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ANTIULCER ACTIVITY OF *CANAVALIA VIROSA (ROXB) W&A* LEAVES IN ANIMAL MODEL

A.LAVANYA*[1], M.PITCHIAH KUMAR[1], J.ANBU[2], ASHWINI ANJANA[2] AND S.AYYASAMY[1]

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

ABSTRACT

Canavalia Virosa (Roxb) W&A Leaves has been widely used in *Siddha* system of medicine for various diseases. The powder of *Kozhi Avarai Ilai Chooranam* showed a significant inhibitory effect was screened at 200 mg/kg, for the in vivo antiulcer activity on chemical induced ulcer in rats. Ranitidine (60mg/kg) used as reference standard. Single dose (200 mg/kg) treatment with the siddha drug *Kozhi Avarai Ilai Chooranam* produced 30% antiulcer effect. The trial drug showed a significant antiulcer activity ($P<0.01$) and were comparable with that of standard thus validating the traditional claim of the plant.

Key words : *Kozhi Avarai Ilai Chooranam*, Siddha system, *Canavalia Virosa (Roxb)*, Antiulcer, Ranitidine

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries[1]. 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[2]. 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[3]. 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.[4, 5] Peptic ulcers are illnesses that affect a considerable number of people in the world. Some of the causes of these disorders are: stress, smoking, nutritional deficiencies and ingestion of nonsteroidal anti-inflammatory drugs.[6,7]The pathogenesis of gastro duodenal ulcers are influenced by various aggressive and defensive factors, such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (Prostaglandins and epidermal growth factor).[8]*Canavalia virosa (Roxb) W&A* is a Perennial climber belongs to the family *Papillonaceae*, it is used in the treatment of Peptic ulcer in traditional practice. In the present study an attempt was made to investigate the anti-ulcerogenic effects of *Kozhi Avarai Ilai Chooranam* in animal models of Peptic ulcers induced by acid-alcohol in rats. The acute toxicity of the drug was also investigated in mice.

MATERIALS AND METHODS

(i) Drugs Collection and Authentication

The plant material used in this study was collected during the month of June (2011) from Tankbund of Rarapuram, Pudukkottai Dist, Tamilnadu, India and authenticated from the Gunapadam experts in Department of PG Gunapadam, Govt. siddha medical college, Chennai 600106 and Certified from Botanist, Central Research Institute For Siddha, Arumbakkam, Chennai-600106.The preparation was selected from *Patharthaguna chinthamani* published by Rathnanayagan & sons**.**

(ii) Preparation of trial drug

The plant leaves were well rinsed in water to remove the impurities. Then the leaves were cut into pieces and dried in shade.The well dried *Canavalia virosa leaves* (*Kozhiavarai Ilai*)were made into fine powder. To get the finest physical form of this drug, the powdered material is sieved through a white cotton cloth *(Vashthirakayam)*. 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 tied cloth. The mouth of the pot closed with another mud pot. The gap between the two mud pots was tied with a wet cloth to avoid evaporation. Then this arrangement was put on fire and boiled until water level gets reduced in the lower pot. Then the powder was taken, dried, powdered finely and preserved for usage.

(iii) Drugs and chemicals

The powdered form of *Kozhi Avarai Ilai Chooranam* was filtered through cheesecloth and was mixed uniformly in 2% CMC solution to achieve 100mg/ml as main stock solution and used in this study.

(iv) Animals

Mice of either sex weighing 25-30gm and male Wistar rats weighing 150-200gm were obtained from the animal house of Vels University. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of humidity, temperature (20-24°C) and light (12-h light: 12-h dark cycle). The rats were randomly assigned to control and different treatment groups, six animals per group. The study was conducted in accordance with CPCSEA (Committee for the Purpose of control and supervision of Experiment on Animals) guidelines and the study was approved by the Institutional Animal Ethical Committee (Registration no. XIII/VELS/COL/06/CPCSEA/IAEC/23.09.11). The animals were acclimatized for one week under laboratory conditions. All animal experiments were carried out in accordance with institutional Ethical Committee acts.

(v) Acute Toxicity Study

The albino mice weighing between 22-28 gm were selected to ascertain the toxicity range of the test drug *Kozhi Avarai Ilai Chooranam*. The starting dose administered to the test group of animals was 200mg/kg. The animals were segregated into six groups consisting of six mice each. The increasing doses were administered upto 2000mg/kg. The toxic dose was determined by observing the mortality rate in the drug treated groups. From this the therapeutic dose was selected for the further study.[9]

(vi) Antiulcer activity evaluation

Hcl, Ethanol induced Gastric Ulceration

In Hcl, Ethanol induced gastric ulcer protocols, rats were starved of food but not for water 24 hours. Negative control group received saline and test group received *Kozhi Avarai Ilai Chooranam* at 200mg/kg, p.o. 120 minutes before receiving Hcl Ethanol and positive control group received ranitidine orally at 60 mg/kg 30 min prior were administered Hcl Ethanol.[10] Ethanol was administered orally to these five groups at 1ml/200g.[11] The volume of the saline, *Canavalia virosa (Kozhi Avarai Ilai Chooranam*) in suspension and ranitidine was 0.5ml/100g of body weight.

The ulcer index score for intensity of the gastric lesions was measured where score 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transdermal necrosis, and 3 = perforated or penetrated ulcer. Ulcer index = 1 O/X where,

$$X = \frac{\text{Total area of stomach mucosa}}{\text{Total ulcerated area}}$$

(vii) Gastric Secretion

The gastric juice was collected 4hrs after ulcerogenic administration and centrifuged for 5 minutes at 2000 rpm and the volume of supernatant was noted. The pH of the gastric juice was recorded by the pH meter. Then the contents were subjected to analysis for free and total acidity. Free acidity and total acidity were determined using 0.01N NaoH and Topfer's reagent containing phenolphthalein as indicator. The acidity level was calculated by using the following formula:

$$\text{Acidity} = \frac{\text{Volume of NaoH X Normality}}{0.1} \text{ X } 100$$

(viii) Statistical analysis

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

RESULTS AND DISCUSSION

From the acute toxicity study was performed as per OECD guidelines-425, it was concluded that the test drug *Canavalia virosa(Kozhiavarai Ilai chooranam)* has no lethal effect upto 2g/kg body weight after oral administration in mice. From the maximum tolerable dose (2000mg/kg) one-tenth of the dose was considered for the experiments. Administration of Diluted 10% Hcl-absolute ethanol to fasted rats resulted in severe gastric damage visible from the outside of the stomach as thick reddish-black lines. After opening, the gastric lesions were found in the mucosa and consisted of elongated bands, 1–10mm long, usually parallel to the long axis of the stomach. They were located mostly in the corpus (the portion of the stomach secreting acid and pepsin). Ranitidine (60mg/kg, p.o.) protected the animals from ulceration significantly. The pretreatment of rats with *Canavalia virosa leaves (Kozhiavarai Ilai Chooranam) powder* rendered a protection from ethanol-induced ulceration. When compared to the ulcer control animals, *Kozhi Avarai Ilai Chooranam* treatment provided more or less 42% protection at 200 mg/kg dose. Although ranitidine, the reference drug used in the study provided the animals with the highest ulcer protection (63%), the effect of 200 mg /kg dose of *Kozhi Avarai Ilai Chooranam* was moderately comparable (30%) with the reference drug. Gross pathological studies of the stomachs removed from animals that were not pre-treated with either reference drug or *Kozhi Avarai Ilai* Chooranam showed complete ulceration.

Table 1

Effect of Kozhi Avarai Ilai 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
Normal	144±1.55	126.07±0.36	0.72±0.07	1.28±0.02
CMC control	489.29 ± 3.35	374.0 ± 4.1	1.08±0.04	24.19 ± 0.28
KAIC (200mg/kg)	254±2.20**	151.10±0.81**	1.02±0.03	14.44 ± 0.24**
Ranitidine (60mg/kg)	341.11±2.60**	171±3.46**	1.02±0.03	10.84 ± 0.14**

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

However, a preventive effect against ulceration (in terms of ulcer area) was noticed in animals pretreated with ranitidine and *Kozhi Avarai Ilai Chooranam* 200 mg /kg. Histopathology of stomach show that test drug significantly reduced gastric lesion formation and sub-mucosal edema similar to the ranitidine treated animals but it was remarkable as standard drug treated group. Careful evaluation revealed that the mucosa of ulcer control animals have hemorrhagic erosion, discontinuity in the lining of epithelium cells and significant damage in sub-mucosa. Normal mucosa with small strophic gland, mild hyperplasia and no edema were observed for animals treated with ranitidine. Similarly, mucosa of animals treated with 200 mg/ kg. *Kozhi Avarai Ilai Chooranam* was identified with have hemorrhagic erosion, discontinuity in the lining of epithelium cells and significant damage in sub-mucosa.

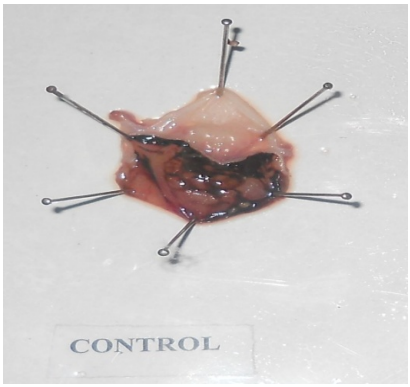

Figure 1: ***Control***

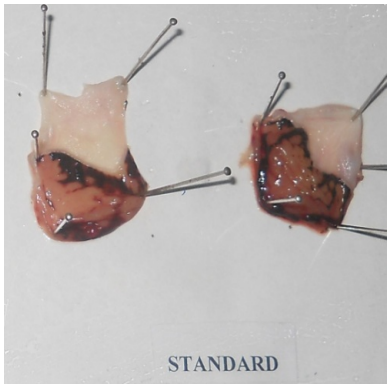

Figure 2: ***Standard***

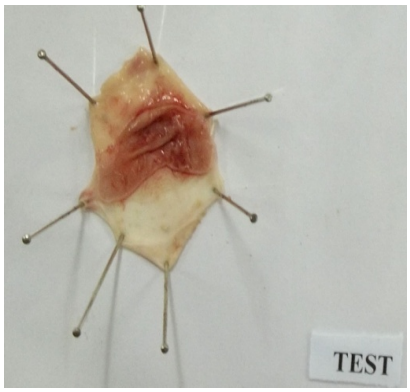

Figure 3: ***Test***

CONCLUSION

From this study, it is clearly evident that Canavalia virosa (Kozhi Avarai Ilai) Chooranam has significant action as anti-ulcer activity in animal models at the dose level of 200mg/kg^{-1}. But it has no muco protective activity and moderate gastric anti-secretary when compared with that of reference drug.

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