATPase inhibition. Protein content is found out according to Bradford's method where BSA

are used as standard. Assessment of H+/K+ ATPase inhibition: Per-incubated for 60 min at 37 ºC for the reaction mixture of the sample containing 0.1ml of enzyme extract (300µg) and plant extract with different concentration (20µg, 40µg, 60µg, 80µg, 100µg)[10].

The reaction was initiated by adding substrate 2 mM ATP (200µL), in addition to this 2mM MgCl2 (200µL) and 10mM KCl (200µL) has added. After 30 min of incubation at 37 ºC the reaction was stopped by 4.5% ammonium molybdate, and 60% perchloric acid was added and centrifuged at 2000rpm for 10 min, and in spectrophotometrically inorganic phosphate was released and measured at 660nm by following the Fiske-Subbarow method. Briefly, at 10 min at room temperature, 1ml of supernatant 4ml of Millipore water, 1ml of 2.5% of ammonium molybdate, 0.4ml of ANSA was added. At 660nm inorganic phosphate, absorbance has been measured at various doses of the extract; the enzyme activity has been calculated as micromoles of Pi released per hour. Results were compared with the known anti-ulcer PPA inhibitor Omeprazole and expressed as Mean ± SEM % enzyme inhibition was calculated using the formula [11]:

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100

RESULTS AND DISCUSSION:

Acid Neutralizing Capacity: The neutralizing effect of the extract was studied for four concentration (F1, F2, F3 and F4) and standard Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2](500mg). The results obtained envisage that the extract at concentration F1, F2, F3 and F4 showed a significant reduction in acid-neutralizing capacity (ANC), *i.e.*, 39.08, 26.52, 23.56 and11.94, respectively, as compared to standard Al (OH)3+Mg(OH)2 (500 mg) which is 19.76.The extract at a concentration of 1500 mg in F4 formulation has been found to neutralize acid more significantly as compared to standard. The results have been tabulated in **Table 2**.

Table No. 2: Effect of aqueous extract of seed of *Trigonella foenum-graecum*on acid neutralizing capacity

S. No.	Formulation / Concentration of extract (mg)	Volume of NaOH Consumed(ml)	MEq of Acid Consumed	ANC per gram of Antacid
1.	F1	53.5	3.908	39.08
2.	F2	52	3.304	26.52
3.	F3	56	1.352	23.56
4.	F4	58	0.376	11.94
5.	$Al(OH)_3$+$Mg(OH)_2$ 500mg	49	3.978	19.76

H+/K+ - ATPase Inhibition Activity: The H+/ K+ - ATPase inhibition activity of aqueous extract at a various concentration (20µg, 40µg, 60µg, 80µg, 100µg) was compared with Omeprazole as standard. The extract significantly showed activity in a dose-dependent manner. Maximum percentage inhibition of 59.56% has been observed for extract at a concentration of 100µg, and standard Omeprazole showed 66.98%. The results have been tabulated in

Table 3.

Table No. 3: Effect of aqueous extract on In-vitro H+/K+ - ATPase Inhibition Activity

S. No.	Concentration (µg)	Percentage Inhibition (%)	
		Standard	Extract
1.	20	34.56	28.95
2.	40	48.07	30.54
3.	60	50.89	31.47
4.	80	56.36	46.57
5.	100	66.98	59.56

CONCLUSION:

The acid-neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize, and it has been measured by a process known as back titration. In ANC, the aqueous extract at 100µg concentration showed a significant reduction in ANC 39.08 of aqueous extract of seed of *Trigonella foenum-graecum*. Also, Maximum percentage inhibition of 59.56% has been observed for extract at a concentration of 100µg, and standard Omeprazole showed 66.98% for the H+/ K+ - ATPase inhibition activity. The results indicate that the seed of *Trigonella foenum-graecum*has the potential to be effective antiulcer agent.

In this study, the ability of aqueous extract to inhibit H^+ -K^+ ATPase in vitro isolated from goat stomach was studied. In vitro studies are considered necessary in order to evaluate the potential of phytochemicals to enter in the cell and additionally to exemplify their interaction with the gastric ATPase. Enzyme H^+ -K^+ ATPase is an important enzyme system located on apical secretory membrane of partial cell. In this study, dose-dependent inhibition of enzyme by omeprazole and extract was observed, suggesting that the aqueous extract of seed of *Trigonella foenum-graecum* was significantly able to inhibit enzyme H^+ - K^+ ATPase, responsible for the secretion of acid and effect was comparable to omeprazole. Therefore, it could be concluded that the inactivation of H^+ - K^+ ATPase is the major gastroprotective mechanisms of action of the seed of *Trigonella foenum-graecum*which indicates its protective role against inhibiting gastric proton pump and opens a door for isolation and characterization of active compounds responsible for it.

References

[1] Ilya Raskin DMRSKNIAPNBABD a MCRNYJMOTCIP, F. Bertold, Plants and human health in the twenty-first century Review, Trends Biotechnol. (2002) 2012522–2012531, https://doi.org/10.1016/S0167-7799 (02)02080-2 2002.

[2] G.M. Cragg, D.J. Newman, Natural products: a continuing source of novel drug leads, Biochim. Biophys. Acta Gen. Subj. 1830 (6) (2013) 3670–3695, https://doi. org/10.1016/j.bbagen.2013.02.008.

[3] T.L. Srinivas, S.M. Lakshmi, S.N. Shama, G.K. Reddy, K.R. Prasanna, Medicinal plants as anti-ulcer agents, J. Pharmacogn. Phytochem. 2 (4) (2013) 91–97.

[4] Y. Syed, M. Khan, Chromatographic profiling of ellagic acid in Woodfordiafruticosa flowers and their gastroprotective potential in Ethanol-induced ulcers in rats, Pharmacogn. Res. (2016),

https://doi.org/10.4103/0974-8490.178649.

[5] C. Mancuso, R. Santangelo, Ferulic acid: pharmacological and toxicological aspects, Food Chem. Toxicol. (2014), https://doi.org/10.1016/j.fct.2013.12.024.

[6] J. Kanski, M. Aksenova, A. Stoyanova, D.A. Butterfield, Ferulic acid antioxidantprotection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies, J. Nutr. Biochem. 13 (5) (2002) 273–281, https://doi.org/10.1016/S0955-2863(01)00215-7.

[7] R. Rukkumani, K. Aruna, P. Suresh Varma, V.P. Menon, Influence of ferulic acid on circulatory prooxidant - antioxidant status during alcohol and PUFA induced toxicity, J. Physiol. Pharmacol. 55 (3) (2004) 551–561.

[8] M. Srinivasan, R. Rukkumani, A.R. Sudheer, V.P. Menon, Ferulic acid, a natural protector against carbon tetrachloride-induced toxicity, Fundam. Clin. Pharmacol. (2005), https://doi.org/10.1111/j.1472-8206.2005.00332.x.

[9] P.K. Prabhakar, R. Prasad, S. Ali, M. Doble, Synergistic interaction of ferulic acid with commercial hypoglycemic drugs in streptozotocin induced diabetic rats, Phytomedicine 20 (6) (2013) 488–494, https://doi.org/10.1016/j.phymed.2012. 12.004.

[10] M.J. Chung, S.H. Lee, N.J. Sung, Inhibitory effect of whole strawberries, garlic juice or kale juice on endogenous formation of N-nitrosodimethylamine in humans, Canc. Lett. (2002), https://doi.org/10.1016/S0304-3835(02)00076-9.

[11] K.L. Khanduja, P.K. Avti, S. Kumar, N. Mittal, K.K. Sohi, C.M. Pathak, Anti-apoptotic activity of caffeic acid, ellagic acid and ferulic acid in normal human peripheral blood mononuclear cells: a Bcl-2 independent mechanism, Biochim. Biophys. Acta Gen. Subj. 1760 (2) (2006), https://doi.org/10.1016/j.bbagen.2005.12.017.