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GASTRO-PROTECTIVE EFFECT OF SIDDHA FORMULATION SHANMUGA CHOORANAM AGAINST ASPIRIN-INDUCED GASTRIC ULCER IN WISTAR RATS

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ABSTRACT

Gastroesophageal reflux disease (GERD) is a common disease that can cause troublesome symptoms and have a significant impact on quality of life. which posed a major threat to the world's population over the past two centuries with a high morbidity and mortality. It is known that numerous pharmaceutical agents such as proton pump inhibitors, anticholinergics, antacids, antimicrobial agents, H2-receptor antagonists, sucralfate, and bismuth are not fully effective, and produce numerous adverse effects such as impotence, arrhythmia, hematopoietic alterations, hypersensitivity, and gynecomastia. Despite many advances in the therapeutic management of GERD, the prevalence of this disease is still high. Many phytotherapeutic studies have shown that the bioactive phytocomponents possess an important protective role in the prevention of acid reflux. Siddha drug offers extensive benefits in treating GERD without major complication as of happened with conventional therapy. The main aim of the present study is to evaluate the gastro protective potential of the siddha drug Shanmuga Chooranam (SC) aspirin ulcerated rats. Results of the study clearly indicates that ulcerative score of the rats belongs to aspirin group has shown the increased intensity and severity of the ulcer induction. There was significant decrease in the ulcerative score observed in trial drug SC treated rats at both the dose level of 500 and 1000 mg/kg which reveals the gastro protective activity of the formulation. It was concluded from the result observation of the present investigation that the siddha drug SC possess excellent gastro protective activity against aspirin in experimental animals which advocate efficacy of the drug upon clinical application.

KEY WORDS: ***Gastroesophageal reflux disease, Siddha drug, Shanmuga chooranam, Aspirin, Ulcerative score.***

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1. Introduction

GERD is a common gastrointestinal disorder in the Western countries (10-20%) and Asia (2-20%) [1]. According to a previous study, the most common cause of physician visits in GI clinics of Iran has been GERD [2]. Dietary factors [3], obesity, smoking, stress, major life events, and alcoholism have been implicated in increasing the risk of GERD during past decades. Furthermore, people in suburban areas and those with a positive family history are at a higher risk of GERD. Socioeconomic status and westernized diet, have been postulated as risk factors, even though their roles have not been confirmed yet. Underlying diseases such as diabetes mellitus, hypertension, cardiovascular diseases, hyperlipidemia, metabolic syndrome, and drug consumption have been proven as risk factors in some studies [4-6].

Gastroesophageal reflux disease (GERD) broadly includes the whole spectrum of reflux disease, from intermittent symptoms like heartburn or acid regurgitation to endoscopic reflux esophagitis and Barrett's esophagus [7]. During the recent decade, several studies about prevalence of symptom-based GERD and endoscopic reflux esophagitis have revealed generally higher number of patients compared to other previous Asian studies. Time trend studies have also shown the increase of prevalence both in symptom based-GERD and endoscopic reflux esophagitis [8].

Proton pump inhibitors (PPIs) (i.e.,omeprazole, esomeprazole, lansoprazole, and pantoprazole) are widely used to treat GERD. PPIs work by blocking the adenosine triphosphatase, an important enzyme in the H + and K + exchange process in the final steps of the acid secretory process within the gastric parietal cell. The use of conventional medicine like PPIs is often associated with side effects; for example, peripheral neuropathy is detected as a result of omeprazole therapy [9].

Natural medicinal and herbal preparations have been used for millennia for the treatment of multiple ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently a resurgence of interest in the use of natural bioactive products by the general public, with many healthy subjects and patients taking them for the prevention and treatment of multiple conditions, including gastrointestinal disorders and postoperative recovery [10]. Unfortunately, current evidence of the scientific validity of many of these traditional and commercial compounds is severely limited [11].

The development of new gastro protective drugs from medicinal plants is an attractive proposition, because diverse chemical compounds with gastro protective activities have been isolated from these herbs [12], and they have shown to produce promising results in the treatment of gastric ulcers [13].The bioactive molecules (generally alkaloids, glycosides, lupeols, essential oils, e.t.c) isolated from crude extracts have been used directly as therapeutic agents or as starting materials for the synthesis of useful drugs or serve as a model for pharmacologically active compounds in the process of drugs in synthesis [14].

Siddha drug Shanmuga chooranam is a shasthric preparation drug which is mentioned in literature Kosayee anuboha vaidhiya brahma ragasiyam it comprises of versatile combination of therapeutics such as Indhuppu (Rock salt), Thippili (Piper longum), Seeragam (Cuminum cymium), Milagu (Piper nigrum),Perungayam (Ferula asafetida) and Kariveppilai thool (Murraya koenigii). Still now there is no evidence on ascertaining the potential of this formulation hence the main aim of the present study is to evaluate the gastro protective property of the siddha formulation Shanmuga chooranam against aspirin induced ulcer in rat 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/X/095/2018.

2.1. Animal grouping and Methodology [15]

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline, Group II – Ulcer control rats received 200mg/kg of Aspirin, p.o for the period of 7 days (Day 1 to 7). Group III (Low dose treated group): Aspirin ulcerated rats was treated with 500mg/kg of SC,p.o for the period of 07 days 1 hr prior to the administration of aspirin. Group IV (High dose treated group): Aspirin ulcerated rats was treated with 1000mg/kg of SC,p.o for the period of 07 days 1 hr prior to the administration of aspirin.

2.2. Sample Collection [16,17]

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

Ulcer index was measured by using the following formula

$$UI = UN + US + UP \times 10^{-1}.$$

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

Percentage inhibition of ulceration was calculated as follows:

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

There was a low percentage of ulcer in the study drug treated animals.

2.3. Histopathological Analysis [18]

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 SC on ulcer severity score on aspirin ulcerated rats

It was observed from the results of the present analysis that ulcerative score of the rats belongs to group II rats has shown the intensity and severity of the ulcer induction by aspirin 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 SC at both the dose of 500 and 1000 mg/kg. As shown in table 1 and figure 1.

Table 1: Effect of SC on Ulcer Index of Aspirin ulcerated rats

Group	Treatment and Dose	Aspirin Induced Ulceration		Percentage of Ulcer Protection
		Ulcer Severity Score	Ulcer Index	
I	Normal Saline	0.0 ± 0.0	-	100
II	Aspirin 200mg/kg	2.83 ± 0.16	11.47 ± 0.04	-
III	Aspirin + 500 mg/kg of SC	1.33 ± 0.42*	9.23 ± 0.19*	19.5*
IV	Aspirin + 1000 mg/kg of SC	0.66 ± 0.33*	5.685 ± 0.30*	50.47*

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

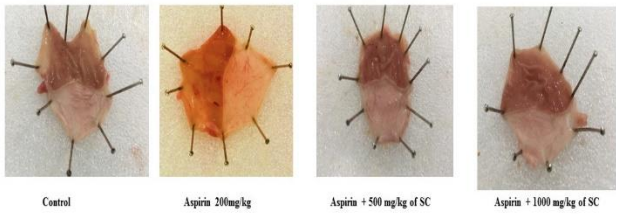

Figure 1: Effect of SC on gross anatomy of rat stomach aspirin ulcerated rats

3.2. Effect of SC on Histopatholgy of aspirin ulcerated rats under low and high power magnification

Histopathological analysis report has revealed that promising regular histology of gastric mucosa containing intact gastric gland cells, parietal cells which are spherical cell with deeply stained dark nucleus were observed in sample belongs to group I animals. Distorted gastric glands with damaged mucosal epithelium and Focal necrosis of gastric mucosa were observed in sample belongs to group II rats. Marked decrease in inflammatory cells infiltration, with reduction in the level of sub-mucosal edema were observed in group III rats. Regular arrangement of Sub-mucosa, gastric glands and muscularis externa were observed in sample belongs to group IV rats. As shown in figure 2.

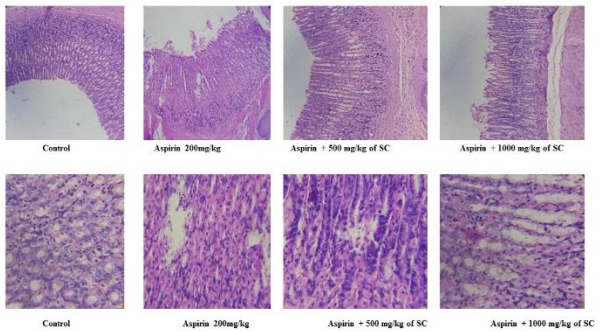

Figure 2: Histopathology of rat stomach belongs to control , aspirin ulcerated and treatment group rats under low and high power magnification

4.Discussion

Gastroesophageal reflux disease (GERD) is a disorder of gastrointestinal motility associated with reflux of stomach contents into the esophagus and oral cavity [19]. It is identified when reflux of stomach contents causes troublesome symptoms and/or complications [20]. GERD is characterized by two cardinal esophageal symptoms: 1) heartburn (reflux); and 2) regurgitation. Extra-esophageal symptoms such as non-cardiac chest (epigastric) pain, dental erosion, cough, laryngitis, and asthma are associated with, but are not specific for, GERD. Recently, one study reported a GERD prevalence of 16.2 % among employees of a large hospital in Northern India [21]. However, there is a paucity of epidemiological data on the prevalence of GERD in general populations in Southern India.

The inhibitory actions of aspirin on prostaglandin synthesis coupled with free radical formation have been reported in previous studies to be critical biochemical events in the pathogenesis of gastric ulceration [22]. Prostaglandins normally protect the gastrointestinal mucosa from damage by maintaining blood flow and increasing mucosal secretion of mucous and bicarbonate. Its inhibition in gastric mucosa by NSAIDs, such as aspirin, caused elevation in gastric acid secretion and reduced mucosal blood flow, mucus, and bicarbonate secretion [23]. Due to that, investigations of the new pharmacologically active agents through the screening of different herbs led to the discovery of effective and safe drugs with gastroprotective activity. Especially, herbs with antioxidant capability as the main mechanism are used as the herbal reservoir for the treatment of ulcer disease [24].

In the present investigation it was observed that the ulcerative score of the rats belongs to group II rats has shown the increased intensity and severity of the ulcer induction by aspirin 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 SC at both the dose of 500 and 1000 mg/kg

Aspirin-induced gastric ulcer models have been widely used for the evaluation of gastroprotective activity. Aspirin-induced ulcer is mediated through tissue damaging free radicals which are produced from the conversion of hydroperoxyl to hydroxy fatty acids, which leads to cell destruction [25]. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing the ulcer [26]. Histopathological analysis of stomach reveals

promising regular histology of gastric mucosa containing intact gastric gland cells, parietal cells which are spherical cell with deeply stained dark nucleus were observed in sample belongs to group I animals. Distorted gastric glands with damaged mucosal epithelium and Focal necrosis of gastric mucosa were observed in sample belongs to group II rats. Marked decrease in inflammatory cells infiltration, with reduction in the level of sub-mucosal edema were observed in group III rats.Regular arrangement of Sub-mucosa ,gastric glands and muscularis externa were observed in sample belongs to group IV rats.Therefore, effective management of GI ulceration would primarily depend on (i) reduction of the aggressive factors (ii) improved generation of protective factors or (iii) a combination of both. Advances in natural product chemistry have led to the purification and characterization of a number of chemical compounds with potent anti-ulcer activity [27].

5.Conclusion

GERD is precipitated by excess secretion of hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species. Drugs used for clinical management of GERD results in potential life threatening adverse effects. Siddha formulation shanmuga chooranam provided adequate protection against aspirin induced ulcer and further restoration of cyto architecture were observed in histological analysis. Hence by considering these outcomes it was believed that clinical application of this formulation may establish new therapeutic strategy in managing GERD at clinical level.

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