



Safety profile of *Alpinia calcarata* Roscoe, used in traditional medicine in Sri Lanka

[Perfil de seguridad de *Alpinia calcarata* Roscoe usada en medicina tradicional en Sri Lanka]

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Abstract

The aim of the present study was to investigate whether *Alpinia calcarata* Roscoe (Family: Zingiberaceae) rhizomes have any toxic effects in rats. Wistar rats were used as the experimental model and orally administered hot water extract (HWE) and hot ethanolic extract (HEE) of *A. calcarata* rhizomes at a dose of 1500 mg/kg respectively for 42 consecutive days. Administration of the HWE or HEE to rats did not result in any chronic toxic effects as evident from their effects on (a) liver function (b) kidney function, (c) hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count and hemoglobin (Hb) concentration (d) external morphology and wet weights of selected organs. Further, the HWE and the HEE did not appear to mediate any unacceptable effects on food and water intake, % weight gain, consistency of faeces and color of urine. In conclusion, the results of this study have revealed that the HWE and the HEE of *A. calcarata* at the doses tested do not produce any serious toxic side effects in rats.

Keywords: *Alpinia calcarata*, renotoxicity, hepatotoxicity, hematology, organs, morphology

Resumen

El objetivo del presente estudio fue investigar si los rizomas de *Alpinia calcarata* Roscoe (Familia: Zingiberaceae) tienen algún efecto tóxico en las ratas. Se utilizaron ratas Wistar como modelo experimental y administrado por vía oral de extracto de agua caliente (EAC) y el extracto de etanol caliente (EEC) de *A. calcarata* rizomas a una dosis de 1500 mg / kg, respectivamente, durante 42 días consecutivos. La administración de la EAC o EEC a las ratas no produjo ningún efecto crónico tóxico como se desprende de sus efectos sobre la (a) función hepática (b) la función renal, (c) los parámetros hematológicos, como conteo de los glóbulos rojos (GR), conteo de glóbulos blancos celular (GB) y hemoglobina (Hb), (d) morfología externa y el peso húmedo de los órganos seleccionados. Además, el EAC y el EEC no parecen mediar efectos inaceptables en la ingesta de alimentos y agua, porcentaje de aumento de peso, la consistencia de las heces y el color de la orina. En conclusión, los resultados de este estudio han revelado que los extractos EAC y la EEC de *A. calcarata* en las dosis utilizadas no producen graves efectos secundarios tóxicos en ratas.

Palabras Clave: *Alpinia calcarata*, nefrotoxicidad, hepatotoxicidad, hematología, órganos, morfología.

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List of abbreviations: ALT - alanine aminotransferase, AST - aspartate aminotransferase, EDTA - ethylenediamine tetra-acetic acid, Hb - hemoglobin, HEE - hot ethanolic extract, HWE - hot water extract, PVP - polyvinylpyrrolidone, RBC - red blood cell, WBC - white blood cell

INTRODUCTION

Many of the drugs used today are based on folk remedies and subsequent ethnopharmacological studies. There are more than 100 drugs of known structures that are extracted from higher plants and used in allopathic medicine (Cox, 1994; Farnsworth, 1990). Different pharmaceutical dosage forms or preparations including decoctions, aromatic waters, extracts, infusions, ointment, powders, tinctures, etc. were originally designed to extract and concentrate the active drug principles like alkaloids, glycosides and volatile oils (Koul et al., 2005). The active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects (De Smet, 2004).

Alpinia calcarata Roscoe (Family: Zingiberaceae), is a rhizomatous perennial herb which is commonly used in the traditional medicinal systems in Sri Lanka. The mature rhizomes are branched and dense with a light to dark brown color (Jayaweera, 1982). Kong and co-workers (2000; 2002) have isolated some diterpenes such as calcaratarins A-E, sesquiterpenes such as shyobunone and coumarins such as herniarin from the rhizomes of *A. calcarata* grown in China. According to Arambewela and co-workers (2005a), 18 volatile constituents were identified in essential oils of *A. calcarata* rhizomes, roots and leaves. Further, 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.

A. calcarata rhizomes are known to possess a broad spectrum of medicinal properties. In Sri Lankan Traditional Ayurveda medicine, rhizomes of *A. calcarata* is recommended as an aphrodisiac and a decoction is widely used in the treatment of bronchitis, cough, respiratory ailments, diabetics, asthma and arthritis (Jayaweera, 1982; Ramanayake, 1994). For the preparation of decoction, 60 g of rhizomes in dried form is introduced into a clay pot and gently boiled in 1920 mL of water for about 3 h and the final volume reduced to 240 mL using a low flame. The dosage recommended for administration to an adult is a half a cup (120 mL) two times per day. Experimentally, *A. calcarata* rhizomes of Sri Lankan origin have shown

to possess antinociceptive (Arambewela et al., 2004), antioxidant (Arambewela and Arawwawala, 2005b), aphrodisiac (Ratnasooriya and Jayakody, 2006), gastroprotective (Arambewela et al., 2005c and 2009a) and antidiabetic (Arambewela et al., 2009b) activities of hot water extract (HWE) and hot ethanolic extract (HEE) of *A. calcarata* rhizomes. However, no extensive safety studies have been conducted on extracts of *A. calcarata* rhizomes to date. Therefore, the aim of the present study was to investigate whether hot water extract (HWE) and hot ethanolic extract (HEE) of *A. calcarata* rhizomes have any toxic effects in rats.

MATERIALS AND METHODS

Plant material

Fresh *A. calcarata* rhizomes were collected from home gardens in Western Province of Sri Lanka between the period of August - November. 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 the 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 of distilled water (DW) for 4 h. The hot water extract was concentrated under vacuum at 60° C and freeze-dried at - 20° C (yield 15.6 % w/w dry weight basis) and stored at 4° C until use.

Preparation of the hot ethanolic extract (HEE)

Fresh *A. calcarata* rhizomes were cut into small pieces and air dried for 5-6 days 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 at 50° C (yield 18.5% w/w dry weight basis) and stored at 4° C until use. Polyvinylpyrrolidone (PVP; MW-44,000) co-precipitate of the extract was prepared by mixing crude ethanolic extract and PVP in the ratio of 1:1 (w/w).

Animals

Healthy adult male Wistar rats (weighing 200-225 g) were used throughout the investigation. They were housed under standardized animal house conditions (temperature: 28-31° C, photoperiod: approximately

12 h of natural light per day, relative humidity 50–55%) and fed with standard rat and water ad libitum. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guide lines and rules of the Faculty of Science, University of Colombo, for animal experimentation.

Experimental design

Thirty six male rats were weighed and randomly assigned into 4 equal groups (n = 9/group). The rats in group 1 were orally treated with 1500 mg/kg of HWE in 1 mL of DW while the rats in group 2 orally treated with 1 mL of DW (served as the control group 1). Rats in group 3 were orally treated with 1500 mg/kg of HEE while the rats in group 4 orally treated with 1500 mg/kg of PVP in 1 mL of DW (served as the control group 2) for 42 consecutive days between 10.00 h – 11.00 h.

In our previous investigations we have used doses ranging from 100 – 1000 mg/kg from HWE and HEE of *A. calcarata* to investigate antinociceptive (Arambewela et al., 2004), aphrodisiac (Ratnasooriya and Jayakody, 2006), gastroprotective (Arambewela et al., 2005c and 2009a) and antidiabetic (Arambewela et al., 2009b) activities *in vivo*. Therefore, in the present study, tested dose level is 2 to 6 fold higher than that have been used in investigation of biological activities.

Determination of general toxic effects

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.

Determination of effects on hematological parameters, renal and hepatic functions

On day 1 post treatment, approximately 4 mL blood was collected from the tail of the treated rats under mild ether anesthesia and divided into two equal parts. To one part ethylenediamine tetra-acetic acid (EDTA) was added and red blood cell (RBC) counts, white blood cell (WBC) counts and hemoglobin (Hb) concentration were determined using standard

procedures (Ghai, 1993). 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. Finally, serum samples were analyzed for concentrations of urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Effects on external morphology and wet weights of selected organs

On day 1 post treatment rats were sacrificed with over dose of ether 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.

Effects on histopathology of selected organs

Small pieces of organs such as heart, intestine, kidney, liver were fixed in 10% formalin and routinely processed for histopathology. These pieces were embedded in paraffin and cut into 7 µm thick sections. Sections were stained with hematoxylin and eosin and mounted between a slide and cover slip for microscope.

Statistical analysis

Statistical comparisons were made using one way ANOVA followed by Tukey's family error test. A *P* value ≤ 0.05 was considered as significant.

RESULTS

General toxic effects

There were no treatments – related deaths with both extracts. Further, HWE and HEE treated rats showed normal food intake, water intake, and their % weight gain were not significantly (*P* ≥ 0.05) altered (Table 1). The consistency of faeces and colour of urine of HWE and HEE treated rats were similar to that of respective control groups. There were no overt signs of toxicity, stress or aversive behaviors in *A. calcarata* extracts treated rats.

Effects on hematological parameters, renal and hepatic functions

Compared to the respective controls neither HWE nor HEE had significant (*P* ≥ 0.05) effects on RBC and

WBC counts, concentrations of Hb, creatinine, urea, ALT or AST (Table 2 and Table 3).

Table 1

Effects of the hot water extract (HWE) and the hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes on food and water consumption and body weight gain in rats treated for 6 weeks (mean \pm S.E.M., n = 9)

Treatment	n	Food intake/week (g)	Water intake/week(mL)	Body weight gain (%)
C ₁ (1 mL of DW)	9	12.7 \pm 1.0	22.0 \pm 1.8	28.2 \pm 2.0
HWE	9	14.5 \pm 0.9	27.1 \pm 2.3	26.4 \pm 2.3
C ₂ (1500 mg/kg of PVP in 1 mL of DW)	9	13.4 \pm 1.1	24.5 \pm 3.0	26.1 \pm 2.5
HEE	9	15.3 \pm 1.8	26.7 \pm 2.7	27.5 \pm 3.1

C₁: Control for the HWE; C₂: Control for the HEE; DW: Distilled water; PVP: Polyvinylpyrrolidone
Not significant with the respective control groups; $P \geq 0.05$

Table 2

Effects of the hot water extract (HWE) and the hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes on some selected blood parameters in rats treated for 6 weeks (mean \pm S.E.M., n = 9)

Treatment	n	RBC count $\times 10^6 \text{ mm}^3$	WBC count $\times 10^3 \text{ mm}^3$	Hb concentration g/dL
C ₁ (1 mL of DW)	9	6.90 \pm 0.08	8.00 \pm 0.14	18.27 \pm 0.19
HWE	9	6.99 \pm 0.07	8.16 \pm 0.12	18.11 \pm 0.21
C ₂ (1500 mg/kg of PVP in 1 mL of DW)	9	6.81 \pm 0.07	8.10 \pm 0.28	18.16 \pm 0.11
HEE	9	6.87 \pm 0.11	8.12 \pm 0.21	18.22 \pm 0.11

Hb: Hemoglobin; RBC: Red blood cell; WBC: White blood cell; C₁: Control for the HWE; C₂: Control for the HEE; DW: Distilled water; PVP: Polyvinylpyrrolidone. Not significant with the respective control groups; $P \geq 0.05$

Table 3

Effects of the hot water extract (HWE) and the hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes on renal function and hepatic function in rats treated for 6 weeks (mean \pm S.E.M., n = 9)

Treatment	n	Creatinine (mg/dL)	Urea (mg/dL)	ALT (U/L)	AST (U/L)
C ₁ (1 mL of DW)	9	0.74 \pm 0.05	31.9 \pm 2.7	16.8 \pm 1.0	37.0 \pm 2.3
HWE	9	0.71 \pm 0.04	34.7 \pm 3.9	17.5 \pm 1.1	41.1 \pm 3.5
C ₂ (1500 mg/kg of PVP in 1 mL of DW)	9	0.78 \pm 0.03	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

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; C₁: Control for the HWE; C₂: Control for the HEE; DW: Distilled water; PVP: Polyvinylpyrrolidone. Not significant with the respective control groups; $P \geq 0.05$

Effects on external morphology and wet weights of selected organs

All the organs examined appeared normal in treated rats. There were no significant ($P \geq 0.05$) alternations in the organ weights between the treated groups except for the spleen (Table 4). In both treatment groups, significant ($P \leq 0.05$) increase in weight of the spleen was evident when compared with respective control groups (HWE: control₁ vs. treatment: 0.23 \pm 0.00 vs 0.42 \pm 0.02 g/100 g body weight, by 83%; HEE: control₂ vs. treatment: 0.25 \pm 0.01 vs. 0.52 \pm 0.03 g/100 g body weight, by 108%).

Effects on histopathology of selected organs

The observed tissues of the selected organs were devoid of any abnormalities (Figure 1).

DISCUSSION

Nature has provided a complete storehouse of remedies to cure ailments of mankind. Medicinal plants have been used for centuries as remedies for disease because they contain component of therapeutic values (Kumar and Chandrashekar, 2011). However, the usefulness of any drug depends not only on its therapeutic efficacy but also on its lack of toxicity or adverse side effects. In the present study, *A. calcarata* extracts were devoid of unacceptable side effects even after following chronic administration in rats. There

were no overt signs of toxicity, stress, aversive behaviors. The food and water intake, % weight gain, consistency of faeces and color of urine of HWE and HEE treated rats were similar to that of respective controls.

The results obtained for hematological parameters, showed that the blood counts of HWE or HEE treated rats remained similar to that of respective control groups. An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. The most sensitive and widely used liver enzymes are the aminotransferase which include AST and ALT. These enzymes are normally found within liver cells. If the liver is injured, the liver cells spill the enzymes into blood which in turn raises the enzyme levels in the blood and signals the liver damage (Champe and Harvey, 1994). As Compared to the respective control groups, the concentrations of ALT and AST were normal in the treated groups. Therefore, *A. calcarata* extracts were devoid of any hepatic damage. Similar results were obtained with extracts of *Ocimum gratissimum* leaves (Obianime et al., 2011) and *Trichosanthes cucumerina* aerial parts (Arawwawala et al., 2011). However, some plants such as *Albizzia chevalieri* (Saidu et al., 2007) and *Salvia verticillata* (Eidi et al., 2011) may cause liver damage leading to hepatocellular carcinoma.

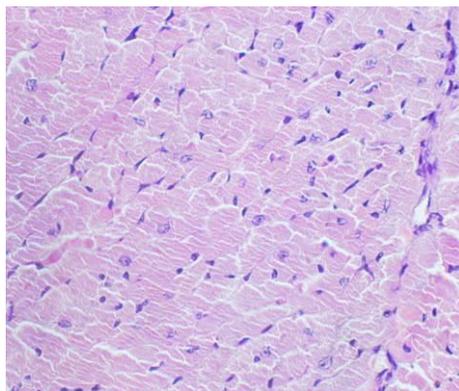
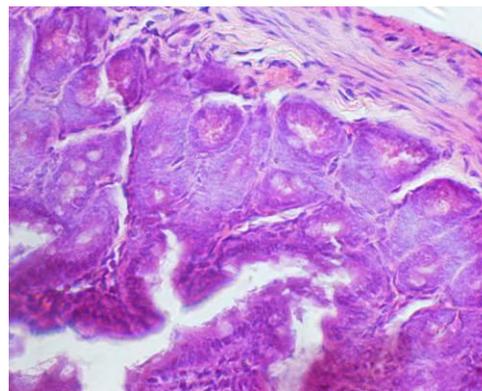
Table 4

Effects of the hot water extract (HWE) and the hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes on some selected wet organs of rats treated for 6 weeks (mean \pm S.E.M., n = 9)

Organ weights g/100g body weights											
Treatment	Liver	Kidneys	Adrenal glands	Testes	Heart	Spleen	Vasa diferentia	Prostate glands	Cauda epid.	Seminal vesicles	Caput \pm corpus epid.
C ₁ (1 mL of DW)	4.13 \pm 0.11	0.35 \pm 0.00	0.01 \pm 0.00	0.51 \pm 0.00	0.30 \pm 0.02	0.23 \pm 0.00	0.03 \pm 0.02	0.25 \pm 0.00	0.07 \pm 0.00	0.61 \pm 0.02	0.10 \pm 0.00
HWE	4.10 \pm 0.11	0.36 \pm 0.01	0.01 \pm 0.00	0.55 \pm 0.02	0.30 \pm 0.00	0.42 \pm 0.02*	0.03 \pm 0.00	0.24 \pm 0.02	0.07 \pm 0.00	0.60 \pm 0.03	0.11 \pm 0.00
C ₂ (1500 mg/kg of PVP in 1 mL of DW)	3.95 \pm 0.09	0.37 \pm 0.01	0.01 \pm 0.00	0.53 \pm 0.02	0.31 \pm 0.00	0.25 \pm 0.01	0.03 \pm 0.00	0.21 \pm 0.00	0.09 \pm 0.00	0.60 \pm 0.00	0.10 \pm 0.00
HEE	3.83 \pm 0.13	0.01 \pm 0.01	0.01 \pm 0.00	0.52 \pm 0.02	0.31 \pm 0.00	0.52 \pm 0.03*	0.04 \pm 0.00	0.25 \pm 0.01	0.08 \pm 0.00	0.62 \pm 0.04	0.11 \pm 0.00

C₁: Control for the HWE; C₂: Control for the HEE; DW: Distilled water; PVP: Polyvinylpyrrolidone; Epid – Epididymides.

*Significant at $P \leq 0.05$ level with respective control group

**(A)****(B)**

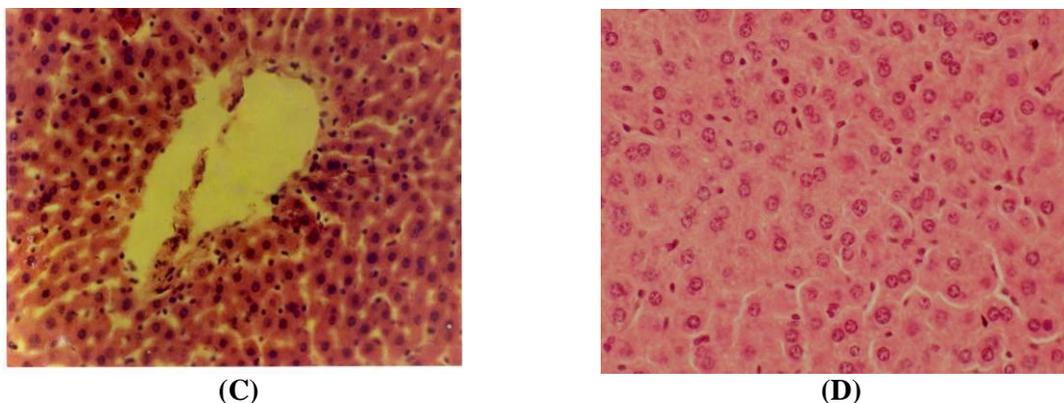


Figure 1

A photomicrograph of a transverse sections of the (A) heart (B) intestine (C) kidney (D) liver of a rat treated with hot water extract (HWE) of *Alpinia calcarata* rhizomes for 42 days
(Similar results were obtained to hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes)

Creatinine is a protein produced by muscle and released into the blood. If kidney function falls, the creatinine level will rise. On the other hand, urea is the major end product of protein nitrogen metabolism. The determination of serum urea nitrogen is an important index of kidney function. Impaired renal function or increased tissue protein breakdown are associated with increased urea nitrogen levels whereas kidney damage or pregnancy are associated with decreased levels (Champe and Harvey, 1994). In this study, the creatinine and urea parameters were normal compared to that of respective controls. Therefore, this indicated that *A. calcarata* extracts were devoid of renotoxicity.

An interesting finding in our study was that both extracts significantly increased the weight of the spleen, which possibly suggests lymphoproliferative activity. This is novel and clinically important finding of the plant. Similar results have also been reported with *A. galanga* (Bendjeddou *et al.*, 2003) a close relative plant of *A. calcarata* and also with *Withania somnifera* (Davis and Kuttan, 2000) which is used in the indigenous system of medicine. In contrast, administration of methanolic extract of *Tylophora asthmatica* leaves for 15 consecutive days in rats significantly decreased the weights of spleen (Malathi and Gomez, 2008). It is generally accepted that the active principles (whether natural or synthesized) may be more toxic than the whole extract or its crude form (Saxena, 1985). In crude preparations, perhaps, the other components that are present in addition to the active components may be influencing the effects of the active components as well as the other toxic

components in the crude preparation (Koul *et al.*, 2005). Therefore, crude plant extracts do not usually demonstrate adverse effects. This may be the reason for the safety of *A. calcarata* extracts indicated in this study.

In conclusion, *A. calcarata* HWE and HEE were found to be safe in terms of hepatotoxicity (as judged by AST, ALT levels), renotoxicity (as judged by urea and creatinine levels) or hematotoxicity (as judged by WBC, RBC counts and Hb concentration), gross morphology, weights of organs, stress or aversive behaviors at a dose level which is 2 to 6 fold higher than that have been used in investigation of biological activities.

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