Gastroprotective activity of hot ethanolic extract of *Alpinia calcarata* rhizome in rats

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Abstract

A study to evaluate the gastroprotective activity of hot ethanolic extract (HEE) of *Alpinia calcarata* Roscoe (Zingiberaceae) rhizomes was carried out in rats. Three doses (500, 750, 1000 mg/kg) of HEE were evaluated for gastroprotective activity against ethanol induced gastric ulcers. Oral administration of HEE provided dose dependent and significant ($P < 0.05$) protection against gastric damage caused by ethanol. The gastroprotective effect of HEE was superior than cimetidine, the reference drug. HEE had strong antihistamine and antioxidant activities, which could have played an active role in inducing gastroprotection. HEE was devoid of unacceptable side effects: no overt signs of toxicity, hepatotoxicity, renotoxicity or haemotoxicity were observed up to 42 days. However, the weight of the spleen was increased in treated groups possibly indicating lymphoproliferative activity. It is concluded that HEE of *A. calcarata* rhizome has gastroprotective activity in rats. No immediate dose related serious toxicity was observed.

Key words: *Alpinia calcarata*, gastro-protection, antihistamine, antioxidant, safety profile.

Introduction

Alpinia calcarata Roscoe (Zingiberaceae, Heen-araththa in Sinhala and Amkolinji in Tamil) is a rhizomatous perennial herb with a tuberous root stock. The mature rhizomes are branched and dense with a light to dark brown color. *A. calcarata* is distributed among the Asian countries including Sri Lanka, India and Malaysia (1,2). Some diterpenes such as calcaratarins A – E, sesquiterpenes such as shyobunone and coumarins such as herniarin were isolated from the rhizomes of *A. calcarata* grown in China (3,4). Merh and co-workers (5) isolated some benzenoids such as protocatechuic acid, vanillic acid, syringic acid, flavonoids and alkaloids from the leaves of *A. calcarata* grown in India. We have isolated 18 volatile constituents in essential oils of Sri Lankan grown *A. calcarata* rhizomes, roots and leaves (6). 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.

The rhizome of *A. calcarata* is known to possess a broad spectrum of medicinal and pharmacological properties (7). In Sri Lankan traditional medicine rhizomes of *A. calcarata* are recommended as an aphrodisiac and a decoction is widely used

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in the treatment of bronchitis, cough, respiratory ailments, diabetes, asthma and arthritis (7,8). Pharmacological investigations carried out on extracts of *A. calcarata* revealed the presence of antibacterial (9), antifungal (10), anthelmintic (11) and antinociceptive (12) activity. There are no available scientific reports in literature on the traditional or experimental claims of this plant as regards to its antiulcer potential. However, it is possible that *A. calcarata* rhizomes possess gastroprotective properties as phenylpropanoids of *A. galanga* rhizome (13) a close relative of the plant, is reported to have gastroprotective properties. Therefore, the present study was carried out to investigate the gastroprotective activity of hot ethanolic extract (HEE) of *A. calcarata* rhizomes. In addition, the safety profile of HEE was also evaluated.

Material and Methods

Fresh *A. calcarata* rhizomes were collected from the Western Province, Sri Lanka between August-November 2003. The plant material was identified and authenticated by Mr. D. H. P. Peramunagama, 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 ethanolic extract (HEE)

Fresh *A. calcarata* rhizomes were cut into small pieces and air dried in the shade. Five hundred grams of powdered rhizomes were extracted with 1.5 L of ethanol using soxhlet extraction apparatus for 4 h. The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure (yield 18.5% w/w dry weight basis). Polyvinyl-pyrrolidone (PVP; MW - 44,000) co-precipitate of the

extract was prepared by mixing crude ethanolic extract (1.0 mg/ mL in ethanol and PVP in the ratio of 1: 1 (w/w).

Administration of extracts

Doses of 500, 750 and 1000 mg/kg of HEE were prepared in 1 mL of distilled water (DW) and given orally to the test groups (n = 9/group) of rats. As the control, 1000 mg/kg of PVP in 1 mL of DW was given orally to a separate group (n = 9/group) of rats. These doses were identical to those used in the investigation of antinociceptive activity of rhizomes of *A. calcarata* (12). Further toxicological studies were carried out using HEE at a dose of 1500 mg/kg.

Phytochemical screening

HEE was subjected for qualitative testing for alkaloids, steroids, coumarins, reducing sugars and flavonoids as described by Farnsworth (14).

Standardization of the extract

Two patterns of TLC fingerprints were developed for HEE using non polar (hexane 1: chloroform 1) and polar (methanol 1: chloroform 2: hexane 3) solvent systems respectively. These TLC fingerprint patterns were quantitatively analyzed at λ 254 nm using a densitometer (CS - 9301PC, Shimadzu, Japan).

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 coprophagy) under standardized animal house conditions with free access to pelleted food (Vet House Ltd., Colombo, Sri Lanka) and tap water.

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 ($n = 9/\text{group}$) and treated orally in the following manner: each rat in group 1 received (1000 mg/kg of PVP in 1 mL of DW), groups 2, 3, 4 (500, 750 and 1000 mg/kg of HEE) and group 5 100 mg/kg of cimetidine, the reference drug (15). 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 killed with an overdose of diethyl ether, their 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. Sliminess of gastric content was observed (subjectively) in both control and treated groups. 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).

Antihistamine activity

Fourteen 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 2 equal groups ($n = 7$) and treated orally in the following manner. Group 1 received 1000 mg/kg of HEE in 1 mL of DW and group 2, 1 mL of DW. After 1 h, 0.05 mL of 200 $\mu\text{g}/\text{mL}$ of histamine dihydrochloride was subcutaneously injected under mild ether anesthesia in the area of the skin where the fur was removed previously (17). The radius of the wheal formed was determined after 2.5 min. and the area was computed.

Evaluation of antioxidant activity

Antioxidant activity of HEE was assessed using thiobarbituric acid reactive substances (TBARS) assay as described by Dorman and co-workers (18). As the positive control, butylated hydroxy toluene (BHT) was used. The absorbance was measured at λ 532 nm and the % antioxidant index (AI %) was calculated.

Toxicological studies

The healthy male rats were randomly divided into two groups ($n = 9/\text{group}$) and treated orally in the following manner. One group was given 1 mL of DW and other group given 1500 mg/kg of HEE per day for 42 consecutive days. The treatments were given between 10.00 h – 11.00 h daily. 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 exophthalmia), aversive behaviors (biting paw and penis, intense grooming behavior, scratching behavior, licking at tail or vocalization) and mortality. Percentage weight gain and, 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 2 mL of blood was collected from the tail of both DW and HEE 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 techniques (19). 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., Antrim, UK) and a spectrophotometer (JascoV500, Jasco Corporation, Tokyo, Japan).

After drawing blood rats were killed 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 $\pm$ 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. ID₅₀ values were determined graphically and dose dependencies were determined by regression coefficient (r).

Results

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, steroids, coumarins, reducing sugars and flavonoids in the extract.

Standardization of extract

Two TLC fingerprint patterns were obtained for HEE using non polar (Figure 1) and polar (Figure 2) solvent systems respectively.

A. TLC fingerprint

B. Densitogram

Figure 1. TLC and densitogram for HEE in hexane: chloroform (1:1) mixture

Figure 2. TLC and densitogram for HEE in methanol: chloroform: hexane (1:2:3) mixture

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

Figure 3. Effects of hot ethanolic extract (HEE) of *A. calcarata* rhizome on the length of gastric lesions (in mm) and number of lesions induced by absolute ethanol (mean $\pm$ S.E.M. $n = 9$)

Experimentally induced gastric lesions

HEE of *A. calcarata* rhizome caused a significant ($P < 0.01$) inhibition of the length and the number of gastric lesions (Figure 3) induced by absolute ethanol in a dose dependent ($r = 0.98$) manner. Among the tested doses, high dose showed the maximum inhibition of the length (by 92%) and number of gastric lesions (by 87%) followed by mid dose (length by 72%, number by 73%) and low dose (length by 39%, number by 27%). The gastroprotective activity of 1000 mg/kg dose of HEE was significantly ($P < 0.01$) higher than cimetidine, the reference drug which only inhibited the length and number of gastric lesions by 14% and 18% respectively. There was no apparent sliminess in gastric contents of treated and control groups.

Antihistamine effect

Compared to the control, there was a significant ($P < 0.01$) reduction in the wheal area of the HEE treated rats (control vs treatment: 69.0 ± 4.3 vs 47.4 ± 2.1 mm²).

Antioxidant activity

AI % of HEE was comparable to synthetic antioxidant BHT (HEE vs BHT: 31.4 ± 0.4 vs 34.7 ± 0.2).

Toxicological studies

There were no treatment related deaths or morbidity. Further HEE treated rats showed normal food intake (control vs HEE: 13.4 ± 1.1 g vs 15.3 ± 1.7 g), water intake (control vs HEE: 24.5 ± 3.0 vs 26.7 ± 2.7 mL) and their % weight gain (control vs HEE: 26.1 ± 2.5 vs 27.5 ± 3.1 %) was not significantly ($P > 0.05$)

altered. The consistency of faeces and colour of urine of HEE treated rats were similar to that of control. There were no overt signs of toxicity, stress or aversive behaviors in HEE extract treated rats.

There was no significant ($P > 0.05$) change in any of the serum parameters (Table 1) and haematological parameters (Table 2) investigated. All organs appeared normal in all treated rats. There was no significant alternation ($P > 0.05$) in the organ weights between the treated groups except for the spleen (Table 3) where there was a significant increase (by 117%) in HEE treated rats (control vs HEE: 0.24 ± 0.01 vs 0.52 ± 0.03 g/100 g body weight, $P < 0.01$). Gastric lesions were not observed in any of the treated rats.

Discussion

The results of this study demonstrate that the HEE of *A. calcarata* rhizomes has gastroprotective activity as evidenced by its significant inhibition in the formation of gastric lesions (in terms of length and number) induced by ethanol. Gastro protective activity of 1000 mg/kg of HEE was superior by 6 fold than the reference drug, cimetidine. The dose response curve was linear and ID₅₀ value was 428.2 mg/kg. As compared with the control, treatment with HEE significantly reduced the area of the wheal formed on the rat skin by the injection of histamine indicating an antihistamine activity. Histamine receptor blockers are used to inhibit gastric acid secretion (20) and are used in the treatment of gastric ulceration. Therefore, it is possible that HEE offers gastroprotection by impairing acid secretion.

Table 1. Effect of 6 weeks of oral administration (1500 mg/kg per day) of hot ethanolic extract (HEE) of *A. calcarata* rhizome on some selected serum parameters of rats (mean $\pm$ S.E.M.)

Treatment	n	Creatinine (mg/dL)	Urea (mg/dL)	ALT (U/L)	AST (U/L)
PVP	9	0.75 $\pm$ 0.04	35.4 $\pm$ 2.4	19.1 $\pm$ 1.2	43.3 $\pm$ 4.7
HEE	9	0.71 $\pm$ 0.04	37.7 $\pm$ 1.3	21.1 $\pm$ 1.3	38.7 $\pm$ 2.1

Not significant at $P \leq 0.05$ level from the control group

PVP - Polyvinylpyrrolidone

ALT - alanine aminotransferase

AST - aspartate aminotransferase

Table 2. Effect of 6 weeks of oral administration (1500 mg/kg per day) of hot ethanolic extract (HEE) of *A. calcarata* rhizome on some selected blood parameters of rats (mean $\pm$ S.E.M.)

Treatment	n	Hb level g/dL	RBC count $\times 10^6 \text{ mm}^3$	WBC count $\times 10^3 \text{ mm}^3$
PVP	9	18.16 $\pm$ 0.1	6.81 $\pm$ 0.1	8.10 $\pm$ 0.3
HEE	9	18.22 $\pm$ 0.1	6.87 $\pm$ 0.1	8.12 $\pm$ 0.2

Not significant at $P < 0.05$ level from the control group

PVP - Polyvinylpyrrolidone

RBC - Red blood cell

Hb - Haemoglobin

WBC - White blood cell

Table 3. Effect of 6 weeks of oral administration (1500 mg/kg per day) of hot ethanolic extract (HEE) of *A. calcarata* rhizome on some selected wet organ weights of rats

Organ weights g/ 100g body weight												
Treatment	n	Liver	Kidneys	Adrenal glands	Testes	Heart	Spleen	Vasa diferentia	Prostate glands	Cauda epid.	Seminal vesicles	Caput + corpus epid.
PVP	9	3.95	0.37	0.01	0.53	0.31	0.24	0.03	0.21	0.09	0.60	0.10
		±	±	±	±	±	±	±	±	±	±	±
		0.09	0.01	0.01	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00
HEE	9	3.83	0.35	0.01	0.52	0.31	0.52*	0.04	0.25	0.08	0.62	0.11
		±	±	±	±	±	±	±	±	±	±	±
		0.13	0.01	0.00	0.02	0.00	0.03	0.00	0.01	0.00	0.04	0.00

*significant at $P \geq 0.05$ level from control group

PVP - Polyvinylpyrrolidone

Epid - Epididymis

Active oxygen species are involved in the pathogenesis of gastric mucosal injury (21) and antioxidants are known to have gastroprotective properties (22). This study has shown that HEE has strong antioxidant activity. Therefore, it is possible that antioxidant mechanisms also play a role in the gastroprotective effect of *A. calcarata* rhizome. Flavonoids (23,24) and alkaloids (25,26) are known to possess potent antioxidant activity and have been previously reported to have potent gastroprotective activity (21,27). Therefore, flavonoids and alkaloids, which are present in HEE, would be likely to contribute to its gastroprotective activity. Gastric mucus layer is considered to be important in the mucosal defense against endogenous aggressors such as acid and pepsin and also as an agent in facilitating its repair (28). Further, drugs that enhance the thickness of mucosal layer are known to exert gastroprotection; examples include, plant extracts such as oleoresin of *Copaifera langsdorffii* (29), hot water extract of *Ruellia tuberosa* root (30) and synthetic drugs such as sucralfate (15). Slimy content of plant extracts adhered to gastric mucosa and facilitate the gastroprotection. However, there was no apparent sliminess in gastric contents of treated groups. This suggests that HEE does not mediate gastroprotection by increasing the thickness of the gastric mucus layer. Slimy liquids could contribute to gastroprotection by adhering to the gastric mucus layer. The extract was not slimy and therefore, such a mechanism is unlikely to play a role in inducing gastroprotection.

HEE was devoid of unacceptable side effects following oral administration; there were no overt signs of toxicity, hepatotoxicity (in terms of AST, ALT), renotoxicity (as judged by serum urea and creatinine) or haematotoxicity (in terms of WBC, RBC counts and Hb concentration). The dose

used in toxicological studies, was more than 3 fold higher to the ID_{50} of HEE that induced gastroprotection. An interesting finding in our study was that HEE significantly increased the weight of the spleen, which suggests lymphoproliferative activity. Similar results have also been reported using *Withania somnifera*, which is used in the indigenous system of medicine (31).

In conclusion, our results demonstrate the gastroprotective activity of *A. calcarata* hot ethanolic extract. It may be possible to develop potential gastroprotective agents from *A. calcarata* rhizome.

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