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ANTI-ULCER EVALUATION OF SIDDHA FORMULATION OF HINGU CHOORANAM (HC) IN WISTAR RAT

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ABSTRACT

Gastric ulcer is a defect in the gastric wall running through the entire mucosal thickness and penetrating the muscularis mucosae. A variety of factors has been implicated in the aetiology of gastric ulcers. These include humoral, genetic, neural, therapy induced (e.g. use of NSAIDs) and Helicobacter pylori infection. Proton pump inhibitors (PPIs) are among the most commonly used and overprescribed medications in the world. The side effects of the PPIs, such as a headache, diarrhoea, constipation, and abdominal discomfort, are minor and easily managed. However, recent studies have suggested an association between PPI use and several serious adverse effects, which has been a source of major concern to patients and physicians. Recent studies have indicated that the percentage of adults using traditional medicine therapies for their gastro intestinal (GI) symptoms ranges from 20% to 26%, but patients with functional GI disorders are more likely to make use of them, as are those with chronic GI conditions. Siddha system of medicine widely utilised globally for its versatile therapeutic potential in treating disease and disorders. The main aim of the present study is to evaluate the anti-ulcer potential of the siddha drug Hingu Chooranam (HC) in Iodoacetamide ulcerated rats. Results of the study clearly indicates that ulcerative score of the rats belongs to group II rats has shown the intensity and severity of the ulcer induction by Iodoacetamide in the experimental animals. There was significant decrease in the ulcerative score observed in test drug HC treated rats which reveals the anti-ulcer potential of the formulation. From the datas of the present investigation it was concluded that the siddha formulation Hingu Chooranam offers promising anti-ulcer potential in the tested in-vivo model, further studies needs to to bextrapolated at clinical level prior to recommendation.

KEY WORDS: ***Gastric ulcer, Siddha drug, Hingu Chooranam, Anti-ulcer, Iodoacetamide, Ulcerative score***

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1. Introduction

Gastric ulcer is precipitated by an imbalance between endogenous aggressive factors in the gastric lumen and cytoprotective factors; these include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins, mucosal blood flow, cell renewal and migration, non-enzymatic and enzymatic antioxidants and some growth factors [1]. Gastric mucosal injury can be exhibited by various forms of macroscopic and histopathological alterations, such as diffuse hyperemia, inflammation, erosion, or even hemorrhagic ulcerations. Several attempts have been made to classify the different types of gastritis, but it is difficult due to the complexity of its pathophysiological mechanisms [2]. There is often no correlation between the symptoms and the macroscopic lesions or histopathological changes [3]. Based on its localization, the injury can be diffuse, antrum- or corpus-predominant, or even multifocal. Regarding the duration, we can differentiate between acute or chronic forms [4]. However, the etiology of the condition is at least as important

Currently, there are several antiulcer drugs such as ranitidine and omeprazole (PPI) that are used to manage NSAIDs-induced gastric ulcers. However, these therapies have major side effects and the search for non-toxic and easily accessible drugs is still in progress [5].

Some of the adverse effects of PPIs are related to their suppression of gastric acid secretion, allowing ingested microbial pathogens that would have been destroyed by gastric acid to colonize the upper gastrointestinal tract and cause infections. Reports are suggesting that the use of PPIs might increase the risk of enteric infections such as Salmonella and Campylobacter, community-acquired pneumonia [6], Clostridium difficile infections [7], and spontaneous bacterial peritonitis [8].

Herbs are potential sources of new therapeutic agents and several drugs have also been introduced for the treatment of gastrointestinal disorders. Herbal medicine refers to folk and traditional medicinal practice based on the use of plants and plant extracts for the treatment of medical conditions. The use of herbs to treat diseases is almost universal among native people. Herbal medicine is one of the most common traditional modalities which offers ultimate cure with less or no side effects [9,10].Siddha formulation Hingu Chooranam (HC) indicated for use in gastro intestinal disorders as per the literature where in still now there is no documentary evidence achieved through proper in-vivo studies hence the main objective of the present investigation is to evaluate the anti-ulcer potential of the formulation in suitable animal model.

2. Materials and Methods

2.1. Experimental Animals

Healthy adult wistar albino rats of either sex weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ¬+ 2o C and relative humidity 50–65%. They were provided with food and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. The IAEC approval number: SU/CLATR/IAEC/XV/160/2020

2.2. Animal grouping and Methodology [11,12]

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline, Group II – Ulcer control rats received Iodoacetamide (IA), intra-gastric administration of 0.1 ml of the IA solution for 7 (Day 1 to 7). Group III (Low dose treated group): Ulcerated rats was treated with 200mg/kg of HC, p.o for the period of 07 days 1 hr prior to the administration of Iodoacetamide. Group IV (High dose treated group): Ulcerated rats was treated with 400mg/kg of HC, p.o for the period of 07 days 1 hr prior to the administration of Iodoacetamide.

2.3. Anti-Microbial Assay [8]

At the end of the study, before sacrifice, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. The stomach was removed and opened along the greater curvature. The stomach was gently rinsed with water to remove the gastric contents and blood clots. The inner surface of free stomach was examined for gastric lesions. The number of ulcers was counted. Ulcer scoring was carried out according to the method by as given below.

The scores were:

0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer and 3 = perforation

2.2.1. Ulcer score [13,14]

Ulcer index was measured by using the following formula

UI = UN + US + UP × 10−1.

Where UI is the ulcer index; UN is the average number of ulcers per animal; US is the average number of severity score and UP is the percentage of animals with ulcers.

2.2.2. Percentage inhibition of ulceration [15]

Percentage inhibition of ulceration was calculated as follows:

$$\%\ \text{inhibition of ulceration} = \frac{\text{UI of Control} - \text{UI of Test}}{\text{UI of Control}} \times 100$$

2.3. Histopathological Analysis [16]

Sample obtained were immersed in 10% formalin for 24 h-48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 µm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

2.4. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett's multiple comparison test. Probability P values < 0.05 were considered as significant.

3.Results

3.1. Effect of HC on ulcer severity score on Iodoacetamide ulcerated rats

Ulcerative score of the rats belongs to group II rats has shown the intensity and severity of the ulcer induction by Iodoacetamide in the experimental animals. There was significant decrease in the ulcerative score observed in trial drug treated rats which reveals the anti-ulcer potential of the formulation. It was further observed that there was a significant increase in the percentage protection offered by the trial drug HC at both the dose of 200 and 400 mg/kg. As shown in table 1 and figure 1.

Table 1: Effect of HC on Ulcer Index of Iodoacetamide ulcerated rats

Group	Treatment and Dose	Iodoacetamide Induced Ulceration		
		Ulcer Severity Score	Ulcer Index	Percentage of Ulcer Protection
I	Normal Saline	0.0 ± 0.0	-	100
II	Iodoacetamide	2.5 ± 0.223	12 ± 0.16	-
III	Iodoacetamide + 200 mg/kg of HC	1.5 ± 0.428*	9.63 ± 0.2*	19.75*
IV	Iodoacetamide + 400 mg/kg of HC	1.33 ± 0.421*	5.58 ± 0.26*	53.5*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

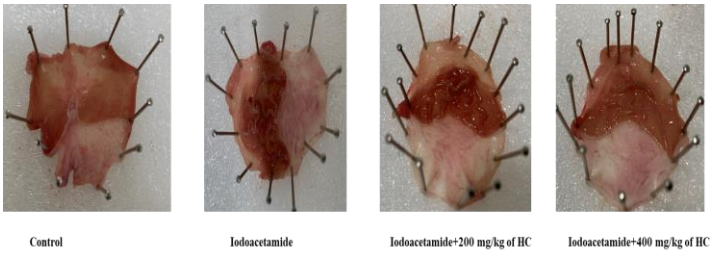

Figure 1: Effect of HC on gross anatomy of rat stomach Iodoacetamide ulcerated rats

3.2. Effect of HC on Histopatholgy of Iodoacetamide ulcerated rats under low and high power magnification

Mucosal wall appears normal with regular arrangement of connective tissue. Regular histology of Inner circular muscle (ICM), gastric pit (GP), and muscularis mucosae (MM) were observed in group I rats. Sever ulceration with glandular degeneration were observed in sample belongs to group II rats. The continuity of mucosa was restored with mild evidence of ulcerative and inflammatory changes in sample belong to group III animals. Regular gastric mucosa containing intact gastric gland cells, parietal were observed in group IV samples. As shown in figure 2.

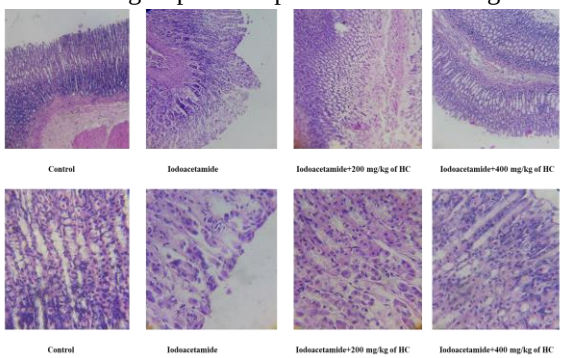

Figure 2: Histopathology of rat stomach belongs to control, Iodoacetamide ulcerated and HC treatment group rats under low and high power magnification

4.Discussion

Gastric ulcer is a break in the tissue lining of the stomach. Most ulcers can be cured without complications; however, in some cases peptic ulcers can develop, such as in penetration, perforation, bleeding (hemorrhage), and obstruction [17,18].

The main therapeutic target is the control of gastric secretion using antacids, H2 receptor blockers like ranitidine, famotidine, anticholinergics like pirenzepin, telezipine or proton pump inhibitors (PPI's) like omeprazole, lansoprazole, etc. The prevention or cure of peptic ulcers is one of the most challenging problem in medicine because gastric ulcer therapy faces drawbacks and most of the drugs currently available in the market show limited efficacy against gastric diseases and are often associated with severe side-effects. The potential of plants as source of new drugs still offers a large field for scientific research. Even if is observed a large number of known plants, a small percentage has already been phytochemically investigated and only a fraction of them has already been assessed to determine its pharmacological potential [19].

Animal models are important for the molecular investigation of gastric injury, since these models may reveal very early biochemical and molecular alterations, much before microscopic or macroscopic lesions can be seen. Good models should have translational relevance. However, in virtually all animal models of gastric injury (e.g., NSAID-, stress-induced) the lesions are well circumscribed (i.e., superficial erosions and/or deep ulcers). Gastritis in humans, on the other hand, is a diffuse inflammatory damage involving all or most parts of the stomach [20]. Iodoacetamide (IAA) is a water soluble sulfhydryl alkylating chemical, which, by depleting sulfhydryl groups, including the protective antioxidant glutathione (GSH) in the gastric mucosa, allows reactive oxygen species production and oxidative tissue damage [21]. Ulcerative score of the rats belongs to group II rats has shown the intensity and severity of the ulcer induction by Iodoacetamide in the experimental animals. There was significant decrease in the ulcerative score observed in trial drug treated rats which reveals the anti-ulcer potential of the formulation. It was further observed that there was a significant increase in the percentage protection offered by the trial drug HC at both the dose of 200 and 400 mg/kg.

Iodoacetamide was added to the drinking water at a concentration of 0.1 % for 7 days, which has been shown to elicit murine gastritis [22] as confirmed by a significant increase in mucosal erosion in the gastric wall. This parameter reflects inflammation-associated infiltration of neutrophils and monocytes into the tissue [23, 24] and is consistent with the iodoacetamide-induced infiltration of inflammatory cells and histological indices of inflammation in the gastric mucosa and submucosa [25].

It was observed from the histological finding of the present study that regular histology of Inner circular muscle, gastric pit and muscularis mucosae were observed in group I rats (control). Sever ulceration with glandular degeneration were observed in sample belongs to group II rats. The continuity of mucosa was restored with mild evidence of ulcerative and inflammatory changes in sample belong to group III animals. Regular gastric mucosa containing intact gastric gland cells, parietal were observed in group IV samples.

5. Conclusion

In conclusion, the data of this study suggest that the siddha formulation Hingu Chooranam can inhibit iodoacetamide induced gastric lesions in rats. The possible mechanisms of antiulcer benefit may be due to its oxygen radicals scavenging by inhibition of lipid peroxidation. Further studies are warranted to evaluate the efficacy of the siddha drug Hingu Chooranam at the pharmacological effective dosage before consideration for clinical trials.

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