Evaluation of anti-ulcer potential of Germinated *Trigonella foenum graecum* ethanolic extract in SD rats

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Abstract

The current experiment was performed to examine the therapeutic action of extract against gastric ulcers in SD rats. Though this experiment explains that the germinated fenugreek seed extract showed significant improvement in gastric mucosa conditions after Indomethacin-induced gastric lesions ($p<0.01$). Further marked improvement in antioxidant enzymes such as SOD, and Catalase was observed ($P<0.01$), if compared to self-healing and untreated ulcer induced groups. Histopathology reports also explain that germinated fenugreek seed extract improved the epithelial layer in treated groups compared to untreated ulcer-induced groups.

***Keywords*:** *Trigonella foenum graeucum*, germination, pharmacological action, gastro-protective activity.

1. INTODUCTION

Herbal medicines are very useful for healthy lifestyle and utilized for the maintenance of variety of diet-related ailments including cancer, hypertension, diabetes, inflammation, and heart related diseases [1]. The marketing and use of dietary supplements have grown rapidly in the United States following enactment of the “Dietary Supplement and Health Education Act” in the year 1994, which states that dietary supplements have no requirement of prior approval from the U.S. Food and Drug Administration (USFDA) before they are marketed. Therefore, toxicologic studies of natural products are crucial for the safe use of dietary supplements. Botanicals, on the other hand, frequently contain a wide range of phytoconstituents with unknown biological effects that can be harmful to human health [2].

Gastric ulcer is characterized by the damage of mucosal membrane, specially affects the superficial or deeper muscularis mucosa of stomach [3]and affects approx 5−10% of people globally [4]. GU is more common in those who have history of hepatic cirrhosis, adult onset diabetes and mental health disorders [5]. Different pathogenic mechanisms are associated in the formation of gastric ulcer, including chronic use of NSAIDs and other drugs such as anticoagulants, corticosteroids, and chemotherapeutic agents, diet, smoking, alcohol, stress, and consumption of fatty foods are also leading causes of peptic ulcers, and will produce free

radicals which and leads to mucosal ischemia. Excess secretion of hydrochloric acid, which decreases local blood flow, and mucus have also been implicated in the etiology of peptic ulcers [6-8].

Fenugreek is a leguminous herb grown commercially in India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, and Argentina [9]. It contains various important bioactive components including carbohydrates, alkaloids, steroidal saponins, amino acids, and minerals which are consumed for nutritional, nutraceutical, medical and therapeutic purposes [10]. The seeds also contain saponins, linoleic, linolenic, oleic acids, polyunsaturated fatty acids, galactomannans, trigonelline, and 4-hydroxy isoleucine. Flavonoids are the chief constituent of *Trigonellafoenum-graecum* specifically apigenin 6,8-di-C-glucoside, apigenin-6-C-glucosyl-8-C-galactoside, apigenin-6-C-glucosyl-8-C-arabinoside, and apigenin-6-Cgalactosyl- 8-C-arabinoside, epigenin, luteolin, orientine, querceitin, and vitexin [11]. Fenugreek is commonly known for taste enhancer in several traditional cuisines and widely utilized for allergic and anti-inflammatory diseases such as seizures, hemorrhoids, palsy, gout, dropsy and has analgesic, hypolipidemic, antibiotic, antiplasmodic, anthelmentic, [12], antioxidant, antidiabetic, hypocholesterolemic, anti-lithogenic, anticarcinogenic, antimicrobial activities [13]. In addition to its medicinal properties, fenugreek has been used as an emulsifier and stabilizer in a range of culinary preparations whereas their extracts or powders have also been utilized in the making of bread and extruded products [14]

2. Material and methods

Collection and authentication of plant

Trigonella foenum graecum seeds were collected from local market of Lucknow, Uttar Pradesh, India. The taxonomic authentication was done by the Scientist of Botany and Pharmacognosy, CSIR, Lucknow, India, and the voucher specimen (accession no. is T025 having reference number CIMAP/Bot-Pharm/2018/11) was deposited to Botany and Pharmacognosy, CSIR, Lucknow.

Germination of seeds

A sufficient quantity of fenugreek seeds cleaned, and rinsed with water and dried in air for 1 hour, then dabble in water for 6 hours at room temperature (27-30 °C). Then steeped seeds were kept in a thick layer of cotton fabric and allowed to sprout in dark at room temperature for 4-5 days. The fabric and seeds were moistened by sprinkling the water. Then germinated seeds were then dried for further processing after 5 days.

Preparation of ethanolic extract of germinated fenugreek seeds

50 g germinated fenugreek seeds were extracted with ethanol (70%) in a Soxhlet apparatus for 5 days. Then extract was filtered and the filtrate was evaporated until it was completely dry. Then, measure the quantity of germinated fenugreek extract in percentage and store it for future work

Chemicals and equipment

Carboxymethylcellulose (CMC), Indomethacin (jagsonpal), ranitidine, , Pyrogallol, Alcian blue, ethanol, ThioBarbituric acid (TBA), trichloroacetic acid (TCA), phosphate buffer, Tris-HCl, hydrogen peroxide, DTNB (5,5-dithiobis-2-nitro benzoic acid), total protein kit (autospan), microscope, pH meter, UV spectrophotometer, homogenizer, centrifuge machine, test tubes, Eppendorf tubes 2 ml, centrifuge tubes 15 ml, beaker, etc.

Experimental animals

Male rats (Sprague Dawley; 150-200 g) were procured from the Animal House of CDRI Facility and kept in the Faculty of Pharmacy, Integral University Lucknow, under a particular laboratory environment with 12 hours light/dark cycle with diet and drinking water. The animals were divided according to the experimental procedure in their respective groups. The temperature and humidity of the animal house were maintained at 22 ± 2ºC and 50 ± 15%. Ethical clearance for animal experimentation according to the protocol was get from the Institutional Animal Ethics Committee (Reg. No. 1213/PO/Re/S/08/CPCSEA).

Protocol for the experiment

The animals were categorized into 7 groups, each containing 6 animals. Indomethacin was selected as an ulcer inducer at 10 mg/kg per oral (Khushtar et al., 2016). Then ulcers were induced in all groups except group-I (normal control) and group-VII (per se group) by 10 mg/kg of Indomethacin. Group-I received 1%, 1 ml/kg/day p.o.carboxymethyl cellulose (CMC) for 10 days. Animals in Group-II (ulcer control) were killed under anesthesia after 6 h of oral administration of Indomethacin on the first day. Then, after 6 hours of oral administration of Indomethacin (10 mg/kg), group III (self-healing) received 1% CMC (1 ml/kg/day/oral) for 10 days. Groups IV and V received ethanolic extracts of germinated fenugreek seeds at 100 and 200 mg/kg, respectively. Group VI rats were given ranitidine at a dose of 50 mg/kg per oral, while Group VII or the perse group received ethanolic extracts of germinated fenugreek seeds at a dose of 200 mg/kg p.o. for 10 days. All groups were allowed food and water ad libidum for 24 hours before the sacrifice. Then all the rats were killed under anesthesia, and the stomach was isolated, cut, and opened along the greater curvature. Then the ulcer lesions were observed and the ulcer index was calculated. Further stomach samples were cut into small portions and stored in formalin solution for further study. The experimental protocol is mentioned in table 1.

Group	**Treatment schedule (n=6)**
Normal control (group-I)	1% CMC (ml/kg/day p.o.) for 10 days then on the 11th day the animal were sacrificed with 36 hours of fasting
Ulcer control (group- II)	Ulcer induction (10 mg/kg/oral) on 1st day then rats were sacrificed after 6 hours of Indomethacin
Self-healing (group-III)	Ulcer induction (10 mg/kg/oral) on 1st day + 1% CMC (1ml/kg/day/oral) for 10 days then on 11th day the rats were sacrificed with 36 hours of fasting.
TG low dose	Ulcer induction (10 mg/kg/oral) on 1st day + 100 mg/kg/oral ethanolic

(group-IV)	TG extract for 10 days then on 11th day the rats were sacrificed with 36 hours of fasting.
TG high dose (group-V)	Ulcer induction (10 mg/kg/oral) on 1st day + 200 mg/kg/oral ethanolic TG extract for 10 days then on 11th day the rats were sacrificed with 36 hours of fasting.
Standard treated (group-VI)	Ulcer induction (10 mg/kg/oral) on 1st day + 500 mg/kg/oral ranitidine for 10 days then on 11th day the animals were slaughtered with 36 hours fasting.
Perse group (group-VII)	200 mg/kg/oral ethanolic TG extract for 10 days then on the 11th day the rats were slaughtered with 36 hours fasting.

Table 1. Treatment schedule for study

Determination of ulcer index

The fore stomach portion of all rats was selected for ulcer lesions and indexed according to severity. The number of ulcer lesions was counted and scoring of ulcers (ulcer index) was made. Normal coloration was assigned (0), red color (0.5), spot ulcer (1), hemorrhagic streak (1.5), deep ulcers (2), and perforation (3). The mean ulcer score for each animal was expressed as an ulcer index. The Ulcer index (UI) was counted using the formula:results shown in figure 1.

$[UI= U_N+U_S+U_P \times 10^{-1}]$

Where, UI= Ulcer Index; U_N= Average number of ulcers per animal; U_S= Average number of severity score; U_P = Percentage of animals with ulcers[15].

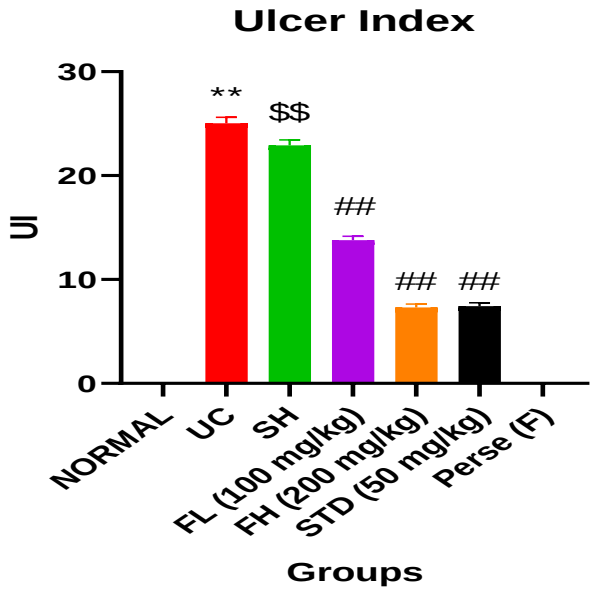

Figure-1.Effect of ethanolic extract of germinated fenugreek seed extract on ulcer index in normal control, ulcer control, self healing and treatment groups.

** ($p<0.01$), as compare to normal control group.

$$ ($p<0.01$), as compare to ulcer control group

($p<0.01$), as compare to self healing group

ns ($p<0.05$) as compare to normal control group.

Percentage of inhibition

The percentage inhibition of gastric ulcers was calculated by the formula:
[(UIcontrol-UItreated)/UIcontrol] × 100
UI = Ulcer index[5]

Determination of gastric wall mucosa

After isolation, stomach tissues were dipped in 0.1% alcian blue solution (dissolved in 0.16 M sucrose and buffered with 0.05 M sodium acetate and maintain pH 5.8 with HCl) for 2 h. Further, the uncomplexed dye was removed by two washes of 0.25 M sucrose at 15- and 45-min intervals. Then dye mix with mucus was diluted by 10 ml aliquots of 0.5 M magnesium chloride for 2 h. The obtained blue solution was shaken with an equivalent volume of diethyl ether and recorded the absorbance of the aqueous phase was at 580 nm. The quantity of mucus was calculated by standard curves of alcian blue (ranging from 20 µg/10 ml) and the finding was pointed as 'µg of alcian blue/g tissue[16]. The results given in figure 2.

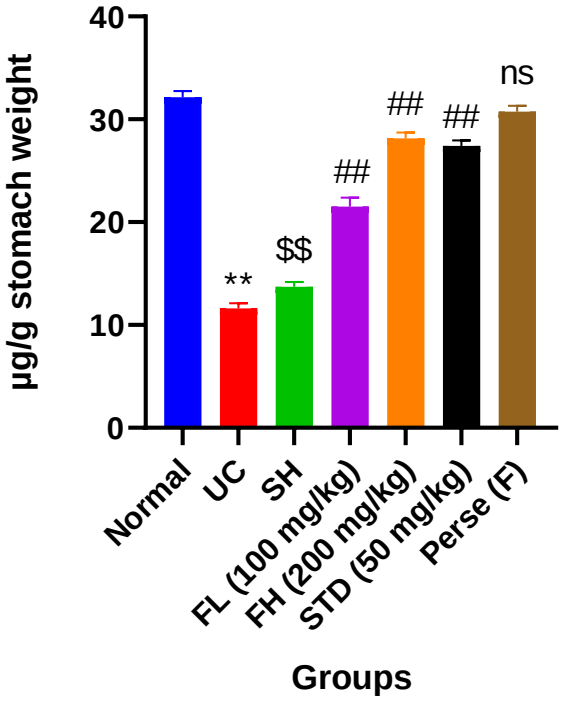

Figure-2: Effect of ethanolic extract of germinated fenugreek seed extract on gastric mucus thickness in normal control, ulcer control, self healing and treatment groups.
**($p<0.01$), as compare to normal control group.
$$ ($p<0.01$), as compare to ulcer control group
($p<0.01$), as compare to self healing group
ns ($p<0.05$) as compare to normal control group.

Determination of lipid peroxidation

Lipid peroxidation was measured by using 1 ml tissue homogenate. In stomach tissue, homogenate added 0.5 ml of 30% solution of TCA, followed by 0.5 ml of 0.8% TBA

solution. Then the tubes were wrapped and kept in an 80°C water bath shaker for 30 minutes, then 30 min later, the tubes were removed and kept in ice for 30 min. Further, the solution was centrifuged for 15 minutes at 3000 rpm. The supernatant was taken for observation at 540 nm against an appropriate blank. The amount of TBARS, that is, the quantity of malondialdehyde in the sample solution was calculated by the formula: [nM of MDA=A540×V/0.156], where, A is the absorbance and V is the volume of the test solution [17].

Determination of superoxide dismutase (SOD)

The supernatant was assayed for superoxide dismutase (SOD) activity based on the inhibition of pyrogallol autoxidation. The 100 µl supernatant was mixed with Tris-HCl buffer (pH to 8.5) and made the solution up to 3 ml with the same buffer. Then, 25 µl pyrogallolsolution was mixed in the previous solution, and variation in absorbance was taken at 420 nm at a 1-min interval for 3 min. SOD in 1 unit was interpreted as the amount of enzyme causing 50% inhibition of auto-oxidative pyrogallol per 3 ml of test mixture. The SOD was calculated by the below-mentioned formula [Unit of SOD per ml of sample = (A – B) ×100/A×50], where, 'A' is the difference of absorbance in 1 min in control, 'B' is the difference of absorbance in 1 min in the test sample [15].

Measure of Catalase

0.05 ml supernatant was added to a small tube containing 2.95 mL of 19mM solution of H_2O_2 prepared in potassium phosphate buffer (pH 7.4). Then observe the absorbance at 240 nm, with an interval of one minute for 3 minutes. The amount of catalase in the supernatant responsible for decomposing H_2O_2, and showed a drop in absorbance. Catalase activity (nanomoles of H_2O_2consumed/minute/mg protein) = (delta A/minute×volume of assay)/(0.081×volume of homogenate × mg of protein)][15].

The result of Antioxidantsa are given in figure 3.

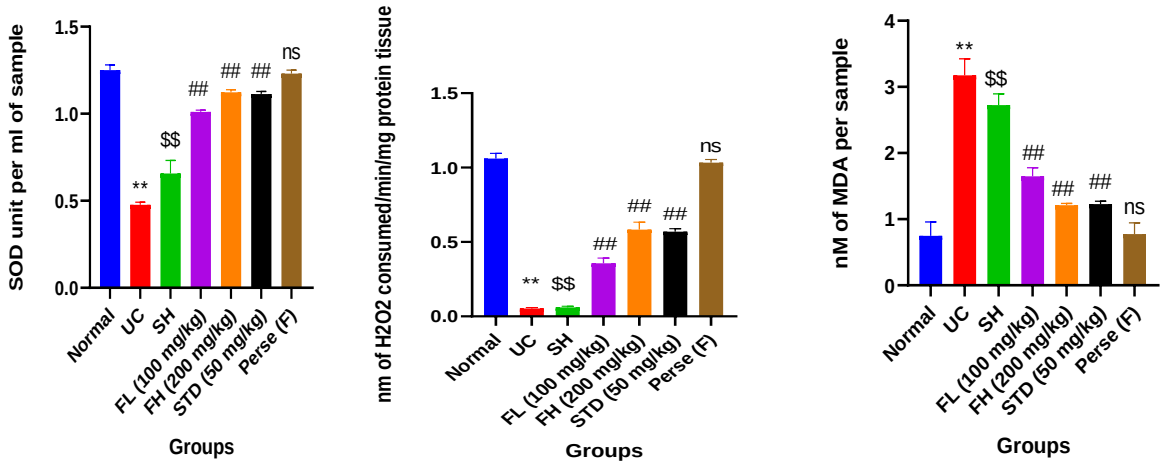

Figure-3: Effect of ethanolic extract of germinated fenugreek seed extract on SOD, TBARS level & Catalase in normal control, ulcer control, self healing and treatment groups.

The measure of tissue glutathione

500 mg rat stomach tissue was homogenized in 5 ml EDTA solution (0.02 molar), then added cold water into the homogenized solution, mixed properly, and then added TCA solution (1ml) and shaken well for 5-10 minutes. Then the whole solution was poured into centrifuge tubes and centrifuged for 10-15 minutes at 6000 rpm. After centrifugation, the supernatant was taken out and stirred with 0.4 molar Tris buffer (4ml, pH 8.9) stirred properly and immediately added DTNB solution and recorded the absorbance at 412nm against a reagent blank. Tissue glutathione (GSH) was calculated using the formula:

[GSH' µg/mg of protein= (A412× 50×3.5×2.25×1)/(0.337×2×mg of protein)] [17].

Histopathological evaluation

A small fraction of the rat stomach was used for the histopathological examination and was stored in a 10% formalin solution.

Statistical evaluation

All experiment was performed 3 times and the outcome was expressed as mean ± standard error of the mean. The results were analyzed by one-way analysis of variance (ANOVA) by using GraphPad Prism 8.0.1 (244). The result was considered statistically significant when $p<0.05$.

3. RESULT

The ulcer index was significantly ($p<0.01$) high in toxic group rats i.e., group-II, and self-healing rats i.e., group-III when compared to normal control rats i.e., group-I. Whereas a significant decrease (($p<0.01$) in ulcer index was noted in two doses that is, 100 mg/kg and 200 mg/kg of germinated fenugreek seed extract (group- IV, V) and standard (ranitidine) treated groups (group-VI) as compared to toxic and self-healing groups. However, no significant difference (($p>0.05$) was observed in per se groups-VII rats treated with germinated fenugreek seeds extract treated with 200 mg/kg dose in contrast with healthy rats.

The percentage inhibition of the self-healing group was calculated 8.5, where the percentage inhibition by 100 and 200 mg/kg dose of germinated fenugreek seed extract was found to be 37.41 and 62, whereas ranitidine treated group rats showed 70.4.

A standard Alcian Blue calibration curve was used to calculate the mean stomach wall mucus thickness. Indomethacin (10 mg/kg) in group II and group III rats significantly causes damage to gastric wall mucus, compared to normal control rats i.e., group-I. In contrast, post-treatment of group IV and group V rats with germinated fenugreek extract and ranitidine respectively for 10 days resulted in a significant increase ($p<0.01$) in gastric wall mucus as compared to that of group III rats. However, no significant ($p>0.05$) change was observed in 200 mg/kg dose of germinated fenugreek seed extract and ranitidine treated rats and found to be same action as ranitidine. Whereas, no significant ($p>0.05$) difference was calculated in per se group treated with germinated fenugreek seeds extract at 200 mg/kg/day dose when matched with normal control group-I rats.

In ulcer control rats (group-II) and self-healing rats (group-III), the level of TBARS was found to be significantly higher ($p<0.01$) than in normal control rats (group-I). A significant ($p<0.01$) decrease in TBARS level was found in germinated fenugreek seed extract-treated

rats i.e. group IV and V as compared to that of ulcer control group-II and self-healing group-III rats. Standard ranitidine (50 mg/kg, p.o.) treated group VI rats also showed a significant ($p<0.01$) decrease in mean TBARS levels as compared to that of ulcer control group II and self-healing group-III rats and show similar effect in 200 mg/kg dose of germinated fenugreek seed extract. However, no significant change was observed in per se groups rats, treated with ethanolic extract of germinated fenugreek seed extract with 200 mg/kg dose as compared to normal control group-I.

A significant ($p<0.01$) decrease was observed in ulcer control group-II and self-healing group-III rats in contrast with normal control group-I rats. After post-treatment with germinated fenugreek seed extract (100 and 200 mg/kg.p.o.) and ranitidine (50 mg/kg.p.o.) significant increase ($p<0.01$) was observed in SOD level as compared to ulcer control and self-healing rats. However, no significant ($p>0.05$) change was observed in per se group rats, treated with ethanolic extract of germinated fenugreek seed extract with 200 mg/kg dose in contrast with normal control group-I

The mean catalase level was decreased ($p<0.01$) in ulcer control (group-II) and self-healing group-III treated with Indomethacin (10 mg/kg p.o.) as compared to control group I. Then after post-treatment, a significant increase ($p<0.01$) was observed in two doses (100 mg/kg/day p.o and 200 mg/kg/day p.o) of germinated fenugreek seed extract and standard drug (ranitidine) treated rats as compared to self-healing group-III rats. No significant ($p>0.05$) change was found in the per se group that is group VII treated with a 200 mg/kg oral dose of germinated *Trigonellafoenum-graecum* seed extract.

The GSH level was decreased ($p<0.01$) ulcer control group-II and self-healing group-III rats as compared to that of the normal control group-I rats. After post-treatment, a Significant increase ($p<0.01$) was observed in germinated fenugreek seed extract and standard (50 mg/kg.p.o.) drug-treated rats i.e. group IV and V as compared to self-healing rats treated with Indomethacin 10 mg/kg p.o dose for 10 days. While no significant ($p>0.05$) change was found in the per se group that is group VII treated with 200 mg/kg oral dose of germinated fenugreek seed extract.

Macroscopic evaluation of rat stomach

Reports of the gross anatomy of the stomach confirmed the large number of ulcers in the Ulcer control group. Further, in the self-healing group and low dose treated groups, few numbers of ulcers were existing, whereas there were no ulcers found in high dose treated and standard (ranitidine) drug-treated groups as given in figure 4.

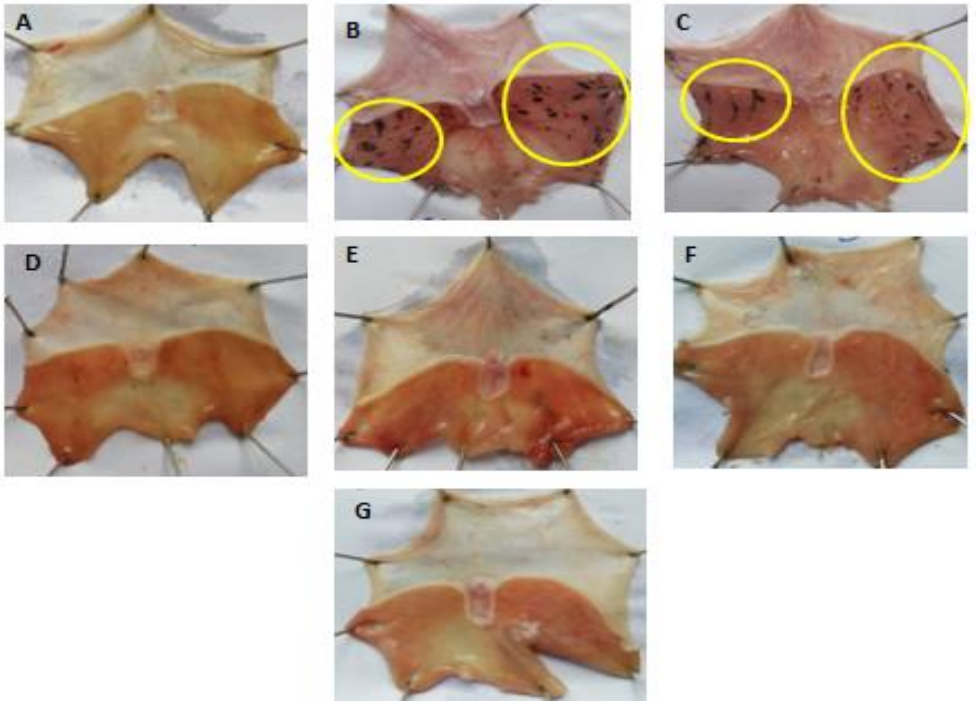

Figure-4: A: Normal control group showing normal apperance, B:Ulcer control (Indomethacin 10 mg/kg) group showing numerous ulcer lesions, C: Self healing group also hasmany ulcer lesion s it receive only CMC, D: Treatment group (Germinated *Trigonellafoenumgraecum* seed extract 100 mg/kg) showing no lesions, only redness is observed, E: Germinated *Trigonellafoenumgraecum* seed extract (200 mg/kg) has no ulcer lesion and showing good effect as compare to self healing group, F: Standard drug (Ranitidine) treated group 50 mg/kg showing normal appearance, G: per se group treated with Germinated *Trigonellafoenumgraecum* seed extract 200 mg/kg.p.o.).

Histology of rat stomach

The Histology report revealed the architecture of the stomach mucosa. The findings are mentioned in figure 8.

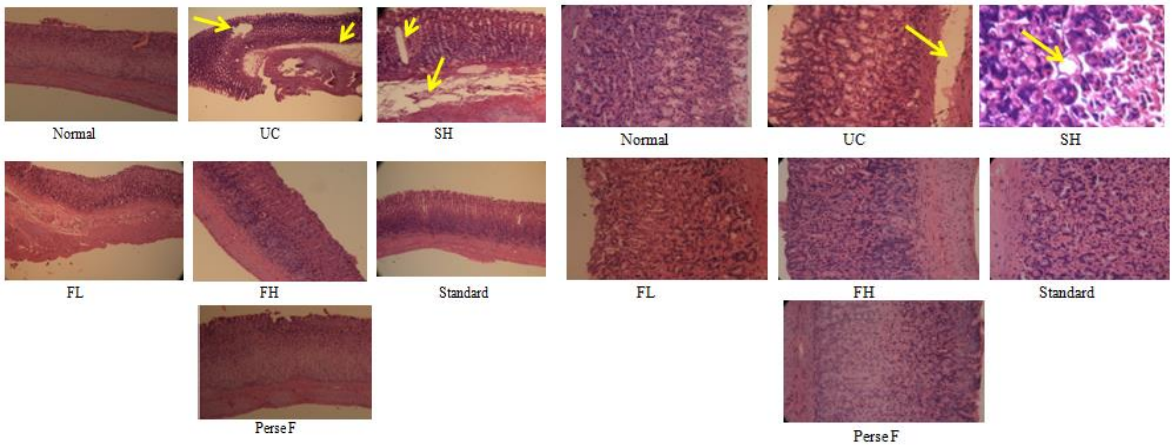

Figure-5: Normal control animals showed normal gastric histology whereas, ulcer control and self healing group showed severe damaged gastric mucosa, significantly high disruption to

the surface epithelium and edema of the submucosal layer. *Trigonellafoenumgraecum*treated animals at dose of 100 and 200 mg/kg.p.o showed less edema and less depleted epithelium as compared to ulcer control group and self healing group and standard drug (ranitidine 50 mg/kg.p.o) treated animals showing comparatively better protection of the gastric mucosa as there is absence of ulcer lesion, submucosaloedema and epithelial cell losses.

4. DISCUSSION

Gastric ulcer is a very general gastrointestinal ailment that estimated to affect 4–5% people in the world. It occurs mostly in the stomach and the proximal duodenum, which characterizes by a significant defect in the mucosal barrier, induction of oxidative stress, infiltration of neutrophils and secretion of pro-inflammatory cytokines [18]. The pathophysiology of gastric ulcer is complicated and unclear but some evidences showed that it is caused by the imbalance between defensive (mucin and peptide secretions, prostaglandin secretion, and blood flow) [19] and offensive (high acid production, reactive oxygen species (ROS), and *Helicobacter Pylori*)infection [20].

Indomethacin is the common choice to induce gastric ulcer because of its higher potential than other NSAIDs. Indomethacin inhibit the COX enzyme, the key of formation of prostaglandins, which have been shown to have a gastroprotective impact not only by decreasing acid secretion but also by boosting gastric mucus levels. Indomethacin increases oxidant parameters, acid secretion and pepsin activity while decreasing antioxidant parameters, mucus strength and bicarbonate secretion. Also increase the lipid peroxidation and produce free radicals in gastric mucosa. These changes lead to increased toxic radicals of oxygen and cause gastric mucousal damages[21-22].

Hence, in present study Indomethacin at 10 mg/kg, was used as a persuader for gastric ulcers in a selected rat model. In our study, Indomethacin (10 mg/kg p.o) produces macroscopic ulceration with high ulcer index in the ulcer control group and self-healing group-III than in normal control rats group-I. Gastric wall mucus is secreted by mucous neck cells and forms a thick layer around gastric mucosa. The mucus protects the gastric wall from hydrogen ion diffusion and enhances the buffering effects of gastric juices, thereby inhibiting gastric ulcer formation. Increased levels of aggressive factors may cause an overproduction of reactive oxygen species such as superoxide radicals, hydroxyl radicals, and H_2O_2, as well as depletion of protective factors like antioxidants (SOD, CAT, GSH, and others), resulting in gastric mucosal erosion and gastric ulcer [23]. So, the findings showed that gastric mucus was reduced ($p<0.01$) in ulcer control and self-healing group III as compared to that of normal control group-I rats. Whereas, gastric mucus wall thickness was increased ($p<0.01$) in different doses of test drugs i.e. germinated fenugreek seed extractand standard (ranitidine) drug-treated group-IV, V, and VI respectively. MDA is the byproduct of lipid peroxidation and is utilized as a pointer to quantify the rate of lipid peroxidation [24]. In our findings, TBARS level was significantly high ($p<0.01$) in ulcer control and the self-healing group than normal control group but declined ($p<0.01$) TBARS level was observed in groups who are post-treated by the fixed doses of ethanolic extract and ranitidine treated rats as compared to the self-healing group.

Many research studies reported that the amount of free radicals, lipid peroxidation and activities of antioxidant enzymes including: SOD, catalase, and GSH is related to gastric mucosal damage induced by Indomethacin. Nevertheless, reactive oxygen and nitrogen species (ROS and RON) produced after treatment of Indomethacin alters the activities of cellular antioxidant enzymes that serve as the first line of defense against oxidative stress [25]. In current study, the antioxidant parameters including superoxide dismutase (SOD), Catalase (CAT), and GSH were decreased ($p<0.01$) in the ulcer control II group and self-healing III group when compared to normal control healthy rats. While, increased ($p<0.01$) level of SOD, CAT, and GSH were found in ethanolic extract-treated and ranitidine-treated rats as compared to self-healing group-III rats. Whereas, no significant difference ($p>0.05$) was found in the per se group i.e. group-VII orally treated with 200 mg/kg dose of germinated fenugreek seed extract when compared to normal control rats.

In India, fenugreek is used as both a herb and a spice and is often used for arthritis, acid reflux, breastfeeding, dysmenorrhea, breast engorgement, bronchial asthma, labor induction, and hormonal abnormalities [26].

Previous studies showed that the soluble gel fraction of fenugreek seed and ranitidine both decreases the ulcer lesions in aspirin induced gastric model[27], while another study was performed in vitro and in vivo models to elucidate the probable mechanism of fenugreek seeds utilising in silico analysis on the H+/K+ ATPase receptor, which is a critical target for mediating the gastro protective action. Here, Insilico analysis was used to assess the flavonoids and saponins in fenugreek extract for potential interactions at the H^+/K^+ ATPase receptor site. The result showed the high protective action against human gastric carcinoma epithelial cells and flavonoids derivative namely vitexin-7-O-glucoside, vicenin-2, orientin and luteolin has great interactions on H^+ /K^+ ATPase while saponinswere not interact with H^+ /K^+ ATPase in in silico analysis. So, the findings suggest the gastro-protective action in both: in vitro and in vivo studies [28]. The study by Pandian et al., proved the gastro-protective effect of fenugreek seed extract as it reduces the lipid peroxidation by increasing the antioxidant potential of gastric mucosa [29]. Another research revealed gastro-protective effect of fenugreek enriched fraction against indomethacin induced gastric ulcer was by increasing the quantity and quality of mucin secretions [30]. The polysaccharide present in fenugreek seeds makes a mucin like layer on the surface of gastric mucosa which works as a barrier against ulcer inducing substances [31].

But no experiment was performed on gastro-protective action of germinated fenugreek seed extract. Germination of seed improves the quality of phytoconstituents such as increases the protein content compared to ungerminated fenugreek seeds. On other hand germinated seeds decrease dietary fiber and starch contents and increases Ca, Fe, and Zn contents [32]. Germinated seeds are good source of flavonoids, phenolic compound, essential amino acids especially leucine, lysine and tryptophan which are effective in improving protein digestibility, as well as fat absorption capacity. It also decreases the levels of total unsaturated fatty acids, total lipid, triglycerides, phospholipids and unsaponifiable matter while those of saturated fatty acids are increased. They are shown to reduce blood sugar and cholesterol in diabetic patients [33]. The results of current experimentsuggest that Indomethacin is a potent drug that cause gastric tissue damage and produce many ulcer lesions. Germinated fenugreek seed extract is effective to protect the gastric mucosa through its antioxidant property and by

reducing ulcer index, and TBARS level and increasing antioxidant enzyme levelssuch as SOD, CAT and GSH. This proves the efficacy of germinated fenugreek seeds extract as an antiulcer agent against Indomethacin induced gastric ulcer in rodent models.

5. CONCLUSION

Germinated seed extract decreases ulcer lesions, and protects the gastric mucosa from further damage due to oxidative stress or other such causes. So, this research confirms flavonoid or polyphenols rich plant extracts could be useful as a therapeutic sword against Indomethacin induced Gastric Ulcer. Secondly, these herbal remedies could be utilized to reduce the risk of development of Gastric Ulcers, in patients undergoing long-term NSAID therapies like Rheumatoid Arthritis, Inflammatory bowel disorders, etc. Future studies could be planned for the identification of specific metabolites responsible for ulcer protective potency of germinated fenugreek seed extract. No doubt we need to extrapolate these preclinical findings to further clinical research to obtain some useful findings.

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DISCLOSURE STATEMENT

All authors have approved the final manuscript and no potential conflict of interest was reported by the author.

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