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Evaluation of Antiulcer Activity of Ethanol Extracts of *Aristolochia krisagathra* Sivarajan and Pradeep and *Aristolochia bracteata* Retz. Whole Plants in Experimental Rats.

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ABSTRACT

Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. Due to various adverse side effects associated with currently available antiulcer drugs, current interest has been shifted towards natural products as a new source of gastroprotective agents. Therefore, the present study was carried out to investigate antiulcer activity of ethanol extract of *Aristolochia krisagathra* and *Aristolochia bracteata* (Aristolochiaceae) whole plants and combined extracts (*A.krisagathra* and *A.bracteata* whole plants) in HCl induced ulceration in the albino rats. In HCl induced ulcer model, various parameters were studied viz; gastric volume, pH, total acidity, ulcer index and percentage of inhibition. Ranitidine at 60mg/kg was used as the standard drug. Pretreatment of ethanol extracts of *A.krisagathra* and *A.bracteata* (300mg/kg) whole plants and combined extracts (100, 200 and 300 mg/kg) showed significant (p<0.05; p<0.01) decrease in the gastric volume and total acidity. However, pH of the gastric juice was significantly (p<0.05; p<0.01) increased only at higher dose, 300mg/kg. It also showed a significant (p<0.05; p<0.01) decrease in number of ulcers and ulcer score index in HCl induced ulceration model. The combined extracts of *A.krisagathra* and *A.bracteata* whole plants possessed significant antiulcer properties in a dose dependent manner. In conclusion the antiulcer properties of the extracts may be attributed to the presence of phytochemicals like flavonoids, alkaloids and tannins present in the plant extracts with various biological activities.

Keywords: Antiulcer, *Aristolochia*, ulcer index, flavonoid

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INTRODUCTION

Peptic ulcer is a chronic, non-malignant inflammatory disease characterized by ulceration in the upper gastro-intestinal tract (stomach and duodenum) where parietal cells are found. 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 [1]. If peptic ulcers are found in the stomach, they are called gastric ulcers. If they are found in the duodenum, they are called duodenum ulcers [2]. Gastic ulcers are also associated with considerable morbidity related to chronic epigastric pain, nausea, vomiting and anemia [3]. Rarely, an ulcer can lead to a gastric or duodenal perforation. This is extremely painful and requires immediate surgery [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, 8, 9].

The genus *Aristolochia* finds a prominent place in different Indian Systems of Medicine. The different ethnic communities in India have used different species of *Aristolochia* in the treatment of various human ailments. Kanikkar tribals of Kalakad-Mundanthurai Tiger Reserve Sanctuary, Tamil Nadu, boiled equal quantity of fresh root and leaves of *A.krisagathra* in coconut oil for about 15-20 minutes over a low flame. The oil is filtered after cooling and applied on the head once in a day as the treatment of rheumatism. The therapy is used to reduce excessive heat of the body [10]. *A.bracteata* is used in traditional medicine as gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites [11]. The whole plant was used as purgative, anthelmintic, antipyretic and antiinflammatory agents [12]. Despite various scientific reports on these plants pharmacological activities, no attempt has been made to study its antiulcer potential. Thus, the objective of the present study was to explore the antiulcer activity of ethanol extracts of *A.krisagathra* and *A.bracteata* whole plants using HCl induced gastric ulcer model in rats.

MATERIALS AND METHODS

Plant Materials

The whole plant of *Aristolochia krisagathra* Sivarajan and Pradeep was collected from the natural forest of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The whole plant of *Aristolochia bracteata* Retz was collected from Vadavalli Coimbatore, Tamil Nadu. The plants were identified with the help of local flora and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. Voucher specimens were preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further reference.

Preparation of Plant Extract

The whole plants of *A.krisagathra* and *A.bracteata* were dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction in a Soxhlet apparatus using ethanol. The ethanol extracts were concentrated in a rotatory evaporator. The concentrated ethanol extracts of whole plants of *A.krisagathra* and *A.bracteata* were used for phytochemical screening [13] and antiulcer activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±20C) and light and Dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Study was carried out as per IAFC approval No: 82 /PHARMA/SCRI, 2010.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), Wistar albino rats/Swiss albino mice (n = 6) of either sex selected by random sampling were used for acute toxicity study [14]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5 mg/kg body weight dose by gastric incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50 mg/kg, 100 mg/kg upto 200 mg/kg body weight.

HCl induced gastric ulcer

Experimental Setup

The animals were divided into seven groups of six rats each.

Group I: Rats treated with 4% w/v aqueous tween 80 (5 mL/kg body weight) for 7 days
Group II: Rats treated with ethanol extract of *A.krisagathra* whole plant, at the dose of 300mg/kg body weight, daily for 7 days
Group III: Rats treated with ethanol extract of *A.bracteata* whole plant, at the dose of 300 mg/kg body weight, daily for 7 days.
Group IV: Rats treated with a combined whole plant ethanol extracts of *A.krisagathra* and *A.bracteata,* at the dose of 100 mg/kg body weight, daily for 7 days.
Group V: Rats treated with a combined whole plant ethanol extracts of *A.krisagathra* and *A.bracteata,* at the dose of 200 mg/kg body weight, daily for 7 days.
Group VI: Rats treated with a combined whole plant ethanol extracts of *A.krisagathra* and *A.bracteata,* at the dose of 300 mg/kg body weight, daily for 7 days.
Group VII: Rats treated with Ranitidine (60 mg/kg body weight, daily for 7 days.

Gastric ulcers were induced in rats by oral administration with 0.06 mL/L HCl to all groups. Animals were fasted for 24 h with free access to water prior to the test. Ethanol extracts of *A.krisagathra* and *A.bracteata* whole plants, combined ethanol plant extracts, 4% Tween 80 (Control) and Ranitidine (Standard drug) were given orally, 30 minutes before administration with HCl.

Determination of pH

An aliquot of 1 ml 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 1 ml gastric juice diluted with 1 ml of distilled water was taken into a 50 ml 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:

$$\text{Total Acidity} = \frac{\text{Vol. NaOH x N x 100}}{0.1}\ \text{mEq/L}$$

Measurement of ulcer index

Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contamination, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI) and the percentage of inhibition (%I) was calculated using the following formula [15]

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$$\% I = (USC - USt) / USc \times 100$$

Where, USc = ulcer surface area in control
USt = ulcer surface area in treated animals.

Statistical analysis

The data was expressed as mean ± standard error mean (S.E.M.). The significance of difference among the group was assessed using one way ANOVA and multiple way analysis of variance. The test followed by Dunnett's test *p* values less than 0.05 were considered as significance.

RESULTS

In the present study, the preliminary phytochemical analysis reveals the presence of alkaloid, anthraquinone, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, glycoside and xanthoprotein. Results of acute toxicity show that, the plants are safe upto a maximum dose 2000mg/kg.

Effect of ethanol extracts of *A.krisagathra* and *A.bracteata* on HCl induced ulceration is shown in Table 1. The HCl induced has caused the accumulation of gastric secretion of 2.80 ± 0.06 mL with pH 2.08 ± 0.02 in a control group. The total acidity of the gastric secretions were found to be 43.90 ± 0.32 mEq/L. Pretreatment with the *A.krisagathra* and *A.bracteata* whole plant extracts separately ($p<0.05$) and combined extracts, significantly. ($p<0.01$) reduced the volume of gastric secretions. pH of the gastric fluid was significantly elevated upto 3.73 only at higher dose of the extract. In addition, total acidity was also reduced significantly in a dose dependent manner. Further it is observed that HCl has caused gastric ulceration and pretreatment with *A.krisagathra* and *A.bracteata* whole plant extracts and combined extracts of *A.krisagathra* and *A.bracteata* extracts have reduced them significantly ($p<0.05$; $p<0.01$) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 50.75%, 59.09%, 45.72%, 65.57% and 74.37% at 300mg/kg (*A.krisagathra* and *A.bracteata)* 100, 200 and 300 mg/kg (combined extracts of *A.krisagathra* and *A.bracteata*) respectively. The gastric protection offered by the test extracts was comparable to that of the standard drug, ranitidine (60mg/kg body weight).

DISCUSSION

An ulcer is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. About 19 out of 20 peptic ulcers are duodenal. Gastric ulcers, found in the stomach wall, are less common. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [16]. A large number of spices and herbs have been evaluated by various researchers for their antiulcer effects to achieve a favorable outcome. A large number of medicinal plants and dietary nutrients have been shown to possess gastro-protective activities such as *Aloe*, *Terminalia chebula*, *Vetiveria zizanioides*, *Capsicum*, Ginseng etc.

Antiulcer activities were performed on wistar albino rats of either sex using 0.6 mol/L HCl induced models. The whole plant ethanol extracts of *A.krisagathra* and *A.bracteata* (300 mg/kg body weight) and combined extracts of *A.krisagathra* and *A.bracteata* (100 mg/kg, 20 mg/kg and 300 mg/kg body weight each) showed significant antiulcer activity.

The percentage of ulcer protection by the plant extracts and the standard ranitidine (60 mg/kg) varies and the percentage of inhibition of ulcer by the whole plant ethanol extracts of *A.krisagathra* and *A.bracteata* (300 mg/kg body weight) is comparatively very less when compared with combined extracts of *A.krisagathra* and *A.bracteata*. The ulcer index is also reduced. Ulcer index parameter was used for the evaluation of antiulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, degrees in free and total activity. Combined ethanol extracts of whole plants of *A.krisagathra* and *A.bracteata* and the standard ranitidine at the doses of 200 and 300 mg/kg and 60 mg/kg body weight

respectively had showed a significant (p<0.001) reduction in the ulcer index.

Table 1: Effect of the whole plant ethanol extracts of *A. krisagathra* and *A. bracteata* separately and in combination of both extracts on HCl induced gastric ulcer in rats

Treatment Groups	Gastric content				% of Inhibition
	Volume of gastric juice (mL/100g)	pH	Total acidity (mEq/L)	Ulcer Index	
Group I	2.80±0.06	2.08±0.02	43.90±0.32	39.80±0.27	---
Group II	1.32±0.01	2.98±0.07*	35.34±0.23ns	19.60±0.28*	50.75
Group III	1.21±0.04*	3.73±0.05*	34.22±0.18ns	16.28±0.36*	59.09
Group IV	1.27±0.07*	2.81±0.07*	38.22±0.66	21.60±0.68*	45.72
Group V	0.96±0.06**	2.96±0.09*	27.93±0.53**	13.70±0.43**	65.57
Group VI	0.74±0.07***	3.26±0.07**	16.84±0.29***	10.20±0.54***	74.37
Group VII	0.87±0.04***	3.69±0.05***	14.22±0.18***	9.60±0.28***	75.87

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests * p < 0.05; ** p < 0.01 and *** p < 0.001 as compared to control (n = 6 in each group)

Ethanol and HCl, the most common necrotizing agents, produce severe gastric erosions when given intragastrically. Oxygen-free radicals that lead to an increased lipid peroxidation and damage to cell and cell membranes are implicated in the pathogenesis of HCl-induced gastric mucosal injury. HCl, in addition to its direct damage of gastric mucosal cells through free radicals formation, also causes solubilisation of mucus constituents and depress tissue levels of proteins, leading to flow stasis in gastric blood. Flavonoids, one of the phytoconstituents in *Brassica oleracea*, have been confirmed to possess antiulcerogenic property through cytoprotection, free radical scavenging and antioxidation also through increased mucus production, antisecretory actions and inhibition of the *Helicobacter pylori* growth [17, 18].

The preliminary phytochemical analysis of *A.krisagathra* and *A.bracteata* whole plants extracts showed the presence of alkaloids, flavonoids, terpenoids, glycosides, phenols, tannins, steroids and saponins. The significant increase in the antiulcer activity of *A.krisagathra* and *A.bracteata* could be attributed to the presence of flavonoids, alkanoids, tannins, saponins and phenolic compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It suggests that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastro intestinal lumen [19]. So the antiulcer activity of *A.krisagathra* and *A.bracteata* may be attributed to its flavonoids content.

CONCLUSION

The results of the present study suggest that the ethanol extracts of *A.krisagathra* and *A.bracteata* whole plants may be beneficial in the treatment of gastric lesions. Further studies to identity the active compounds and elucidation of the mechanism of action is recommended. The investigation on mode of action may pave way for establishment of new antiulcer therapy regimen.

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