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Anti Ulcer Activites of Hydro-Alcoholic Extract of *Trigonella foenum-graecum* in Different Experimentally Induced Ulcer Models in Rats

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Abstract— *The aim of the study was to determine antiulcer activity of whole plant of Trigonella foenum-graecum L on the albino wister rats. Dried whole plant of Trigonella foenum-graecum L was powdered and this coarse powder was extracted with 70% ethanol by soxhlet extraction method to yield an ethanol extract of whole plant of Trigonella foenum-graecum L. The extract was subject to preliminary phytochemical analysis and was evaluated for antiulcer activity against various models such as pylorus ligation, Swimming stress induced and Aspirin induced ulcer models. In acute toxicity study, HATFG was found to be safe till 2000mg/kg. So the doses of HATFG at various concentration of 200 and 400mg/kg body weight was administered orally for prevention of ulcer from Pylorus ligation, Swimming stress and Aspirin induced ulcers. Analytical parameters like percentage of ulcer protection was calculated based on ulcer index and gastric juice volume, pH, Total and free acidity of gastric juice. Preliminary phytochemical analysis of HATFG showed the presence of carbohydrates, amino acids, lipids, alkaloids, flavonoids, tannins, saponins, cardiac glycosides, steroids, and terpenoids. The HATFG has shown significant activity at both 200mg/kg and 400mg/kg dose level in a dose dependent manner. Phytochemicals like tannins, flavonoids and saponins may be responsible for anti ulcer activity of HATFG.*

Keywords— *Phytochemical analysis, Ulcer index, Ulcer protectives, Gastric juice, Tannins, Saponins.*

I. INTRODUCTION

Peptic ulcers are erosion of lining of stomach (or) the duodenum[4]. Peptic ulcer develops due to an imbalance between aggressive factors (*Helicobacter pylori*, NSAIDS, Gastric acid,) and protective factors (mucin, bicarbonates, and prostaglandins), leading to an interruption in the mucosal integrity[5]. Peptic ulcer is one of the world's major gastrointestinal disorders and affecting 10% of the world population[6]. *H-pylori* is the main cause of stomach ulcers, was first identified by the two Australian Scientists in 1982. *H-pylori* is a negative bacillus, micro-aerophilic, motile, flagellated and spiral shaped bacteria, usually found in the stomach. Peptic ulcer may cause due to emotional stress *Helicobacter- pylori*, and regular usage of NSAIDs drugs, alcohol abuse, and smoking are the principal etiological factors associated with peptic ulcer[7]. Patients with gastric ulcers have normal (or) diminished acid production, yet ulcers may occur even in complete absence of acid[8].Medications called proton pump inhibitors (PPI) and H_2-blockers suppress the production of stomach acid and let ulcers heal. H_2-blockers are less effective than PPIs, are usually tried first. Antibiotics are taken simultaneously to treat the *H-pylori* infection. To ensure that *H-pylori* are eliminated, doctors typically prescribe triple therapy 7 to 14 days, which includes a PPI plus two antibiotics[9]. Medicinal plants play a significant role as therapeutics aids in health system in all over the world. Herbal drugs have been used to cure several diseases from ancient period to modern era[1]. Herbal medicine is the oldest form of healthcare known to mankind. It was integral part of the development of modern civilization. Many drugs commonly used today are of herbal origin[2].Scientists and medical professionals have shown increased interest in the field as they recognized the true health benefits of these remedies. "Let food be your medicine and Let medicine be your food" was advised by father of medicine, Hippocrates, over the millennia ago[3]. Fenugreek, *Trigonella foenum-graecum* L. is an annual herb grown in various countries around the world. It was thought to be indigenous to the countries bordering on the eastern shores of the Mediterranean, but now is widely cultivated in India, China, northern and eastern Africa, and parts of Europe and Argentina. In addition, Fenugreek was reported to have gastro protective effect, anticancer effect, used in treatment of arthritis, antimicrobial activities, reducing weight, increasing milk production and may regulate hyperthyroidism. In the view from the literature review the present study is took to evaluate the antiulcer activity of *Trigonella foenum-graecum* L. plant[10,11].

II. MATERIALS AND METHODS

The whole plant of *Trigonella foenum-graecum* L was collected from chintamani, Karanataka (India). The plant was authenticated by a pharmacognosist Dr. Jagadeesh Singh M.Pharm., Ph.D, Department of Pharmacognosy, East Point College of pharmacy, Bidarahalli, Bangalore-49. Ref.No.EPCP/M.Pharm-std/193/2017-18.

Animal

Male wistar albino rats (150-200g) and swiss albno mice (25-30g) were used. They were obtained from the animal house of East Point College of pharmacy and experiments were carried out in accordance with CPCEA guide lines. The study was approved by Institutional Animal Ethics Committee.

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Hydro-alcoholic Extraction

Whole plants were collected and air dried under the shades and powdered. The coarse powder was given to GREEN CHEM, in order to carry out the successive soxhlet extraction by using hydro-alcoholic solvent and stored in desiccators and the HATFG was subjected for preliminary phytochemical analysis.

Acute Toxicity Study (LD_{50})

Acute toxicity studiers were carried out to study acute toxic effects of the drug and to determine minimum lethal dose of the drug extracts. The doses were selected according to the OECD guide lines. Female Albino mice of weighing 20-30gms were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory condition for one week prior to start of dosing. Hydro-alcoholic extract of *Trigonella foenum*-graecum L are dissolved in suitable solvents to prepare a dose of 2000mg/kg. The doses were selected according to the OECD guidelines. The procedure was divided in to two phases. Phase I (observation made on day one) and phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female mice (each set of 3 mice) were used for experiment. First set of animals were divided into three groups, each of three in a group. Animal were fasted for overnight with water *ad libitum*. Animals received a single dose of 2000mg/kg was selected for the test. After administration of the extract, food was withheld for 1-2 hours. All animals were observed for clinical symptoms during the first 30minutes and then at approximately 4 hours after administration of the extract on day 0 and once daily during 1-14 days. The same procedure was repeated with another set of animals to nullify the errors.

Pyloric Ligation Model[13,14]

Albino Wister rats of either sex weighing between (150-200gms) are divided into five groups of animal. Each group contain six (n=6) animal. Albino rats were fasted for 48 hours in individual cages. In this model, Group-I of rats are served as normal distilled water (as a negative control) and Group-II of rats are served distilled water and are pyloric liagted (as positive control). Group-III receives Famotidine (40mg/kg) as reference drug, whereas Group-IV and V animals received hydro alcoholic extract of *Trigonella foenum-graecum* L (200 and 400 mg/kg, respectively) daily for 3 days. Animals were given water *ad libitum*. Pyloric ligation was applied by ligating the pyloric end of the stomach of the rat on the 3rd day under ketamine anaesthesia a dose of 72mg/kg, after 30min of hydroalcoholic extract or famotidine treatment. Animals were allowed to recover and stabilize in individual cage and were deprived of water during the post-operative period, after 4hrs of surgery. Rats were sacrificed with over dose of chloroform, stomach was removed and gastric juice was collected for performing gastric secretion study and ulcer scoring was done in stomach as described in the method of Suzuki et al. The collected gastric juice was centrifuged at 1000rpm for 10mins and the volume is noted. The pH of the gastric juice is recorded by pH meter. Then the collected gastric juice is placed for estimation of pH, free acidity, total acidity and total protein. Stomachs were washed with running water to see for ulcers in the glandular portion of the stomach. Open the stomach along with the greater curvature, clean properly with the running water and found out the ulcer scores with the help of the magnifying glass. Histopathological studies were conducted by fixing stomach tissues in 10% formalin for 24hrs. The formalin fixed specimens are embedded in paraffin and section (3-5cm) and stained with haematoxylin an eosin dye. The histochemical sections are evaluated by light microscopy. Ref Table 1:

TABLE 1. Ulcer Scores for Ulcer Index

Stomach Colour	Ulcer Scores
Normal Colour	0
Red Colour	0.5
Red Spot	1
Hemorrhagic Streaks	2
3>5 ulcer	2.5
5 > ulcer	3

Calculation:
Ulcer Index was calculated as;
$UI=UN+US+UPx10^{-1}$
Where, UI = Ulcer Index
UN = Average number of ulcer per animal
US = Average of severity score,
UP = Percentage of animals with ulcer

Swimming Stress Induced Ulcer[15]

Stress ulcers were introduced by forcing swimming in the glass cylinder (height 45 cm, diameter 35 cm) containing water to the height of 35 cm maintained at 35^0C for 3 hours. Animals were fasted for 24 hours prior to the experiment and divided into 5 groups, 6 animals in each group. Group-I rats received distilled water as negative control, Group-II rats received distilled water as positive control were exposed to swimming stress, Group-III received famotidine (40mg/kg) as reference drug, whereas Group-IV and V received HATFG according to 200mg/kg, 400mg/kg, as test drug. After 30mins of the treatment of drug the treated animals are allowed to swim for 3 hours. After 3 hours the animals were sacrificed and the stomachs were removed. All the stomachs were opened along with the greater curvature, clean properly with running water and ulcer scores were identified by magnifying glass.

Aspirin Induced Ulcer Model[12]

Albino rats of either sex weighing between 150-200gms. The animals were fasted for 24 hours and divided into five groups, each group contain six animals. Group I of rats received distilled water as negative control, Group II of rats received Aspirin (200mg/kg) along with distilled water served as positive control, Group III of rats received Famotidine (40mg/kg) and Aspirin (200mg/kg) as reference drug, and Group IV and V received Aspirin (200mg/kg) and HATFG (200mg/kg, 400mg/kg) will be test drug. Then after 30 minutes aspirin at dose of 200mg/kg was given orally to all the animals. 4 hours later the rats were sacrificed by using the chloroform and their stomach dissected and they were opened along with the greater curvature for the determination of

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gastric lesions. The stomachs were washed off either running water. Ulcer score was identified by magnifying glass.

III. RESULTS

Phytochemical Analysis

On phytochemical analysis of HATFG, the extract has shown the presence of carbohydrates, glycosides, phytosterols, phenolic compounds, tannins and saponins.

Acute Toxicity Studies

The studies were conducted with the hydro-alcoholic extract of *Trigonella foenum-graecum* L of different dosages, belonging to the family Fabaceae were as per OECD guideline (OECD 420). The mortality was not observed till the dose of 2000 mg/kg of body of animal. *Trigonella foenum*-graecum L doesn't show any toxic effect. The dose was selected at a dose of 200 mg/kg, 400 mg/kg.

Pyloric Ligation Model

Gastric pylorus ligation in rats produced a characteristic gastric lesion. The pre-treatment with HATFG has reduced the gastric lesions compared with control. More activity was shown by 400mg/kg body weight compared with other extract (200 mg/kg). The ulcer index in control group was 14.2. Whereas the standard famotidine shown the reduction of ulcer index 2.2. The ulcer index of treated groups like 200mg/kg, 400mg/kg, of *Trigonella foenum-graecum* L were 4.5, 2.8 respectively. The table shows the values with SEM and the figure shows the graph on treatment dependent manner. Ref Table 2:

TABLE 2. Effect of HATFG on Ulcer Index in Pylorus Ligation Model

Group	Treatment	Ulcer Index
Normal	Distilled water (vehicle)	-
Control	Vehicle + Pylorus Ligation	14.2 ± 1.2
Famotidine	40mg/kg body weight + Pylorus Ligation	2.2 ± 0.5*
HATFG	200mg/kg body weight + Pylorus Ligation	4.5 ± 0.5
HATFG	400mg/kg body weight + Pylorus Ligation	2.8 ± 0.4*

Values expressed as mean ±SEM, n=6, ANOVA followed by Dunnett's multiple comparison test. Significant ***P<0.001, **P<0.01, *P<0.05 when compared with control.

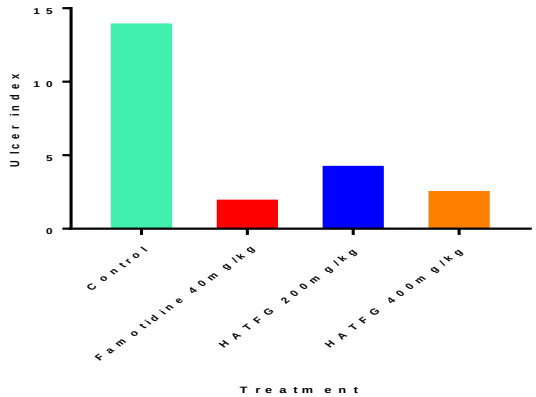
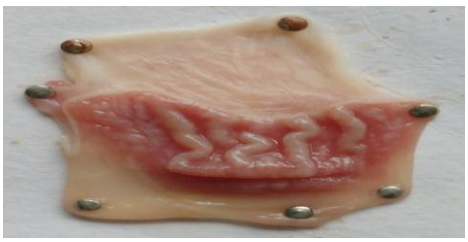
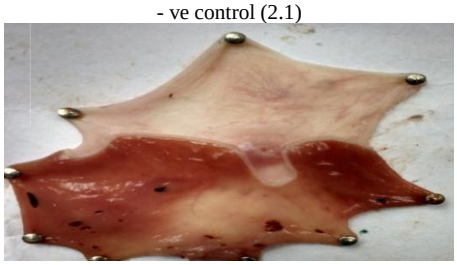


Figure 1. Effect of Different Concentration of HATFG on Ulcer Index

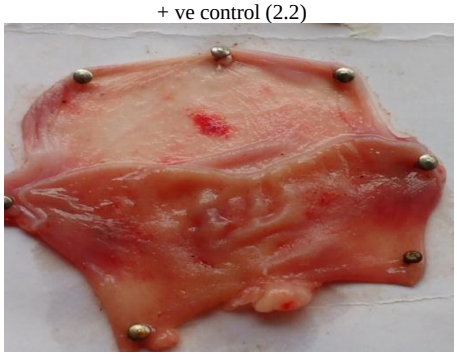
The graph was plotted for treatment verses ulcer index ±SEM



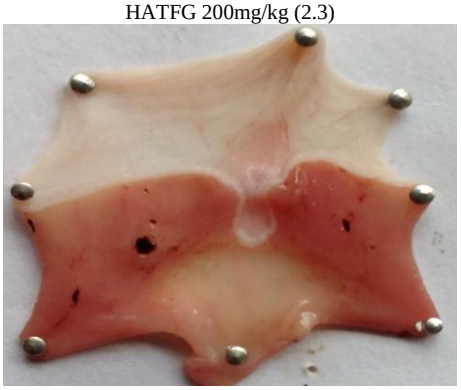
- ve control (2.1)



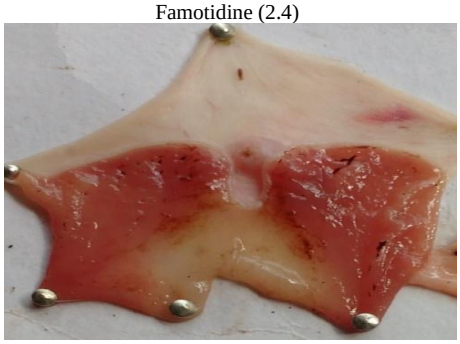
+ ve control (2.2)



HATFG 200mg/kg (2.3)



Famotidine (2.4)



HATFG 400mg/kg (2.5)

Figure 2.1, 2.2, 2.3, 2.4, 2.5. Effect of different concentration of *Trigonella foenum-graecum* L on Ulcer Index in Pylorus Ligation Induced Ulcers in rat's stomach

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TABLE 3. Effects of different dosage of HATFG on ulcer index in Aspirin Induced ulcer model

Group	Treatment	Ulcer Index
Normal	Distilled water (vehicle)	-
Control	Aspirin 200mg/kg + distilled water	13.73 ± 0.10
Famotidine	Aspirin (200 mg/kg) + Famotidine (40 mg/kg)	4.59 ± 0.01*
HATFG	Aspirin (200 mg/kg) + HATFG (200 mg/kg)	6.28± 0.01*
HATFG	Aspirin (200 mg/kg) + HATFG (400 mg/kg)	5.03 ± 0.03*

Values expressed as mean ±SEM, n=6, ANOVA followed by Dunnett's multiple comparison test. Significant ***P<0.001, **P<0.01, *P<0.05 when compared with control.

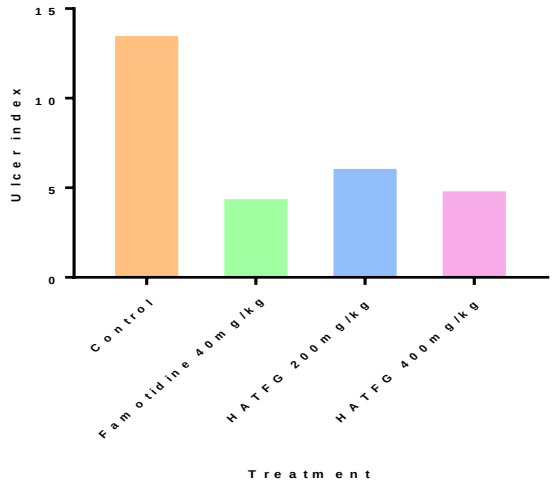
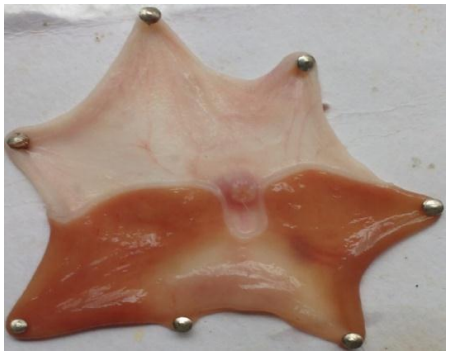
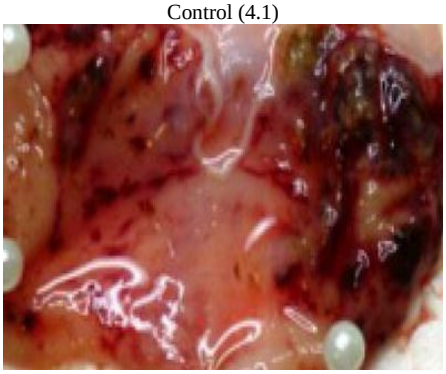


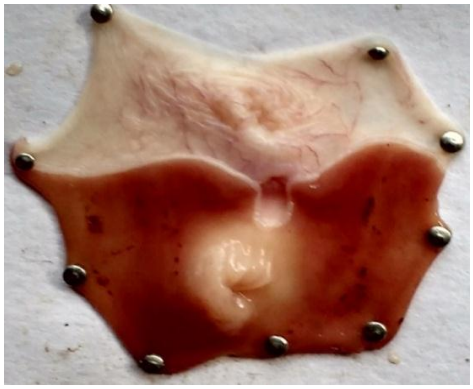
Figure 3. Effect of Different Concentration of HATFG on Ulcer index



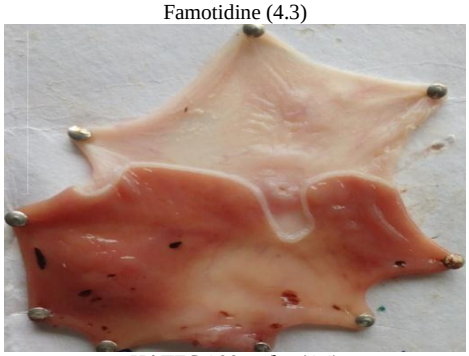
Control (4.1)



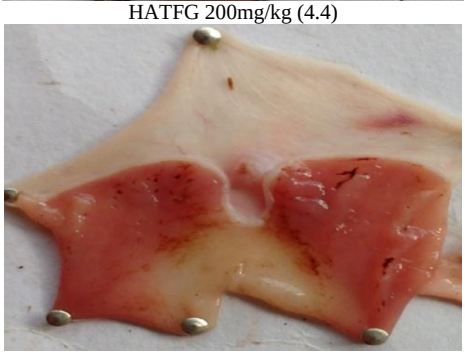
Aspirin (4.2)



Famotidine (4.3)



HATFG 200mg/kg (4.4)



HATFG 400mg (4.5)

Figure 4.1, 4.2, 4.3, 4.4, 4.5. Effect of different concentration of *Trigonella foenum-graecum* L on Ulcer Index in Aspirin Induced Ulcers in rat's stomach

TABLE 4. Effects of different dosage of HATFG on ulcer index in Swim Stress Induced Ulcer Model.

Group	Treatment	Ulcer Index
Normal	Distilled water	-
Control	Vehicle + Swimming	8.88±0.228
Famotidine	40 mg/kg + Swimming	3.77±0.09***
HATFG	200 mg/kg + Swimming	7.13±0.160***
HATFG	400 mg/kg + Swimming	5.70±0.087**

Values expressed as mean ±SEM, n=6, ANOVA followed by Dunnett's multiple comparison test. Significant ***P<0.001, **P<0.01, *P<0.05 when compared with control.

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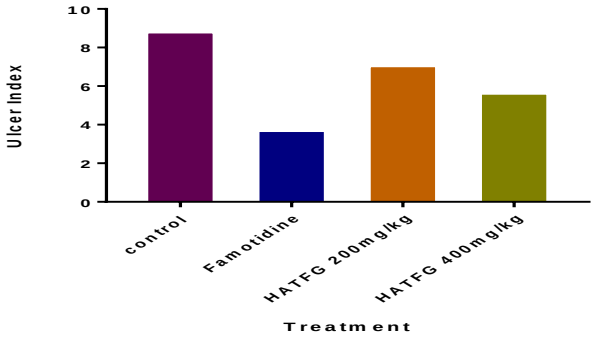
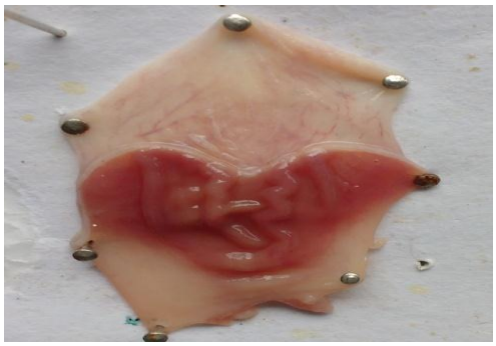


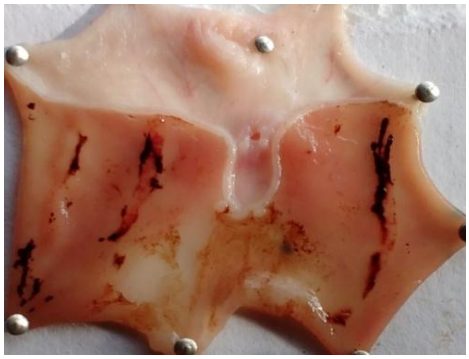
Figure 5. Effect of Different Concentration of HATFG on Ulcer Index



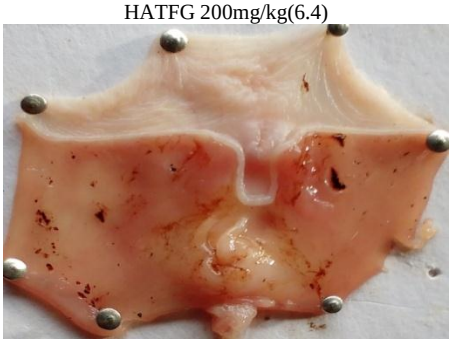
-ve control (6.1)

+ve control (6.2)

Famotidine (6.3)



HATFG 200mg/kg(6.4)



HATFG 400mg/kg (6.5)

Figure 6.1, 6.2, 6.3, 6.4, 6.5. The Effect of HATFG on Ulcer Index in swimming test induced ulcer

IV. DISCUSSION

Antiulcer activities were performed on albino wister rats of either sex using Pylorus ligation, Swimming stress and Aspirin induced ulcer models. The HATFG (200mg and 400mg/kg) showed significant antiulcer activity in all the three models. In Pylorus Ligation Induced Ulcer model, the ulcer is produced by excess secretion of acids and lowest pH. An increase in acid pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion is the known mechanism of the induction of ulcer. The pylorus ligation study is the most important model to screen the antiulcer activity for evaluation of efficiency of extract. The antiulcer activities of HATFG in different concentration in pylorus ligation have shown a remarkable reduction in excess secretion of acids, pH, total acidity, free acidity, gastric volume and ulcer index. Aspirin Induced model is produced ulcer due to the COX-1 blockage as a results the decrease of prostaglandin synthesis. So there will be an enhancement in the lipoxygenase pathway and leukotriens. The leukotriens will cause ulcer in aspirin model. An increase in acid secretion and back diffusion of H+ ion is reasonable for the gastric mucosal lesion induced by aspirin. In the control group of aspirin treatment has shown severe ulcer scores by ulcer index. The different concentration of *Trigonella foenun-graecum* L such as 200 mg/kg, 400 mg/kg were shown a significant reduction in ulcer index in compared to the control and famotidine as standard. Swimming stress induced model is one of the models to produce ulcer by stress. It is probably mediated by histamine release by enhancement in acid secretion and a reduction in mucin production. The enhanced motility of stomach by swimming is also a reason for inducing ulcer. The evidently increased lipid peroxides and mucosal damages cause the increase of ulcer scores in the

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control group. In the present study, the 400mg/kg body weight were shown more active compared with the other concentrations. The reduction of ulcer score by the different concentrations of extracts of *Trigonella foenun-graecum* L was significant in stress induced model. The present study reveals that the hydro-alcoholic extract of *Trigonella foenun-graecum* L plant having the antiulcer activity in experimentally induced ulcer models in rats.

V. CONCLUSION

From present study of different concentration of extracts of *Trigonella foenum-graecum* L for antiulcer activities was concluded with a positive response in ulcer induced models such as pylorus ligation, aspirin induced and swimming stress induced in compared with standard drug famotidine. The remarkable activity was shown at a dose 400 mg/kg body weight. The ulcer scores are reduces while the dose of hydro-alcoholic extract of *Trigonell foenum-graecum* L is increased. In acute toxicity studies the extracts were shown safety up to 2000 mg/kg body weight. The hydro-alcoholic extracts at a dose 400 mg/kg of *Trigonella foenum-graecum* L has better antiulcer activities than the other dose like 200 mg/kg. Pretreatment with hydro-alcoholic extracts of *Trigonella foenum-graecum* L decreases the ulcer index, total acidity, free acidity, gastric volume and pH when compared with the control. The possible mechanism for all the protective action brought by the HATFG may be due to increase in mucous secretion, reduction in gastric acid secretion which minimizes the activity of pepsin and prevents the auto-digestion of mucosal barrier.

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