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Effect of *Alpinia calcarata* rhizomes on ethanol – induced gastric ulcers in rats

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ABSTRACT

A study to evaluate the gastroprotective activity of hot water extract (HWE) of *Alpinia calcarata* Roscoe (Zingiberaceae) rhizomes was carried out. Three doses (500, 750, 1000 mg/kg) of HWE was evaluated for gastroprotective activity against ethanol induced gastric ulcers in rats. Oral administration of HWE provided dose dependent (r^2 = 0.98) and significant (P< 0.05) protection against gastric damage caused by ethanol. The gastroprotective effect of HWE was superior to that of cimetidine, the reference drug. The HWE significantly (P< 0.05) inhibited gastric volume, acidity (total and free) and significantly (P< 0.05) increased the gastric pH. On the other hand, gastric mucosal secretion remained unaltered. Further, HWE possessed significant (P< 0.05) antihistamine activity. The HWE was well tolerated: no overt signs of toxicity, hepatotoxicity (in terms of aspartate transaminase, alanine transaminase) or renotoxicity (as judged by serum urea and creatinie). It is concluded that HWE of *A. calcarata* rhizome has strong and safe gastroprotective activity.

Keywords: *Alpinia calcarata,* gastroprotection, acidity, antihistamine activity, toxicity.

INTRODUCTION

Alpinia calcarata Roscoe (Zingiberaceae), is a rhizomatous perennial herb with a tuberous root stock. The mature rhizomes are branched and dense with a light to dark brown color. The leaf of the plant is simple, alternative, 25 – 32 cm long and 2.5 – 5 cm broad, lanceolate, acuminate, long pointed, glabrous on both surfaces and shining on the upper surface. Leaf stem is slender and approximately 75 – 100 cm tall. Flowers are irregular and bisexual (1 – 2). *A. calcarata* is distributed among the tropical countries including Sri Lanka, India and Malaysia. It is a common medicinal plant found in Sri Lanka and usually cultivated for medicinal purposes in village gardens in the country (1).

In our studies, 18 volatile constituents were identified in essential oils of Sri Lankan grown *A. calcarata* rhizomes, roots and leaves (3). 1, 8 – cineol was found to be the major constituent in the oils of rhizomes and leaves while in the roots, it was α fenchyl acetate (3). Kong and co – workers (4 – 5) have isolated some diterpenes such as calcaratarins A – E, γ - bicyclohomofarnesal, zerumin A, sesquiterpenes such as shyobunone and coumarins such as herniarin from the rhizomes of *A. calcarata* grown in China. Further, benzenoids such as protocatechuic acid, vanillic acid and syringic acid, flavonoids such as quercetin 4 - o –methyl and alkaloids were isolated from the leaves of *A. calcarata* grown in India (6).

Experimentally, rhizomes of *A. calcarata* are shown to possess antibacterial (7), antifungal (8), anthelmintic (9) antinociceptive (10) and antioxidant (11) activities. The rhizomes of *A. calcarata* is known to possess a broad spectrum of medicinal properties and considered to be as aphrodisiac (1,12). It is used in the treatment of arthritis

(13 – 14), bronchitis, cough, respiratory ailments, asthma and diabetes (1,12). According to available literature, *A. calcarata* is not recommended as a gastroprotective agent in traditional medicinal systems. However, it is possible that *A. calcarata* rhizomes to possess gastroprotective properties as rhizomes of *A. galanga* (15) a close relative of the plant, is reported to have gastroprotective properties. Therefore, this study was undertaken to investigate the gastroprotective activity of *A. calcarata* rhizomes using a hot water extract.

MATERIALS AND METHODS

Plant material

Fresh *A. calcarata* rhizomes were collected from home gardens in Western Province of Sri Lanka. The plant material was identified and authenticated by the Curator of National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (AS 01) was deposited in the Industrial Technology Institute, Colombo 7, Sri Lanka.

Preparation of hot water extract (HWE)

Fresh *A. calcarata* rhizomes were cut into small pieces and air dried for 5–6 days in the shade. Five hundred grams of dried rhizomes were boiled with 2.5 L (to cover the raw material) of distilled water (DW) for 4 h. The hot water extract was concentrated under vacuum, freeze dried (yield 15.6 % w/w dry weight basis) and stored at 4 °C until use.

Administration of extracts

Doses of 500, 750 and 1000 mg/kg of HWE were administered orally by gastric gavage (each dose in a volume of 1 mL DW) to separate groups of rats. These doses were identical to those used in the investigation of antinociceptive activity of rhizomes of *A. calcarata* (10).

Animals

Healthy adult cross- bred male albino rats (weighing 200g–250g) were used throughout the experiment. They were housed individually in raised mesh bottom cages (to prevent coprohagy) under standardized animal house conditions with free access to pelleted food (Vet House Ltd., Colombo, Sri Lanka) and tap water. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guide lines and rules of the ethical Committee University of Colombo, for animal experimentations.

Evaluation of gastroprotective activity

The food was withdrawn 36 h and water for 12 h in 45 rats before the commencement of the experiment. These rats were randomly divided into 5 equal groups (n = 9 / group) and treated orally in the following manner: each rat in group1 received (1 mL of DW), 2, 3, 4 (500, 750 and 1000 mg/kg of HWE) and 5 (100 mg/kg of cimetidine, the reference drug). After 1 h of oral treatment, each rat was given 1 mL of absolute ethanol orally and kept for another 1 h. Then the rats were sacrificed with an over dose of ether, stomachs were removed and inflated with 1% formalin solution and immersed in the same solution to fix the outer layer of the stomach. Each stomach was opened along the greater curvature, rinsed with tap water to remove gastric contents and blood clots. The number of haemorrhagic lesions were counted and the lengths of the linear lesions were measured with a vernier caliper. The number and the length of the lesions per rat were calculated (16).

Evaluation of the mode of gastroprotective activity

This was investigated using 1000 mg/kg dose of HWE since the gastroprotective activity of this dose was maximum compared to other tested doses.

Evaluation of the effects on acidity and volume of the gastric juice

Twelve male rats were starved for 36 h as described previously. They were randomly divided into 2 equal groups. Rats in two groups were orally treated with either 1000 mg/kg of HWE or 1 mL of DW per rat respectively. One hour later, these rats were laparotomised under ether anesthesia and at the pyloric end of the stomach were ligated with a cotton thread. The stomachs were then carefully placed back in the abdominal cavities and the rats were sutured and allowed to regain consciousness. Four hours later stomach was excised, gastric juice collected and centrifuged at 3500 rpm for 15 min. The volume of gastric juice from each rat was measured and acidity (total and free) was determined by titration with 0.01 M NaOH according to the method described by Reitman (17).

Determination of mucus content of stomach

Alcian blue binding to gastric wall mucus was determined by a modified method of Corne (18). In this experiment, twelve male rats were starved for 36 h as described previously. They were randomly divided into 2 equal groups. Rats in two groups were orally treated with either 1000 mg/kg of HWE or 1 ml of DW per rat respectively. One hour later, these rats were laparotomised under

ether anesthesia and at the pyloric end of the stomach was ligated with a cotton thread. The stomach was then carefully placed back in the abdominal cavities and the rats were sutured and allowed to regain consciousness. After 4 h, the rats were sacrificed with over dose of ether, each stomach was opened along the greater curvature, rinsed with 0.25 M sucrose solution. These stomachs were incubated in 10 mL aliquots of 0.1 % alcian blue solution for 2 h at room temperature (30 °C). After 2 h stomachs were removed, washed with 0.25 M sucrose solution and separately incubated in 10 mL aliquots of 0.5 M magnesium chloride solution for 2 h at room temperature while shaking at 30 min. intervals to elute the alcian blue bound to the mucosa of the stomachs. Two hours later, the stomachs were removed and 5 mL of each aliquot of magnesium chloride solution containing the alcian blue eluted from each stomach was shaken with 5 mL of diethyl ether. The aqueous phase was separated out, centrifuged at 3200 rpm for 5 min. and the absorbance of the supernatant was measured at λ 605 nm. The amount of alcian blue bound per stomach in micrograms was determined using a standard calibration curve.

Antihistamine activity

Twenty one male albino rats were selected and their fur on posterior left lateral side was shaved under ether anesthesia. Twenty four hours later, these rats were randomly divided into 3 equal groups (n = 7) and treated orally in the following manner. Group1 received (1000 mg/kg of HWE in 1 mL of DW), 2 (chlorpheniramine, antihistamine receptor antagonist., 0.67 mg/kg) and 3 (1 mL of DW). After 1 hour 0.05 mL of 200 µg/mL of histamine dihydrochloride was subcutaneously injected under mild ether anesthesia in the area of the skin where the fur was removed previously (19). The radius of the wheal formed was determined after 2.5 min. and the areas were computed.

Toxicological studies

The healthy male rats were randomly divided into two groups (n =9/ group) and treated orally in the following manner. Group 1(1500 mg/kg of HWE in 1 mL of DW) and 2 (1 mL of DW) per day for 7 consecutive days between 10.00 h – 11.00 h. Rats were checked twice daily (9.00 h and 16.00 h) for overt signs of toxicity (salivation, diarrhoea, lacrymation, tremors, ataxia, yellowing of hair, loss of hair, postural abnormalities or behavioral changes), stress (fur erection or exopthalmia) and aversive behaviors (biting paw and penis, intense grooming behavior, scratching behavior, licking at tail or vocalization), morbidity and mortality. Percentage weight gain, food and water intake were determined weekly during the period of treatment for each group. The consistency of faeces and color of urine were noted daily.

On day 1 post treatment, approximately 1 mL of blood was collected from the tail of the treated rats under mild ether anesthesia and divided into two equal parts. To one part EDTA was added and red blood cell (RBC) counts, white blood cell (WBC) counts and hemoglobin (Hb) concentration were determined using standard procedures (20). Other part was allowed to clot (25–30 min.) at room temperature (28–30 °C) and subjected to 15 min. centrifugation at 3200 rpm for the collection of serum. Serum samples were analyzed for concentrations of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine using Randox enzyme kits (Randox Laboratories Ltd., Antrium, UK) and a spectrophotometer (JascoV500, Jasco Corporation, Tokyo, Japan). After drawing blood rats were sacrificed with chloroform and weighed. The liver, kidneys, testes, adrenal glands, heart, spleen, vasa deferentia, prostate glands, seminal vesicles together with coagulating glands, cauda epididymides and caput plus corpus epididymides were examined for gross external pathological abnormalities. These organs were removed, blotted free of blood and wet weights were recorded. Weights of the organs were expressed as a percentage of the body weight. The stomachs were also removed, opened along the greater curvature and observed for any gastric lesions.

Statistical analysis

Data are given as means ± S.E.M. Statistical comparisons were made using one way ANOVA followed by Tukey's family error test. A P value ≤ 0.05 was considered as significant. Dose dependencies were determined by regression coefficients (r^2).

RESULTS

Experimentally induced gastric lesions

A. calcarata HWE caused a significant (P< 0.01) inhibition of the length (Figure 1) and the number (Figure 2) of gastric lesions induced by absolute ethanol in a dose dependent (r^2 = 0.98) manner. Among the tested doses, high dose showed the maximum inhibition of the length (by 90 %) and number of gastric lesions (by 91 %) followed by mid (length: by 62 % ; number: by 65 %) and low (length: by 23 %; number: by 27 %) doses. The ID_{50} value of the HWE was 705 mg/kg. The gastroprotective activity of HWE was significantly (P< 0.01) higher than

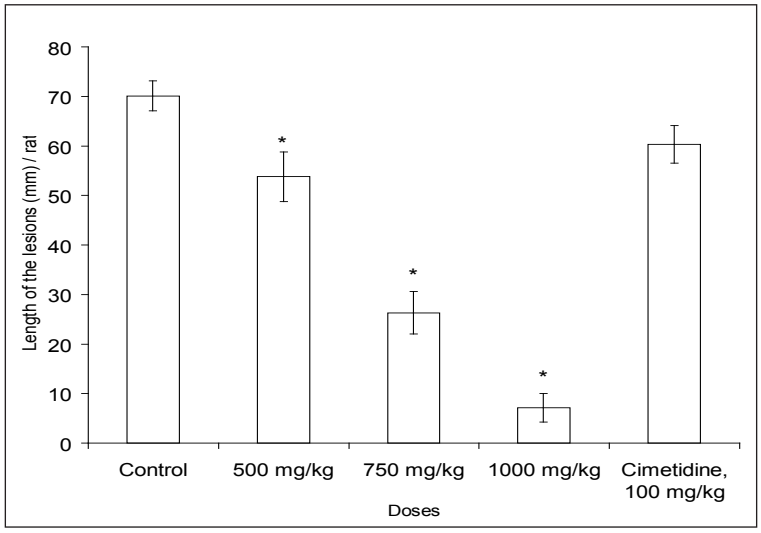

Figure 1: Effects of hot water extract of *Alpinia calcarata* rhizome on the length of gastric lesions induced by absolute ethanol (means ± SEM, n = 9) ; * Significant at P < 0.05 as compared with control.

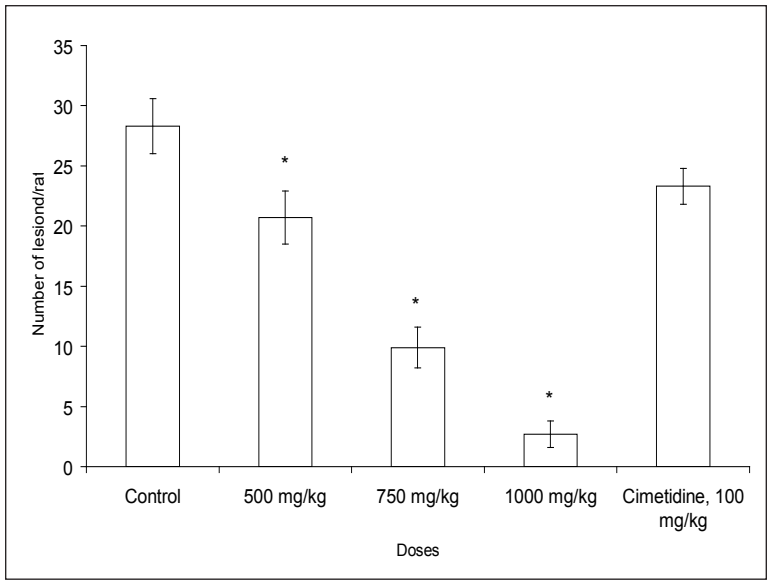

Figure 2.Effects of hot water extract of *Alpinia calcarata* rhizome on number of the gastric lesions induced by absolute ethanol (means ± SEM, n = 9) ; * Significant at P < 0.05 as compared with control.

that of cimetidine, the reference drug which only inhibited the length and number of gastric lesions up to 14 % and 18 % respectively.

Acid secretion studies

In 4 h pylorus ligated rats, HWE caused a significant (P< 0.01) reduction in volume of gastric juice (by 41 %), free acidity (by 49 %) and total acidity (by 21 %) and significant (P< 0.01) increase in pH value (by 45 %) (Table 1).

Gastric mucus studies

There was no significant (P > 0.05) effect of HWE on changes in the amounts of mucus content that adhered to gastric mucosa in 4 h pylorus ligated rats (control vs treatment: 151.0 ± 9.1 vs 161.8 ± 10.9 µg/stomach).

Antihistamine effect

Compared to the control, there was a significant (P< 0.01) reduction in the wheal area of the HWE treated rats (by

Table 1. Effect of hot water extract of *Alpinia calcarata* rhizome on gastric juice volume, pH, free acidity and total acidity from pylorous ligated rats (means SEM, n = 6)

Treatment	Volume of gastric juice (ml)	pH	Free Acid ($molL^{-1}$)	Total Acid ($molL^{-1}$)
Control (1 mL of DW)	4.5±0.31	3.5±0.19	$0.043 \pm 4.7 \times 10^{-3}$	$0.076 \pm 3.9 \times 10^{-3}$
Hot water extract	2.5±0.30*	6.0±0.17*	$0.022 \pm 2.0\ 10^{-3}$*	$0.060 \pm 3.5 \times 10^{-3}$*

*Significant at $P < 0.05$ as compared with control

Table 2: Antihistamine activity of hot water extract of *Alpinia calcarata* rhizomes as indicated by the area of the wheal (means SEM, n = 7)

Treatment	Area of the wheal (mm^2)
Control (1 mL DW)	69.0 ± 4.3
Hot water extract (1000 mg/kg)	51.3 ± 2.7 *
Chlorpheniramine (0.67 mg/kg)	40.3 ± 2.8 *

*Significant at $P < 0.05$ as compared with control

26 %). However, this effect was inferior to that of the antihistamine drug, chlorpheniramine by 15 % (Table 2).

Toxicological studies

There were no treatments – related deaths or morbidity or any overt signs of toxicity, stress or aversive behaviors with HWE. Further, HWE treated rats showed normal food intake, water intake, and their % weight gains were not significantly ($P > 0.05$) reduced (data not shown). The consistency of faeces and color of urine of HWE treated rats were essentially similar to that of control. There was no significant ($P > 0.05$) change in any of the serum or haematological parameters investigated and all the organs examined appeared normal in treated rats. There was no significant difference ($P > 0.05$) in the wet organ weights between the treated and control groups (data not shown).

DISCUSSION

The results of this study demonstrate that the HWE of *A. calcarata* rhizomes possesses marked gastroprotective properties as evidenced by its significant inhibition in the formation of gastric lesions (in terms of length and number) induced by absolute ethanol. Gastroprotective activity of HWE was superior to that of reference drug, cimetidine. The dose response curve was linear and ID_{50} value was 705 mg/kg. It is generally believed that enhanced acid secretion is the most important factor for the induction of gastric lesions. In this study, HWE caused significant inhibition in the volume of gastric fluid, acidity (both total and free) with an elevation in gastric pH. Similar mode of action has been reported with some plant extracts. For example, gastroprotective activity of *Mammea americana* (21), *Anisomeles indica* (22) and *Jasminum grandiflorum* (23). As compared with the control, treatment with HWE also significantly reduced the area of the wheal formed on the rat skin by the injection of histamine indicating an antihistamine activity. These suggest that inhibition of total and free acidity by HWE mediated via histamine receptors; histamine receptor blockers are therapeutically used to inhibit acid secretion in the gastric mucosa (24).

Mucus layer is considered to play a key role in gastroprotection as well as in facilitation of gastric mucosal repair (25). Further, drugs that enhance mucosal layer are known to exert gastroprotection, for example, plant extracts such as oleoresin of *Copaifera langsdorffii* (26) and hot water extract of *Ruellia tuberosa* root (27). However, the highest dose of HWE failed to significantly increase the mucus content adhered to gastric mucosa. This suggests that HWE does not mediate gastroprotection by this mechanism.

Active oxygen species are involved in the pathogenesis of gastric mucosal injuries. Antioxidants are known to induce gastroprotection (28). HWE also has antioxidant activity (11). Flavonoids (29 – 30) and alkaloids (31 – 32) are known to possess potent antioxidant activity in plants. *A. calcarata* rhizomes are reported to possess flavonoids and alkaloids (10). Therefore, it is possible that antioxidant mechanisms also play a role in mediation of gastroprotective effect of *A.calcarata* rhizome. HWE was devoid of unacceptable side-effects following sub chronic administration; there were no overt signs of toxicity, hepatotoxicity (in terms of AST, ALT) or renotoxicity (as judged by serum urea and creatinie).

In conclusion, our results demonstrate the gastroprotective activity of *A.calcarata* rhizomes for the first time and indicate its therapeutic potential to be used as a cost effective safe herbal gastroprotective agent.

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