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Hypoglycemic and Antihyperglycemic activities of the aqueous and the ethanolic extracts of *Alpinia calcarata* rhizomes in rats

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ABSTRACT

Rhizomes of *Alpinia calcarata* Roscoe (Family: Zingiberaceae) is a common medicinal plant cultivated in Asian countries including Sri Lanka. The aim of this study is to evaluate the hypoglycemic and antihyperglycemic activities of *A. calcarata* which are not investigated so far. This was tested in normoglycemic and streptozotocin (STZ) – induced diabetic rats using oral administration of the hot water extract (HWE) and the hot ethanolic extract (HEE). In normoglycemic rats both HWE and HEE significantly lowered the blood glucose levels in a dose - dependent manner. Further, both HWE and HEE markedly improved the oral glucose tolerance in rats. The hypoglycemic activity of the HEE was generally higher than that of the HWE. However, the HWE or the HEE failed to reduce blood glucose levels of STZ – induced diabetic rats. Further, the HEE significantly inhibited the glucose absorption from the small intestine and increased the glycogen accumulation in both liver and skeletal muscle. It is concluded that *A. calcarata* rhizomes possess strong hypoglycemic and antihyperglycemic activities.

Keywords: *Alpinia calcarata,* hypoglycemic and antihyperglycemic.

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder affecting approximately 4% population worldwide and is expected to increase by 5.4 % in 2025 (1). It is characterized by abnormalities in carbohydrate, lipid and lipoprotein metabolism, which not only leads to hyperglycemia but also cause many complications such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis (2–3). Before the discovery of insulin in 1922, the only treatment options for diabetes were those based on traditional practices. Ethnobotanical knowledge played a particularly important role in historical diabetes therapies, with over 1200 species of medicinal plants recognized throughout the world for their ability to treat diabetic indications (1, 4). In Sri Lanka too, aqueous extracts of several plant species are recommended for the control of blood glucose levels in diabetic patients (5), despite paucity of evidence from scientifically controlled trials to validate the claimed therapeutic effects or to determine potential risks of treatment with such products.

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. The leaf of the plant is simple, alternative, 25 – 32 cm long and 2.5 – 5 cm broad (6, 7). *A. calcarata* is cultivated in tropical countries including Sri Lanka, India and Malaysia (7). 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 (8, 9). Further, benzenoids such

as protocatechuic acid, vanillic acid and syringic acid, flavonoids and alkaloids were isolated from the leaves of *A. calcarata* grown in India (10). We have isolated 18 volatile constituents in essential oils of Sri Lankan grown *A. calcarata* rhizomes, roots and leaves (11). 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 (11).

Experimentally, rhizomes of *A. calcarata* are shown to possess antibacterial (12), antifungal (13), anthelmintic (14), antinociceptive (15), antioxidant (16), aphrodisiac (17) and gastroprotective (18) activities. The rhizomes of *A. calcarata* is considered as aphrodisiac (19) and used in the treatment of arthritis (20) bronchitis, cough, respiratory ailments, asthma and diabetics (19). The present study was conducted to evaluate the hypoglycemic and antihyperglycemic potential of *A. calcarata* rhizomes, using rats as the experimental model. Both normoglycemic and streptozotocin (STZ) – induced diabetic rats were used for the investigation.

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 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 12 –14 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 (1.0 mg/ mL in ethanol) and PVP in the ratio of 1: 1 (w/w).

Administration of extracts

Doses of 250, 500, 750, 1000 and 1500 mg/kg of the HWE and the HEE were orally administered by gastric gavage (each dose in a volume of 1 mL DW) to separate groups (n = 12 or 6/group/extract) of rats. The doses tested for the hypoglycemic and antihyperglycemic activities were similar to those used in the investigation of antinociceptive activity of rhizomes of *A. calcarata* (15).

Animals

Healthy adult cross- bred male albino rats (weighing 180 – 200 g) were used throughout the experiment. They were housed under standard environmental 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.

Phytochemical screening of the HWE and the HEE

Qualitative testing of the HWE and the HEE for alkaloids, polyphenols, flavonoids, steroids, saponins and tannins was carried out according to the method described by Farnsworth (21).

Effects of the HWE and the HEE on fasting blood glucose levels

Seventy two rats were fasted overnight for 12 h, but water was allowed. Using aseptic precautions, under light ether anesthesia blood was collected from their tails. Immediately afterwards, these rats were divided randomly into 6 groups and treated orally (n = 12/group) in the following manner. Each rat in group 1 received 1 mL of DW while rats in groups 2, 3, 4, 5 and 6 received 250, 500, 750, 1000 mg/kg of HWE and 22.5 mg/kg of tolbutamide (the reference drug) respectively. Blood samples were collected from the tails 2 h, 4 h and 6 h post treatment either with DW or HWE for the determination of serum glucose levels. To investigate the effect of HEE on fasting blood glucose levels above mentioned methodology was followed using the same four doses. Instead of DW, 1000 mg/kg of PVP in 1 mL of DW was given to the control group (n = 12/group).

Effects of the HWE and the HEE on oral glucose tolerance

This was investigated using 500 mg/kg of both HWE and HEE since the maximum hypoglycemic activity was

evident with this dose in normoglycemic fasted rats. In brief, sixty rats were fasted for 12 h and assigned randomly into 5 equal groups (n=12/group). These rats were orally treated in the following manner. Each rat in group1 and 2 received 1 mL of DW and 500 mg/kg of PVP in 1 mL of DW respectively and served as control groups. Rats in groups 3, 4 and 5 received 500 mg/kg of HWE, 500 mg/kg of HEE and 22.5 mg/kg of tolbutamide respectively. One hour later, all these rats were orally loaded with 5 mL/kg of 50% (w/v) glucose solution. Blood samples were collected from the tails of these rats immediately prior to commencement of treatment and at hourly intervals up to 3 h after glucose challenge.

Effects of the HWE and the HEE on blood glucose levels of streptozotocin (STZ) - induced diabetic rats

STZ (Sigma Chemical Company St. Louis MO, USA) was dissolved in 0.1M cold citrate buffer (pH=4.5). Immediately afterwards, 50 mg/kg dose of STZ was injected to the tail vein of the rat under mild ether anaesthesia with aseptic precautions (22). Seventy two hours later, blood samples were collected from tails of these rats and glucose levels were determined. Twenty four rats having blood glucose level > 200 mg/dL and showing polydipsia and polyuria were selected. These rats were assigned randomly to four equal groups (n=6/group) and treated in the following manner. Each rat in group 1 and 2 received 1 mL of DW and 500 mg/kg of PVP in 1 mL of DW respectively and served as control groups. Rats in groups 3 and 4 received 500 mg/kg of HWE and 500 mg/kg of HEE respectively. Blood samples were then collected from tails of these rats 2 h and 4 h post treatment and serum glucose levels were determined.

Blood sampling

In all these experiments approximately 1 mL blood was drawn each time from the tail using aseptic precautions and serum was separated immediately by centrifuging at 3000 rpm for 15 min. The glucose concentration in the serum samples was analyzed immediately by the glucose oxidase method using Randox assay kit (Randox Laboratories Ltd., Co. Antrium, UK) and a spectrophotometer (V500 Jasco Cooperation, Tokyo, Japan).

Determination of the mode of hypoglycemic and antihyperglycemic activities

This was investigated using the HEE since the hypoglycemic and antihyperglycemic activities were higher compared to the HWE. Further, 500 mg/kg was selected because the maximum hypoglycemic activity was evident with this dose.

Effect on glucose absorption from intestine

Twenty four male rats were fasted for 16 h and divided randomly into two equal groups (n=12/group). The HEE at a dose of 500 mg/kg was orally administered to one group and 500 mg/kg of PVP in 1 mL of DW to the other group. Thirty minutes later, 10 mL/kg of 50% glucose solution was given orally. Following 2 h, these rats were sacrificed and their small intestines were exposed. Fifty milliliters of DW was then infused from one cut end of the intestine and the content was collected at the other end. This was centrifuged at 3000 rpm for 5 min. and supernatant discarded (23). Glucose level in the supernatant was then estimated using Randox kit (Randox Laboratories Ltd., Co. Antrium, UK) spectrophotometer (V500 Jasco Cooperation, Tokyo, Japan).

Effects on liver and skeletal muscle glycogen content

Twelve rats were assigned randomly into two equal groups (n=6/group) and treated in following manner. Each rat in group 1 and group 2 received 500 mg/kg of HEE and 500 mg/kg of PVP in 1 mL of DW daily for 42 consecutive days. On day 1 post treatment, these rats were sacrificed by over exposure to diethyl ether and portions of their livers and skeletal muscles were removed and blotted free of blood. Glycogen content was determined using a spectrophotometric method as described in detail by Borst and co – workers (24). Briefly, 100 mg of each organ was digested with 2 mL of 30 % boiling KOH, and cooled. Three milliliters of 95 % ethanol was added and heated until bubbles were formed. These were cooled and centrifuge (at 1000 rpm for 5 min.) and supernatant discarded. The residue was dissolved in 5 mL of DW. Four milliliters of anthrone reagent was added and immersed in an ice bath, to prevent excessive heating. Tubes were incubated at 100 °C for 4 min. for color development and immersed in an ice bath. Absorbance was measured at λ 620 nm using a spectrophotometer (V500 Jasco Cooperation, Tokyo, Japan).

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).

Table 1: Effects of the hot water extract (HWE) and the hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes on blood glucose levels in normoglycemic rats (means ± SEM, n = 12/group)

Treatments	Glucose concentration (mg/dL)			
	Pre treatment	2 h	4 h	6 h
C_1(1 mL of DW/rat)	91.9 ± 2.1	91.2±1.9	86.7±1.5	88.2±1.7
HWE				
250 mg/kg/rat	88.7 ± 2.7	77.5±1.9*	83.3±1.6	87.5±1.6
500 mg/kg/rat	90.3 ± 1.5	60.1±1.3*	67.6±1.7*	72.4±1.8*
750 mg/kg/rat	88.1 ± 1.6	67.0±1.3*	73.3±1.5*	76.5±1.4*
1000 mg/kg/rat	89.6 ± 2.4	66.2 ±1.5*	73.2 ±1.7*	76.1±1.5*
C_2(PVP;500mg/kg/rat)	90.6 ± 2.4	89.0±1.9*	91.6±2.1*	93.2±1.4*
HEE				
250 mg/kg/rat	91.6 ± 2.1	70.9±1.6*	74.2±1.8*	83.8±2.0*
500 mg/kg/rat	90.8 ± 1.5	53.1±1.7 *	59.2±1.2*	62.6±1.1*
750 mg/kg/rat	95.1 ± 1.4	57.3±1.6*	62.5±1.5*	71.8±1.1*
1000 mg/kg/rat	89.6 ± 1.7	61.8±1.0*	69.6±1.6*	76.2±1.2*
Tolbutamide (22.5 mg/kg/rat)	91.1 ± 1.8	65.1±1.7*	71.9±1.5*	77.4±1.4*

*Significant when compared with respective controls: $P < 0.05$

C_1- Control for the HWE; C_2 - Control for the HEE

DW: Distilled water; PVP: Polyvinylpyrrolidone

RESULTS

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, steroids, saponins and tannins in the HWE and the HEE.

Effects on fasting blood glucose levels

The effects of the HWE and the HEE on the fasting blood glucose levels are shown in Table 1. All doses of the HWE significantly ($P < 0.05$) reduced the blood glucose levels up to 6 h except the lowest dose, which impaired the blood glucose level only up to 2 h. On the other hand, all doses of HEE significantly ($P < 0.05$) impaired blood glucose levels up to 6 h. This impairment of blood glucose levels of both extracts were marked and dose dependent (HWE: r^2; 2nd h: 0.7, 4th h : 0.7, 6th h: 0.8; HEE: r^2; 2nd h: 0.9, 4th h : 0.9, 6th h: 0.7) at each time points. The maximum hypoglycemic activity was induced by 500 mg/kg dose of both extracts at 2 h (HWE by 34%; HEE by 40%). Hypoglycemic activity of tolbutamide, the reference drug, was comparable to that of 500, 750 and 1000 mg/kg doses of HWE. However, hypoglycemia induced by 500 mg/kg (by 12, 18 and 21% at 2, 4 and 6 h respectively) and 750 mg/kg (by 7, 15 and 11% at 2, 4 and 6 h respectively) of HEE was superior to that of tolbutamide.

Effects on glucose tolerance test

Both HWE and HEE significantly ($P < 0.05$) improved the glucose tolerance test up to 3 h (Table 2). The HWE and the HEE showing approximately 16%, 18%, 20% and 21%, 19%, 23% reduction in glycemia from control values at the 1, 2 and 3 h respectively. Tolbutamide also improved the glucose tolerance in rats upto 3 h. This impairment was comparable to that of the HWE but was inferior to the HEE.

Table 2: Effects of the hot water extract (HWE) and the hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes on oral glucose tolerance in normoglycemic rats (means ± SEM, n = 12)

Treatments	Glucose concentration (mg/dL) Time following 50% oral glucose load			
	Pre treatment	1 h	2 h	3 h
C_1(1 mL of DW)	88.3 ± 2.2	159.2 ± 4.4	145.5 ± 4.1	134.0 ± 4.6
HWE 500 mg/kg/rat	92.3 ± 2.8	134.1 ± 2.3*	119.8 ± 2.4*	109.4 ± 2.7 *
C_2(PVP; 500mg/kg/rat)	88.0 ± 1.4	153.7 ± 2.2	144.5 ± 2.2	136.5 ± 1.9
HEE 500 mg/kg/rat	88.7 ± 1.2	121.7 ± 1.6 *	116.3 ± 2.1*	101.5 ± 2.7 *
Tolbutamide (22.5 mg/kg/rat)	89.4 ± 2.5	135.2 ± 2.7 *	124.9 ± 2.3*	106.6 ± 3.3 *

*Significant when compared with respective controls: $P < 0.05$

C_1- Control for the HWE; C_2 - Control for the HEE

DW: Distilled water; PVP: Polyvinylpyrrolidone

Effect of blood glucose levels on STZ - induced diabetic rats

As shown in Table 3, acute antidiabetic activity was not observed in STZ – induced diabetic rats treated with HWE and HEE.

Effect on glucose absorption from intestine

The HEE markedly (by 83 %) and significantly (P(0.05) inhibited the glucose absorption from the lumen of the intestine (control vs treatment: 33.4 ± 3.2 vs 61.2 ± 2.6 mg/ dL).

Effects of liver and skeletal muscle glycogen content

The HEE significantly ($P \leq 0.05$) increased the glycogen content of both skeletal muscle (by 77 %; control vs treatment: 69.4 ± 3.2 vs 122.9 ± 4.9 μg/100 mg) and liver (by 98 %; control vs treatment: 67.0 ± 4.1 vs 132.5 ± 7.1 μg/100 mg).

DISCUSSION

Overall results show that both HWE and HEE of *A. calcarata* rhizome possess marked hypoglycemic activity (when tested in fasted normoglycemic rats) and antihyperglycemic activity (by improvement of glucose tolerance in rats). The hypoglycemic and antihyperglycemic activities of the HEE was superior to that of the HWE and tolbutamide, reference hypoglycemic drug of sulphonylurea type (25). The hypoglycemic and antihyperglycemic activities of the HWE were comparable to that of the tolbutamide. On the other hand, both HWE and HEE failed to reduce blood glucose levels of STZ - induced diabetic rats at a dose which is known to irreversibly damage the insulin secreting β cells of the pancreas (22). This suggests that an intact endocrine pancreas and insulin are essential for antidiabetic activity of *A. calcarata* extracts. Inability of both extracts to drop blood glucose levels of STZ - induced diabetic rats also suggests that these extracts do not have insulinomimetic activity. However, it is possible that both HWE and HEE may act as a insulin secretagoue and/or sensitize insulin receptors as proposed for some sulphonylureas (26) and some plant extracts. Interestingly, similar mode of hypoglycemic activity is reported with methanolic and aqueous extracts of *A. galanga* rhizomes (27), a close relative of *A. calcarata*.

The HEE caused marked inhibition of glucose absorption from the lumen of the intestine. This could be one of the main mechanism through which the HEE induced hypoglycemia. Some herbal extracts (28 – 30) and biguanides (26) show a similar mode of action. Both extracts had gummy appearance and it is possible that impairment of intestinal glucose absorption is mediated via "fiber effect" as reported for *Sizigium cumini* (28), *Cassia fistula* (29) and *Syzygium jambos* (30). Alternatively, it may result from inhibition of intestinal Na^+- glucose cotransporter as reported with synthetic phlorizin derivatives (31). However, additional studies are required for conformation. Furthermore, the HEE induced an increase in the glycogen content both in the liver and skeletal muscle. This could be another mechanism by which the HEE impaired the blood glucose level. This increased glycogenesis may result from enhanced glucose up take into liver and skeletal muscle by sensitization of insulin receptors and/or inducing the activity of enzymes involved in glycogen synthesis. Some other plants such as *Hygrophila longifolia* (32), *Piper betle* (33), *Cinnamomum zeylanicum* (34) and *Trichosanthes cucumerina* (35) have also been reported to exert similar effects.

Polyphenols are plant compounds that can exert significant antioxidant activity, mainly due to their redox properties (36 – 37), which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Phenolic compounds (e.g. flavonoids, tannins) have also been reported to exhibit antidiabetic activity (38–40). Further,

Table 3. Effects of the hot water extract (HWE) and the hot ethanolic extract (HEE) of *Alpinia calcarata* rhizomes on blood glucose levels of streptozotocin – induced diabetic rats (means ± SEM, n = 6)

	Glucose concentration (mg/dL)		
Treatment	**Pre - treatment**	**2 h**	**4 h**
C_1(1 mL of DW)	228.0 ± 13.2	239.3 ± 10.8	242.9 ± 17.9
HWE 500 mg/kg/rat	236.0 ± 7.6	238.6 ± 10.2	248.6 ± 6.8
C_2(PVP; 500mg/kg/rat)	220.0 ± 8.2	232.3 ± 11.3	240.3 ± 12.8
HEE 500 mg/kg/rat	230.3 ± 6.1	235.6 ± 10.5	244.4 ± 9.4

Not Significant when compared with respective controls: $P > 0.05$

C_1- Control for the HWE ; C_2 - Control for the HEE

DW : Distilled water ; PVP : Polyvinylpyrrolidone

A. calcarata extracts had profound antioxidant activity *in vitro* (16). Therefore, antioxidant compound/s present in the HWE and the HEE may also play a major role in mediating the hypoglycemic and antihyperglycemic activities of *A. calcarata*. As reported in previous studies (18), *A. calcarata* extracts were devoid of unacceptable side - effects even following chronic administration: There were no overt signs of toxicity, hepatotoxicity (in terms of AST, ALT) or renotoxicity (as judged by serum urea and creatinine).

In conclusion, our results demonstrate the hypoglycemic and antihyperglycemic activities of *A. calcarata* rhizomes for the first time and show its potential to be used in the treatment of diabetes mellitus.

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