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Research Article

Anti-Ulcer Activity of *Hingu Chooranam* against Aspirin and Pylorus Ligation Induced Gastric Ulcer in Rats

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ABSTRACT

In this modern era, gastrointestinal disorders are the universal problem. Peptic ulcer is one of the major diseases affecting the human population. It develops due to the imbalance between aggressive factors like acid, pepsin, *H. pylori* and bile salts and defensive factors like mucous, bicarbonate, blood flow, epithelial cell restoration and prostaglandins. The anti-ulcer activity of *Hingu Chooranam* (HC) was evaluated by using the experimental models of acute gastric lesions induced by aspirin and pylorus ligation in rats. Animals pre-treated with doses of 100 mg/kg and 200 mg/kg of HC were statistically analyzed and compared to the standard and control group with the parameters like volume of gastric secretion, PH, free acidity, total acidity and ulcer index. The results suggested that the HC significantly decreased the volume of gastric acid secretion, free acidity, total acidity and ulcer index in comparison with standard drug Ranitidine. HC shown significant reduction in lesion index, total affected area and percentage of lesion in comparison with control group in aspirin induced ulcer in experimental models. The gastric mucosal protective effect of HC is brought by inhibiting the gastric secretion, which shows it may act like a proton pump inhibitor. Further, the anti ulcer activity of HC which reduced gastric volume and total acidity in pylorus ligation ulcer model reveals that HC may act as a H2 receptor antagonist. Thus the present study indicates that HC has anti-ulcerogenic potency in aspirin induced and pylorus ligation induced ulcers in rats.

Keywords: Anti –ulcer, aspirin, *hingu chooranam*, peptic ulcer, pylorus ligation.

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INTRODUCTION

Peptic ulcer is also known as Acid peptic disease (APD), an ulceration of the mucous membrane of the stomach, duodenum or esophagus. An ulcer is a sore or erosion that forms when the lining of the digestive system is corroded by acidic digestive juices and thus extremely painful [1]. It is produced by an imbalance between gastro duodenal mucosal defense mechanisms and the aggressive factors, particularly gastric acid and pepsin [2].

In this modern world gastrointestinal disorders are the universal problem. Nowadays people are subjected to increase in stress due to the modern life style and they often consume fast foods. These factors lead to many kinds of gastro intestinal disorders. About 10% of the population may develop peptic ulcer in their life time [3]. It affects 9.5% among women and 10.5% among men. Duodenal ulcer is most frequent in the individuals of age group 30 to 55 years. In the general population 20-50% is infected with H. pylori, its prevalence increases with age and 15-20% of the infected individuals will develop peptic ulcer [4].

Various anti ulcer drugs are available in the market such as H_2 receptor antagonist, Proton pump inhibitors, 5-HT4 receptor agonist, cytoprotectant, healing agents etc. The adverse effects of these drugs are cardiac arrhythmias, blood dyscrasias, hypertension, central nervous system and gastro intestinal disturbances, nephritis, impairment of sexual drive, hepatitis,

pancreatitis, increased liver enzyme activity and triglycerides, leucocytopenia and thrombocytopenia, pharyngitis, pruritis and electrolyte imbalance [5]. So, there is a necessity to discover a potent and safe anti ulcer drug with less or no side effect.

In this present scenario, Siddha system of medicine plays a vital role in the treatment of many diseases. Siddha system of medicine is an ancient medical system of Dravidian origin. It flourished and being practiced in Tamil Nadu from time immemorial. It emphasizes the treatment of both body and soul by balancing the principal humours. It is based upon writings of *Siddhars* who are eminent scholars in all fields of medicine and attained spiritual enlightenment.

Siddha medicine gives prime importance to herbal based formulations. In Siddha system of medicine, *Chooranam* is one among the thirty two types of internal medicines used. *Chooranam* is a kind of medicine in which all the raw drugs are purified and powdered well.

The present study involves *Hingu Chooranam* (HC), one of the herbo- mineral formulations, is a compound drug containing eight ingredients. This medicine is traditionally used for ailments of upper gastro intestinal tract, especially for heart burn, gastric regurgitation, bloating and burps. So far no scientific studies were carried out to evaluate its medicinal values. Therefore, an attempt had been made to validate its traditional claim for its anti ulcer properties by using the models of acute gastric lesions induced by aspirin induced and pylorus ligation induced in rats.

MATERIALS AND METHODS

Preparation of *Hingu Chooranam* (HC)

The preparation of HC was based on the Siddha classical literature *'Sarabenthirar Vaidhyamuraigal – Gunmaroga Sigichai'* [6].

Collection and authentication of the test drug:

The ingredients of HC are *Indhuppu* (Sodium chloride impura – **Fig. 1A**), *Perunkayam* (*Ferula asafoetida* – **Fig. 1B**), *Chukku* (*Zingiber officinale* – **Fig. 1C**) *Seeragam* (*Cuminum cyminum* –**Fig. 1D**), *Kodiveli* (*Plumbago zeylanica* **Fig. 1E**) *Koshtam* (*Costus speciosus* **Fig. 1*F***), *Vasambu* (*Acorus calamus* **Fig. 1G**), *Kadukkai* (*Terminalia chebula* **Fig. 1H**). All these raw materials were collected from country drug shop at Chennai. They were identified and authenticated by *Gunapadam* (Pharmacology) experts and botanist of Government Siddha Medical College, Chennai-106. The specimen samples have been preserved in PG Department of Gunapadam for future reference.

Purification of raw drug

These ingredients were purified by classical methods, as mentioned in Siddha literatures [7-9]. The dry rhizomes of *Zingiber officinale* was purified in the following manner. The outer skin was peeled off and it is dried in sunlight. The fruits of *Cuminum cyminum* was dried in sunlight for six hours and mildly fried. *Ferula asafoetida* was fried till the water content gets completely evaporated. The rhizomes of *Acorus calamus* was burnt in fire and charred. Sodium chloride impura was purified by soaking *in Kaadi* (Vinegar) and then dried in sunlight. The inner root of *Plumbago zeylanica* was removed and the root bark was powdered well. The powdered drug was placed in a cloth and tied in the mouth of a pot filled with milk. Then, the pot was closed with suitable lid and boiled for three hours. Then it is allowed to dry completely and ground well.

The above drugs were powdered and mixed well until the homogenous mixture was obtained. In order to get the finest physical form, the powdered material was sieved through a white cotton cloth *(Vashthirakayam)*.

Then the *Chooranam* was moistened with cow's milk. The pot was half-filled with milk and water. The mouth of the pot was covered and tied with white cotton cloth. The *Chooranam* (moistened by milk) was placed above the cloth. The mouth of the pot was closed with another mud pot. The gap between the two mud pots was sealed with a wet cloth to avoid evaporation. Then, this arrangement was placed on fire and boiled until the water level gets reduced in the lower pot. Then the powder was taken, dried, powdered finely and preserved for usage.

Fig. 1: Showing the Ingredients And Final Product of *Hingu Chooranam*. A) Sodium Chloride Impure, B) *Ferula Asafetida,* C) *Zingiber Officinale,* D) *Cuminum Cyminum,* E) *Plumbago Zeylanica,* F) *Costus Speciosus,* G) *Acorus Calamus,* H) *Terminalia Chebula,* I) Trial Drug - *Hingu Chooranam*.

Pharmacological activity

Chemicals

All chemicals used in the present study were analytical grade and purchased from SD fine chemicals Ltd (Mumbai, India). Aspirin was obtained from BD Pharmaceutical Works and Ranitidine (Reference drug) was obtained from Ranbaxy Laboratories.

Stock solution preparation

The powdered form of *Hingu Chooranam* (HC) was mixed uniformly in 2% CMC solution to achieve 100 mg/ml as main stock solution and used in this study.

Animals

Wistar albino rats of either sex, weighing 150-200 g were used for the study. They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24±28^{0}C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum. Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. The animals were acclimatized for one week under laboratory conditions. The present study was approved by the Institutional Animal Ethical Committee (IAEC) obtained from Vels University, Chennai with Approval no. XIII/ VELS/ PC OL/ 45/ 2000/ CPCSEA/ IAEC/ 08.08.2012.

Acute toxicity studies

Acute oral toxicity test was carried out as per OECD Guidelines 425 [10]. Female albino rats were used for acute toxicity study. The animals were kept on overnight fasting and provided water *ad libitum*. The trial drug HC was administered in a single dose by gavage using a stomach tube. The dose of the trial drug was determined by the fasted body weight. After the drug has been administered, food was withheld for a further period of two hours. The dose administered was assigned as toxic dose if mortality was observed in two out of three animals. The animals were observed carefully for toxic symptoms for 72 hours.

ANTI-ULCER EVALUATION

Aspirin-induced gastric ulcer:

Animals were divided into four groups, with each group containing six animals each (n = 6). The first group served as a control and were administered vehicle (CMC) only, second group served as a positive control and were treated with standard drug Ranitidine (30 mg/kg) third and fourth group served as test groups and were administered HC at the dose level 100 mg/Kg (*HC 1*), 200 mg/Kg (*HC 2*) respectively. All the above drugs and vehicle were administered 30 minutes before the administration of aspirin (400mg/kg) orally [11]. After six hours, the animals were sacrificed and the stomachs were removed and 2% formalin was injected into the stomach. The stomach was opened along with greater curvature and immersed in 2% formalin solution. The length of each lesion was measured under the dissecting microscope. The sum of the length (mm) of all lesions for each rat was used in lesion index.

The ulcer score was determined by using a 10 × magnifying hand lens. The scoring of severity of ulceration was as follows: 1 mm (pin point) = 1; 1-2 mm = 2; > 2 mm = 3; > 3 mm = 4 [12]. The mean ulcer score was determined by dividing the total ulcer indices in a group by the total number of animals in that group.

Ulcer Score = Total ulcer index (UI) in a group / Total number of animals in that group [13].

Pylorus- ligation induced gastric ulcer:

Male albino rats weighing 150-200g were selected for pyloric ligation ulcer model [14]. Rats were divided into four groups, each group consisting of six animals. Animals were fasted for 24 hours. One group received normal saline 2 ml/kg (negative control), the second group received Ranitidine 30 mg/kg by oral route (positive control) and the third group received *Hingu Chooranam* (*HC 1*) at dose level of 100 mg/kg and fourth group received *Hingu Chooranam* (*HC 2*) at the dose level of 200 mg/kg by oral route, 30 min prior to pyloric ligation. 4 hours later all animals were sacrificed by decapitation and the stomach was opened to collect the gastric contents.

The total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as;

Acidity = Volume of NaOH x Normality x 100 mEq/1. 0

STATISTICAL ANALYSIS

The statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparison tests. All the results obtained in the study were compared with the vehicle control group. P values <0.05 were considered as statistically significant.

RESULTS

Acute Toxicity study

In acute toxicity study, no mortality or toxicity was observed during the experimental period. The trial drug HC was considered safe orally up to the dose level of 2000 mg/kg body weight. No major behavioural changes were noted during the study.

Aspirin-induced gastric ulcer

The results of this study were summarized in (**Table 1**) and (**Fig 2**). The

administration of two doses of HC (100 mg/kg and 200 mg/kg bodyweight) thirty minutes before the administration of aspirin produced a significant reduction ($p<0.05$) of ulcer index observed in *HC 1* group, whereas highly significant reduction of ulcer index ($p<0.01$) was noted in the higher dose treated groups (200 mg/kg body weight) as compared to the control group. The standard drug ranitidine also produced highly significant decrease in ulcer index as compared to the control ($p <0.01$). HC at the dose level of 200 mg/kg has protected the gastric mucosa against ulcerogenic effect of aspirin with dose dependent manner.

Table 1: Effect of *Hingu Chooranam* on Ulcer Index

Groups	Ulcer index
CMC control	8.36 ± 0.35
Ranitidine (30mg/kg)	2.21 ± 0.36
HC 1 (100mg/kg)	4.55 ± 0.39*
HC 2 (200mg/kg)	3.28 ± 0.38**

Results are mean ±S.E.M. (n=6) Statistical comparison was performed by using ANOVA followed by Dunnet's multiple comparison test. P<0.05, **P<0.01were considered statistically significant and highly significant when compared to control group.*

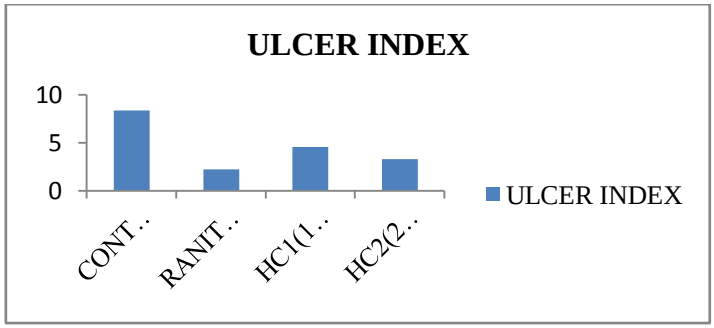

Fig. 2: Effect of *Hingu Chooranam* on Ulcer Index

Pylorus- ligation induced gastric ulcer

The result of pyloric ligation induced gastric ulcer model was summarized in (**Table 2**). In this method, HC at both doses (100 mg/kg and 200 mg/kg) produced a reduction in the ulcer score, gastric volume, free acidity, total acidity and raised gastric pH significantly in comparison with control group. Reference drug ranitidine produced significant reduction in total acid output as compared to control group. *HC 2* 200 mg/kg was found to possess remarkable ulcer protective properties and almost exhibited similar effects as that of ranitidine (30mg/kg) in reducing the gastric volume (**Fig. 3**).

Table 2: Effect of HC against Pylorus Ligation Induced Gastric Ulcer in Rats

Group	Treatment and Dose mg/Kg	Gastric volume (ml)	pH	Free acidity (mEq/l)	Total acidity (mEq/l)	Ulcer Score	% Inhibition
I	Control	6.83± 0.37	2.18± 0.34	27.58± 0.33	59.61±0.42	4.46± 0.39	--
II	Standard	2.78± 0.35	4.78±0.34	10.65±0.29	22.53±0.37	0.86±0.08	80.71***
III	*HC* 1	4.80 ±0.37	3.48± 0.35	19.56±0.32	39.48±0.34	1.73±0.34	61.21*
IV	*HC* 2	3.36± 0.30	3.81± 0.34	17.38± 0.37	32.6±0.37	1.26± 0.34	71.74**

Results are mean ±S.E.M. (n=6) Statistical comparison was performed by using ANOVA followed by Dunnet's multiple comparison test. P<0.05, **P<0.01, ***P<0.001 were considered statistically significant,highly significant and extremely significant when compared to control group.*

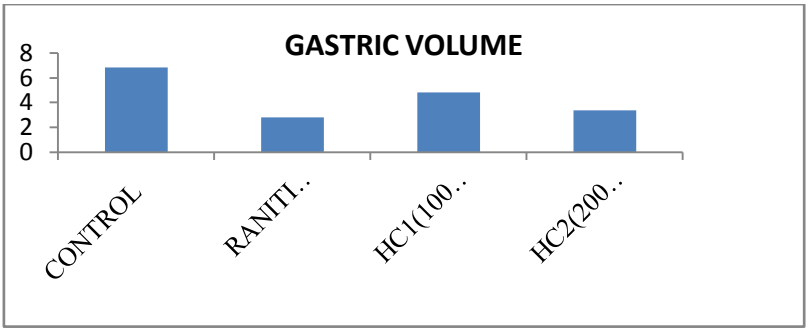

Fig. 3: Effect of Hc on Gastric Volume

Compared to control group the entire test showed elevation in pH indicating their capacity to reduce the acidity of the gastric juice. The *HC 2* indicated almost equi-potent effect as that of ranitidine (**Fig. 4**). Gastric free acidity is increased in control animals due to pylorus ligation. Higher dose of HC at 200 mg/kg decreased the gastric free acidity significantly (**Fig. 5**).

Total acidity was decreased with both doses of HC administration (**Fig. 6**) when compared to control. The HC at 200 mg/kg reduced the mean ulcer score and percentage curative ratio of *HC* at 200 mg/kg was almost comparable to that of standard drug ranitidine (**Fig. 7**).

The percentage of inhibition of ulcer was 80.71%, 61.21% and 71.74% produced by the treatment of standard drug ranitidine, trial drug *HC* at dose level 100 mg/kg and 200 mg/kg respectively (**Fig. 8**).

The stomach of rats of control group, standard, *HC 1* (100 mg/kg) and *HC 2* (200 mg/kg) on aspirin induced gastric ulcer was shown in the (**Fig. 9 A, B, C and D**) which appeared to have beneficial ulcer protective effects of standard and trial drugs.

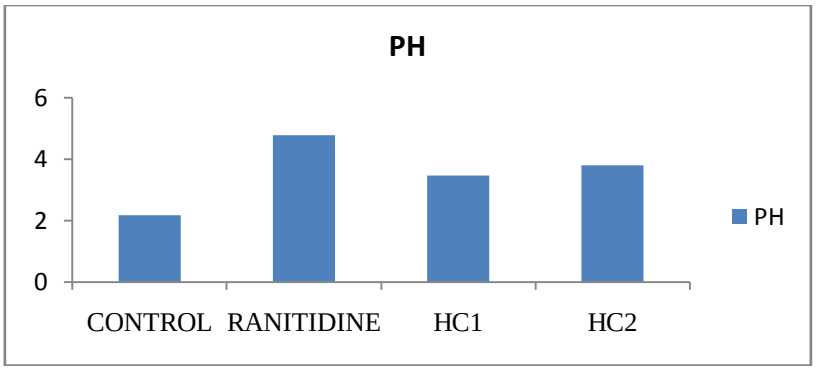

Fig. 4: Effect of HC on Ph

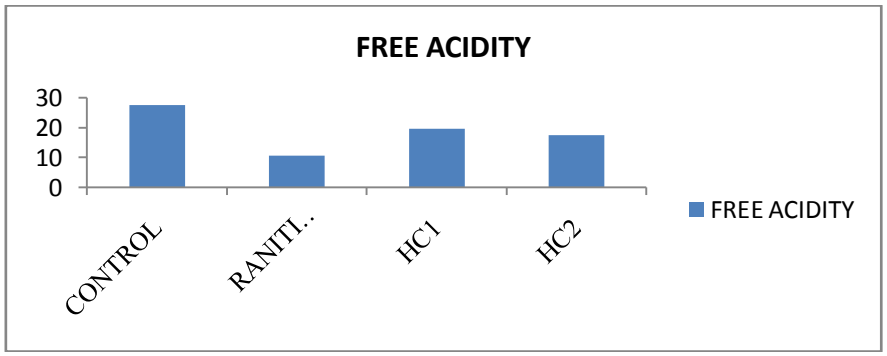

Fig. 5: Effect uf HC on Free Acidity

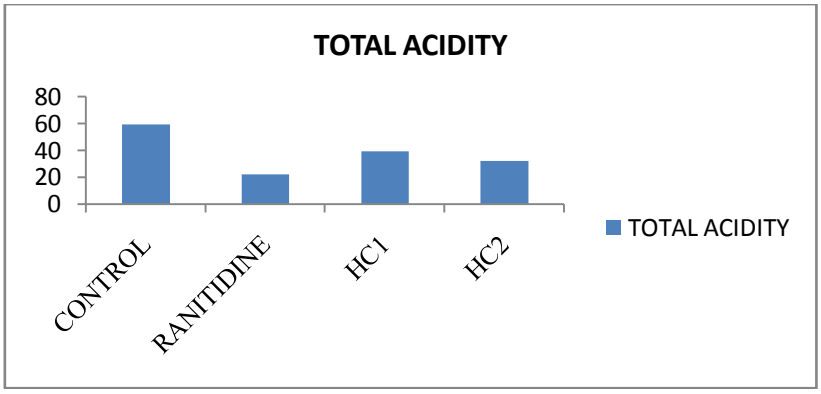

Fig. 6: Effect of *HC* on Total Acidity

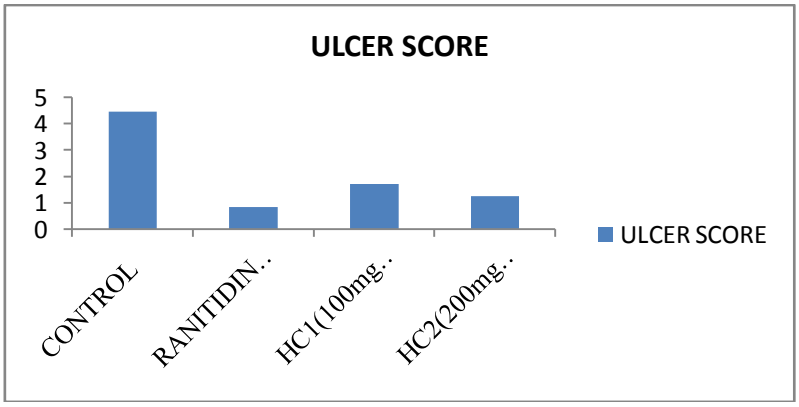

Fig. 7: Effect of Hc on Ulcer Score

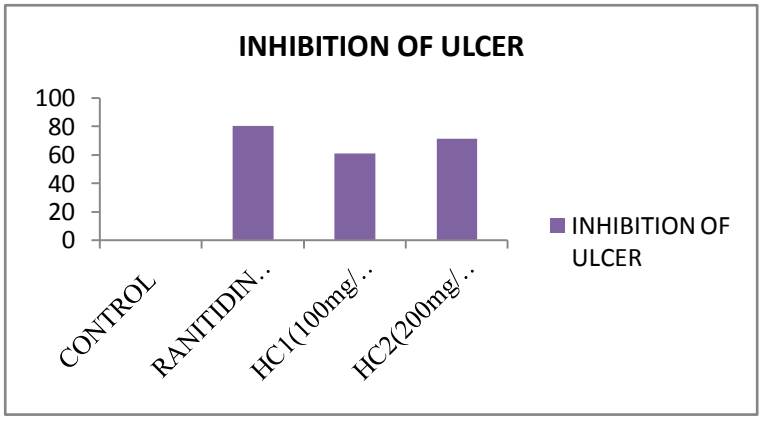

Fig. 8: Effect of HC on % Inhibition of Ulcer

DISCUSSION

Peptic ulcer is one of the major ailments effecting humans and develops because of imbalance between aggressive factors (acid, pepsin, *H. pylori*, and bile salts) and defensive factors (mucous, bicarbonate, blood flow, epithelial cell restoration and prostaglandins [15].

This study revealed that HC has a significant anti-ulcer effect on experimental animals with ulcer induced by aspirin. Aspirin inhibits two forms of cyclo-oxygenase enzymes that is cyclo-oxygenase-1 (COX-I) and cyclo-oxygenase-2 (COX-2) thus inhibiting synthesis of prostaglandins (PGs). The important role of prostaglandins (PGE2 & PGI2) is to stimulate the secretion of mucus and bicarbonate and to bring out vasodilatation and inhibit acid secretion [16]. We hypothesize that the gastric mucosal protective effect of HC may be due to the biosynthesis of prostaglandins.

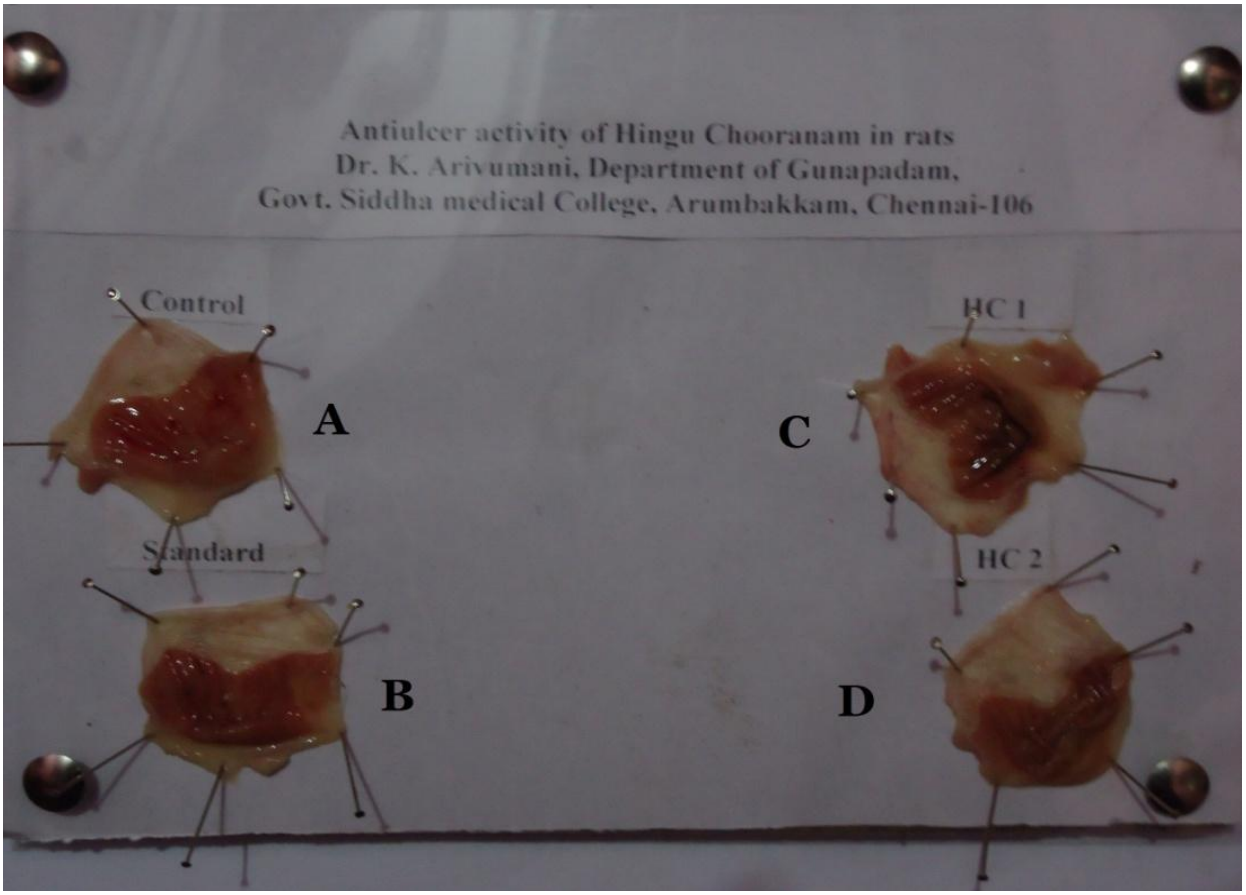

Fig. 9: A- Stomach of Control Rat; B- Standard Drug Ranitidine Treated Rat; C- Effect Of Hc (100mg/Kg) On Aspirin Induced Gastric Ulcer In Rat; D- Effect of HC (200mg/Kg) On Aspirin Induced Gastric Ulcer In Rat.

These Proton pumps inhibitors are preventing the ulcerogenic action of aspirin by inactivating the enzyme H –K-ATPase irreversibly. It thereby inhibits gastric secretion. We hypothesize that the mechanism of HC is as like that of proton pump inhibitor.

HC showed a dose dependent protection against aspirin (400 mg/kg body weight) induced ulcers in rats (**Table 1**). HC produced a significant reduction of ulcer index only in the higher dose treated groups (100 and 200 mg/kg body weight); all the tested doses produced a decrease in ulcer index as compared to the control. HC produced statistically significant P value < 0.05 and <0.01 at dose level 100 mg/kg and 200 mg/kg respectively as compared to control.

H_2 receptor antagonists bind to the histamine (H_2) receptors on the parietal cells of stomach and competitively inhibit the action of histamine on these receptors and thereby reduce gastric secretion. It decreases both volume and acid content of gastric juice without any effect on pepsin secretion. The result of anti ulcer activity of HC in pylorus ligation model is evident (**Table 2**) from its significant reduction in gastric volume, total acidity. So HC acts by H_2 receptor antagonist.

The anti-ulcer activity of HC in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer score and increase in pH of gastric juice in the higher dose treated groups (100 and 200 mg/kg) as compared to the control. Statistically significance of 100mg/kg HC are P value < 0.05 and 200mg/kg P value <0.01 as compared to control.

Distinguished ulcers along with hemorrhagic stripes and the damage of mucosal layer were observed in control group (**Fig 9A**), whereas in rats treated with both doses of HC (**Fig. 9C and Fig. 9D**) and the reference drug ranitidine (30mg/kg) (**Fig. 9B**) showed significant

reduction in ulcer formation. This reveals the protective effect of test drug against ulcer due to pyloric ligation. Marked response was seen in standard group and *HC 2* groups.

Hingu Chooranam is one of the Siddha drug used in the present study to evaluate the anti-ulcer activity in aspirin induced and pylorus ligation induced ulcers in rats. From the above study, it is inferred that there is statistically significant decrease in ulcer index, gastric volume, total acidity, free acidity, ulcer score and also increase in pH of gastric juice. Thus, the present study exhibit that the *Hingu Chooranam* has significantly decreased the ulceration in aspirin induced and pylorus ligation induced ulcers in rats.

CONCLUSION

Finally, the experimental studies on animal model demonstrated the protective and curative activities of the *Hingu Chooranam* against gastric ulceration compared with those of ranitidine, used as a standard drug. HC of both doses (100 mg/kg and 200 mg/kg) shown anti-ulcer activity, but marked response was observed at the dose level of 200 mg/kg body weight and the result was more similar to that of the reference drug ranitidine (30mg/kg). Hence it is concluded that the *Hingu Chooranam* significantly decreased the ulceration in Aspirin and pylorus ligation induced ulcer in rats. Further studies are needed to determine the degree of protection and mechanism of action of *Hingu Chooranam.*

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