Int J Pharm Bio Sci 2012 Oct; 3(4): (P) 638 - 644

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ULCER PROTECTIVE ACTIVITY OF *GUNMATHI CHOORANAM* ON PYLORIC LIGATED METHOD

S. ARULSORUBI,*[1] P.PARTHIBAN[1], K. KANAGAVALLI[1], J. ANBU[2] AND ASHWINI ANJANA[2]

[1]Department of Medicine, Govt. Siddha Medical College, Arumbakkam, Chennai-600106, India
[2]Department of Pharmacology, School of Pharmaceutical Sciences, (VISTAS)
Vels University, Pallavaram, Chennai-600117 India

ABSTRACT

The present investigation was undertaken is to explore the antiulcer activity of the *Gunmathi Chooranam* in rats by pyloric ligation method. The experimental parameters used were ulcer index, gastric pH parameters. The animals groups were divided in five controls, standard (Ranitidine 100 mg/kg) and *Gunmathi Chooranam* in 125, 250 and 500 mg/kg. The siddha formulation produced the significant action against the antiulcer group's animal in all parameters. The *Gunmathi Chooranam* was found to be good antiulcer drug in siddha system.

KEYWORDS: ***Gunmathi Chooranam*, Pyloric ligation, Ranitidine, Antiulcer, Siddha System**

S. ARULSORUBI
Department of Medicine, Govt. Siddha Medical College, Arumbakkam, Chennai-600106, India

Corresponding author

INTRODUCTION

A localized loss of gastric as well as duodenal mucosa leads to the formation of peptic ulcer. It arises when the normal mucosal defensive factors such as mucus, mucosal blood flow, formation of HCO_3^- and PGE2 are impaired or over powered. Also by the aggressive factors includes acid, pepsin, NSAIDS and helicobacter pylori. A number of drugs are available for the treatment of peptic ulcer but its clinical evaluation shows the incidence of relapses, side effects and drug interactions. [1] Since it has well established that the nonsteroidal anti-inflammatory agents are potentially ulcerogenic, because of the fact that these drugs inhibit the synthesis of cytoprotective prostaglandins PGE2 and PGI2 and might also cause overproduction of leukotrienes (as NSAIDs block the cyclooxygenase pathway, arachidonic acid metabolism is shifted to the lipooxygenase pathway) which in turn might be damaging to the gastric mucosa. Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, funaecomastia and hematopoietic changes) of modern medicine. [2] Indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer.

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes the high rate of morbidity particularly in the population of non-industrialized countries.[3] Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors.[4] Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage.[5] Hence,herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors.[6, 7] Though the *Gunmathi Chooranam* have been used in the treatment of gastrointestinal disorders in the traditional siddha system of medicine, there are no scientific evidences available on its anti-ulcer potential. Hence, the present work was undertaken to investigate the anti-ulcer activity of *Gunmathi Chooranam* in experimental animals.

MATERIALS AND METHODS

(i) Drug and Preparation of Stock solution

The *Gunmathi Chooranam* was collected from the Tamilnadu India. The aqueous suspension of *Gunmathi Chooranam* was prepared in 0.5 % carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals. It was used within seven days and stored at 8^{o}C while for further use, freshly prepared solution was used. The vehicle alone served as control. All the drugs and chemicals were of analytical grade. Ranitidine (Osaka), Ethanol (Research lab) were used.

(ii) Experimental animals

Albino rats (Wistar strain) of eithersex, weighing 180-200g and Swiss albino mice (20-22g) of either sex were procured from animal housing facility, School of pharmaceutical Sciences, Vels university, Chennai. All the animals were placed in polypropylene cages in a controlled room temperature 22±1°C and relative humidity of 60-70% in animal house. The animals were maintained on standard pellet diet (Sai meera foods Pvt limited, Bangalore, India) and water *ad libitum*. They were acclimatized to laboratory condition for seven days before commencement of the experiment. Ethical clearance was

obtained from Institutional Animal Ethical Committee.

(iii) Acute oral toxicity study

Acute oral toxicity study of *Gunmathi Chooranam* was carried out in Swiss albino mice of both sexes (20-22 g) according to OECD guidelines no 423. *Gunmathi Chooranam* at different doses up to 2000mg/kg, p.o. was administered and animals were observed for behavioral changes, any toxicity and mortality up to 48 h.[8]

(iv)Anti ulcer activity

Pyloric ligation induced gastric ulceration

Albino rats of either sex were divided into five groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Group I: treated with vehicle alone (2ml/kg, p.o.) and was kept as control. Group II: treated with Ranitidine (100mg/kg, p.o.) and was kept as standard. Group III: treated with the *Gunmathi Chooranam* (125mg/kg, p.o.). Group IV: treated with the *Gunmathi Chooranam* (250mg/kg, p.o.). Group V: treated with the *Gunmathi Chooranam* (500mg/kg, p.o.). After 1h of drug treatment, they were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al.[9] avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anesthetics ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. The inner surface of free stomach was examined for gastric lesions.

(v) Determination of pH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter. Determination of total acidity
an aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as meq./l by the following formula:
n X 0.01 X 36.45 X 1000
Where n is volume of NaOH consumed, 36.45 is molecular weight of NaOH, 0.01 is normality of NaOH, 1000 is the factor (to be represented in litre).[10]

(vi) Determination of free acidity

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.[10]

(vii) Macroscopic evaluation of stomach

The stomach was opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a X5 magnifier lens to assess the formation of ulcers. The number of ulcers were counted. Ulcer scoring was undertaken according to Vogel et al.[11]

Ulcer index was measured by using following formula. $UI=UN+US+UPX10^{-1}$

UI= Ulcer Index; UN= Average number of ulcers per animal; US =Average number of severity score; UP=percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test})}{\text{Ulcer index Control}} \times 100$$

Ulcer score	Descriptive /observation
0	Normal coloured stomach
0.5	Red colouration
1.0	Spot ulcers
1.5	Hemorrhagic streak
2.0	Ulcers
3.0	Perforation

(viii) Statistical analysis

The results are expressed as the mean± SEM for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnet's t-test. Results were considered to be statistically significant at $P<0.05$.

RESULTS

(i) Acute oral toxicity study

Swiss albino mice of both the sexes treated with *Gunmathi Chooranam* did not show any behavioral changes, toxic reaction or mortality. It was found to be safe at the dose of 2000mg/kg. LD_{50} of the *Gunmathi Chooranam* was found to be >2000mg/kg.

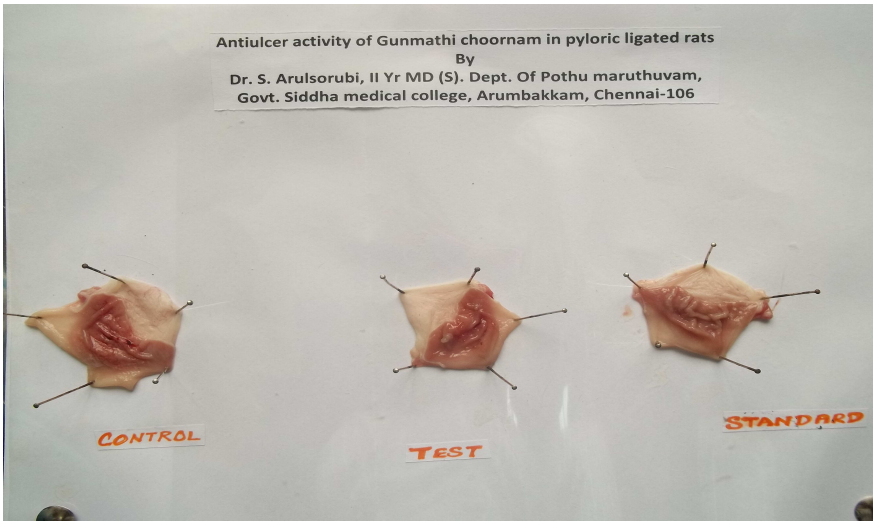
(ii) Pyloric ligation induced gastric ulceration

Effect of *Gunmathi Chooranam* on pyloric ligation induced ulceration is shown in table-1. The pyloric ligation has caused the accumulation of gastric secretions with pH 3.88±0.17 in a control group. The total acidity and free acidity of the gastric secretions were found to be 172±2.28 and 115.11±0.48meq./l respectively. Pretreatment with the *Gunmathi Chooranam*, significantly ($P<0.01$) reduced the volume of gastric secretions 3.00±0.03, 3.51±0.03 and 3.33±0.03ml at the doses of 125, 250 and 500mg/kg respectively. pH of the gastric fluid was significantly ($P<0.05$) elevated up to 4.52±0.46 only at higher dose of the test drug. In addition, total acidity and free acidity were also reduced significantly ($P<0.01$) in a dose dependant manner. Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with *Gunmathi Chooranam* has reduced them significantly ($P<0.01$) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 52, 64 and 66 at 125, 250 and 500mg/kg respectively. The gastro protection offered by the test drug was comparable to that of the standard drug, ranitidine (100mg/kg).

Table 1
Effect of Gunmathi Chooranam on total and free acidity, gastric volume and ulcer index

Groups	Total acidity (mEq/l)	Free acidity (mEq/l)	Gastric Volume (ml/100g)	Ulcer index (% Ulcer protection)
CMC control	172±2.28	115.11±0.48	2.9±0.05	1.15±0.02
Control (Pyloric ligated)	251±2.84	173.23±0.52	4.76±0.04	5.00±0.05
Gunmathi Chooranam (125 mg/kg)	176±2.12**	120.12±0.74**	3.00±0.03**	2.37±0.06** (52.60)
Gunmathi Chooranam (250mg/kg)	185±3.07**	148.16±0.54**	3.51±0.03**	1.79±0.05** (64.20)
Gunmathi Chooranam (500mg/kg)	197±2.88**	132.10±0.77**	3.33±0.03**	1.68±0.05**(66.40)
Ranitidine (100mg/kg)	162±2.19**	123.08±0.53**	2.68±0.07**	1.25±0.05** (75.00)

P values <0.05 as compared to ligation control; Values are the mean ± S.E.M. of six rats /treatment. Significance
p <0.05, **p<0.01 Vs Control



DISCUSSION

Although in most of the cases the etiology of the ulcers is unknown, it is generally accepted that they are a result of an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defensive mechanisms.[12] Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Aspirin, phenylbutazone, indomethacin and some non-steroidal anti-inflammatory drugs are also known to cause duodenal and gastric ulceration. Prostaglandin E_2 and I_2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant - like phospholipids secretion in the gastric epithelial cells is also stimulated by the prostaglandin. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid. The antiulcer property of *Gunmathi Chooranam* in pylorus ligation model is evident from its significant reduction in

free acidity, total acidity, number of ulcers and ulcer index. *Gunmathi Chooranam* treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that *Gunmathi Chooranam* can suppress gastric damage induced by aggressive factors. It is suggested that, the active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen.[13, 14, 15] So the antiulcer activity of *Gunmathi Chooranam* may be attributed to its active principle.

CONCLUSION

The results obtained in the experimental model of pylorus ligation induced ulceration method in rats. The *Gunmathi Chooranam* was found to posses remarkable ulcer protection of 68.2% at 500mg/Kg and standard drug at 68.4%. Pylorus ligation consistently caused hemorrhagic lesions in the mucosa of the glandular stomach, indicating true ulcer formation as stated in histological findings. Pretreatment of rats with *Gunmathi Chooranam* prevented gastric ulcerogenesis significantly. But it is seemed to be less efficient than standard drug. The result of the present study substantiates the traditional claim that the *Gunmathi Chooranam* possesses antiulcer activity. The results of the present study suggest that the *Gunmathi Chooranam* may be beneficial in the treatment of gastric lesions. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

REFERENCES

1. Srinivas K and Celestin Baboo RV, Antiulcer activity of *Ixora Pavetta.* International Journal of Current Pharmaceutical Research, 3, 3, (2011).
2. Prakash Patil, Prasad K, Nitin M, Vijay Kumar M and Sreenivas Rao K. Evaluation of Anti-ulcer and Anti-secretary properties of the *Calotropis procera* root extract. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2 (3); 35, (2011).
3. Falk GW. Disease of the stomach and duodenum. In: Andreoli The, editor. Cecil essentials of medicine. 5th Edn. Edinburgh: WB Saunders Company; 334-343 (2001).
4. Tripathi KD, Gastrointestinal Drugs: Drugs for peptic ulcers. In: Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, 628-642, (1999).
5. Ariyphisi I, Toshiharu A, Sugimura F, Abe M, Matsuo Y and Honda T. Recurrence during maintenance therapy with histamine H2 receptors antagonist in cases of gastric ulcers. Nikon University Journal Medical, 28: 69-74, (1986).
6. Sairam K, Rao Ch V and Goel RK. Effect of *Centella asiatica* linn on physical and chemical factors induced gastric ulceration and secretion. Indian J Exp Biol. 39: 137-142, (2001).
7. Sairam K, Rao Ch V and Goel RK. Effect of *Convolvulus pluricaulis Chois* on gastric ulceration and secretion in rats. Indian J Exp Biol. 39: 350-354, (2001).
8. Guidance document on acute oral toxicity testing" Series on testing and assessment No. 423, 1996. Organisation for economic co-operation and development, OECD Environment, health and safety publications, Paris.
9. Shay H, Komarov SA, Fels SS, Meranze D, Grunstein M and Siplet H. A Simple method for uniform production of gastric ulcers in rats. Gastroenterol 5: 43-51, (1945).
10. Trease GE and Evans WC. Text Book of Pharmacognosy, 13th Edn, 202-205, (1992).

11. Vogel HG and Vogel WH. Activity on the gastrointestinal tract In: Drug discovery and evaluation (Pharmacological assays). Berlin: Springer Verlag Company, 486-487, (1997).
12. Aguwa CN and Mittal GC. Study of antiulcer activity of aqueous extract of leaves of Pyrenacantha standtii (Family I cacinaceae) using various models of experimental gastric ulcer in rats. Eur J Pharmacol. 74: 215-219 (1981).
13. Akah PA, Orisakwe OE, Gamaniel KS and Shittu A. Evaluation of Nigerian traditional medicines: II. Effects of some Nigerian folk remedies on peptic ulcer. J Ethnopharmacol. 62 :123-127 (1998).
14. Gurbaz I, Ustan O, Yesilada E, Sezik E and Kutsal O. Anti-ulcerogenic activity of some plants used as folk remedy in Turkey. J Ethnopharmacol. 88: 93-97 (2003).
15. Sairam K, Priambada S, Aryya NC and Goel RK. Gastroduodenal ulcer protective activity of Asparagus racemosus: an experimental, biochemical and histological study. J Ethnopharmacol. 86: 1-10, (2003).