Digest Journal of Nanomaterials and Biostructures Vol. 6, No. 3, July – September 2011, p. 991 - 997

AMELIORATIVE EFFECTS OF *Alpinia calcarata* IN ALLOXAN-INDUCED DIABETIC RATS

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Rhizomes of *Alpinia calcarata* Roscoe (Zingiberaceae) posses several bio-activities and are used in traditional medicine of India (kulanjan) and Sri Lanka. However, the effect of *Alpinia calcarata* on diabetic activity has not been investigated so far. The aim of this study therefore, was to examine the ameliorative effects of ethanolic extract *Alpinia calcarata* rhizomes (ACRE) in alloxan-induced diabetic rats models. The ethanolic extracts of *Alpinia calcarata* rhizomes (ACRE) significantly reduced the body weight gain, blood glucose level, plasma total cholesterol (TC) and triglyceride (TG) levels when given orally at a dose of 100, 200 and 300 mg/kg/day to the alloxan-induced diabetic rats for 21 days. In this study, the effects of *Alpinia calcarata* on the diabetes of alloxan-induced diabetic rats and possible transcriptional impact are investigated.

(Received March 28, 2011; Accepted June 8, 2011)

Keywords: Alloxan, Diabetes mellitus, Rhizomes of *Alpinia calcarata*, Ethanolic extract

1. Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 1.5% of the total population that continues to present a major worldwide health problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism [1]. As a consequence of metabolic derangement in diabetes, various complications developed, including both macro and micro vascular dysfunctions [2] where complete cure with insulin and oral hypoglycemic agents without side effect has been challenging [3,4]. Broad research on diabetes leads to a number of synthetic oral hypoglycemic agents like biguanides, sulphonylureas and thiozolidinediones being used to treat diabetes. But all have side effects associated with their uses [5]. There is increase in current interest and demand for herbs as worldwide phenomenon, WHO currently encourages, recommends and promotes traditional/herbal remedies in national health care programmes because such drugs are easily available at low cost, and comparatively safe with the people`s faith in such remedies [6]. On the other hand, traditional medicinal plants with their various biological constituents have been used effectively by the communities since long time to treat diabetes. Several natural products such as alkaloids, flavonoids, terpenoids, saponins, polysaccharides and glycosides have been isolated from medicinal plants and are being reported to possess anti-diabetic activities [3]. Extracts of various plant materials with a potential of decreasing the blood sugar have been tested in experimental animal models and their effects confirmed [7].

Alpinia calcarata Roscoe (Zingiberaceae) is one such medicinal plant. In India, Kulanjan in Hindi, Heen-araththa in Sinhala, Amkolinji in Tamil and Kattuchena in Malyalam is a rhizomatous perennial herb. 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, 2.5–5 cm broad. The

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flowers are irregular, bisexual and pendanculate. Terminal densed flowers are found in panicles 8.5 cm long [8, 9]. *Alpinia calcarata* is cultivated in tropical countries including India, Sri Lanka and Malaysia. Especially the rhizomes of *Alpinia calcarata* are used for the medicinal purposes [9]. Kong et al. [10, 11] have isolated some diterpenes such as calcaratarins A–E, sesquiterpenes such as shyobunone and coumarins such as herniarin from the rhizomes of *Alpinia calcarata* grown in China. On the other hand, some benzenoids such as protocatechuic acid, vanillic acid and syringic acid, flavonoids and alkaloids were isolated from the leaves of *Alpinia calcarata* grown in India [12]. Indian researches have shown antibacterial [13], antifungal [14] and anthelmintic activity [15] in extract of *Alpinia calcarata*. However, in Indian traditional ayurveda medicine *Alpinia calcarata* is not generally used. *Alpinia calcarata* is a slender aromatic herb belonging to this genus and in India; it is used in the traditional systems of medicine to treat diabetes, rheumatism, fever and stomachache [16]. However, antidiabetic activity of *Alpinia calcarata* rhizomes has not been investigated using scientifically controlled experiments. Therefore, this study was undertaken to examine whether extract of rhizomes of *Alpinia calcarata* possess antidiabetic activity. This was tested in rats using oral administration of ethanolic extract.

2. Experimental

2.1. Plant material

The rhizomes of *Alpinia calcarata* were collected in the month of July 2009 from ABS Botanical garden Kari Patti, Salem (T.N.).The rhizomes of *Alpinia calcarata* were authenticated by Mr.A.BalSubramanian from Department of Botany, Consultant Central Siddha Research, Salem, (T.N.) and voucher specimen was deposited in our research laboratory for the future reference.

2.2. Animals

Male wistar rats weighing 200±20g were provided by the animal house of TIT pharmacy, Bhopal. All animals were maintained in standard propylene cages and with free access to standard diet and continues water supply. The animals were housed at room temperature (20±2°C) on a normal day-night cycle (0600 h to 1800 h). All animal experimentation was carried out after approval of the protocol by the Institutional Ethical Committee of RGPV University. The guidelines of CPCSEA, India, were strictly followed

(Reg no.TIT/IAEC/831/P'cog/2010/01).

2.3. Chemicals

Alloxan was purchased from Sigma Aldrich Chemicals Pvt, Ltd. One touch glucometer (Accu-chek sensor) of Roche Diagnostics, total cholesterol (TC) and triglyceride (TG) kits were purchased from Roche Diagnostics GmbH, Mannheim, Germany. All other chemicals and reagents used were of analytical grade.

2.4. Preparation of Alpinia calcarata extract

Fresh *Alpinia calcarata* rhizomes were cut into small pieces and air dried for 12–15 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 miscella was filtered and the filtrate was evaporated to dryness under reduced pressure at 55°C (yield 19.5%, w/w, dry weight basis) and stored at 4 °C until use. The extract (1%, w/v) was dissolved in carboxy methyl cellulose (CMC) for oral administration to experimental animals.

2.5. Phytochemical screening of ACRE

Ethanolic extract of *Alpinia calcarata* rhizomes (ACRE) was subjected to phytochemical screening [17] for the detection of various phyto-constituents.

2.6. Determination of effective dose of ACRE

The *ACRE* was administered at different doses of 50, 100, 250, 500, 750 and 1000 mg/kg/day orally for 4 days of six groups of rats (six in each group) and the animals were observed for mortality during the course of treatment or on the fifth day were tested again at lower dose levels and dose showing no mortality in rats was selected as effective dose.

2.7. Evaluation of antidiabetic activity

2.7.1. Experimental induction of diabetes

All animals were allowed to adapt to cages for 3 days, after which they were fasted overnight and 120 mg/kg of alloxan monohydrate freshly dissolved in normal saline was injected intra-peritoneally. After alloxan treatment, all animals were given free access to food and water. Blood glucose levels were measured 2 days after alloxan injection and used as parameters to obtain matching pairs of rats with diabetes of similar level of severity [18].

2.7.2. Experimental Design

Male Wistar albino rats were divided into 6 groups each comprising six rats (n=6).The following treatment was given to animals of different groups:

Group I - Normal rats received vehicle solution (1% CMC)

Group II - Diabetic control rats received vehicle solution (1% CMC)

Group III - V - Diabetic rats treated with ethanolic extract *Alpinia calcarata* rhizomes (ACRE) 100, 200 and 300 mg/kg body weight in (1% CMC), respectively.

Group VI - Diabetic rats treated with standard drug Glibenclamide 600 µg/kg body weight in aqueous solution.

The vehicles and the drugs were administered orally using oral feeding needle daily for three weeks. Changes in body weight of animals were recorded weekly. Blood samples were collected for the measurement of blood glucose level from the tail vein on 0 day, 7^{th}, 14^{th} and 21^{st} day. The blood glucose level was determined by glucometer (one touch). The values of sample treated were compared with that of the standard group which was treated with Glibenclamide. The blood samples were collected from the tail vein of the overnight fasted rats into micro centrifuge tubes containing heparin (10 µl, 1000 IU/ml). Biochemical parameters were estimated using commercially available diagnostic kits.

2.8. Statistical analysis

Statistical analysis was carried out by using Graph-Pad Instat statistical package (Graphpad Software Inc.). Values are expressed as mean±S.E.M. For multiple comparisons, one way ANOVA was used followed by Tukey test. *P* value<0.001 was considered to be significant.

3. Results

3.1. Phytochemical screening of ACRE

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

3.2. Antidiabetic activity

3.2.1 Effect of ACRE on body weight

Table 1 illustrates the variations in body weight of normal, diabetic control and diabetic treatment groups after 21 days. Alloxan significantly reduced the body weight compared with the controls ($P < 0.001$), which gained significant weight. Although the extract at 100,200 and 300 mg/kg body weight ameliorated this weight loss, the extract at 300 mg/kg demonstrated a significant beneficial effect when compared with the reference drug glibenclamide. The effect of *Alpinia calcarata* at a dose of 300 mg/kg body weight was more significant than at 100 and 200 mg/kg.

Table 1 Effect of ACRE on body weight (g) in normal and diabetic rats.

Group	Treatment	Body weight (g) Onset of study	End of study
I	Normal	212.17±3.88	240.83±11.26
II	Diabetic Control	215.83±3.57	198.17±6.68$^{\#}$
III	ACRE (100 mg/kg)	213.17±3.51	186.33±7.97*
IV	ACRE (200 mg/kg)	215.17±3.36	180.00±4.90**
V	ACRE (300 mg/kg)	219.17±3.36	177.00±4.90***
VI	Glibenclamide (600µg/kg)	216.33±3.12	169.50±5.01***

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at # P<0.001 vs. normal group;$^{***}P$<0.001, $^{**}P$<0.01, $^{*}P$<0.05 vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

3.2.2 Effect of ACRE on blood glucose

Table 2 shows the effect of ACRE on blood glucose in control and diabetic animals. Alloxan caused a significant increase in the blood glucose level of experimental animals compared with control ($P < 0.001$). The blood glucose was significantly reduced after 21 days of treatment in all animals except normal & control animals. The effect of ACRE at a dose of 300 mg/kg was mark significant than 100 mg/kg and 200 mg/kg. However the standard drug glibenclamide markedly exerted the most significant ($P < 0.001$) effects on blood glucose level on 21 days as compared to the diabetic control.

Table 2 Effect of ACRE treatment on blood glucose (mg/dl) in normal and diabetic rats.

Group	Treatment	Blood glucose (mg/dl) Onset of study	End of study
I	Normal	96.16 ± 5.36	95.0 ± 4.61
II	Diabetic Control	241.83 ± 19.0	265.16 ± 7.38$^{\#}$
III	ACRE (100 mg/kg)	250.5 ± 13.25	210.83 ± 9.16*
IV	ACRE (200 mg/kg)	254.0 ± 15.0	201.31 ± 3.55**
V	ACRE (300 mg/kg)	258.4 ± 12.5	189.47 ± 4.45***
VI	Glibenclamide (600µg/kg)	262.33 ± 13.9	174.5 ± 5.46***

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at # P<0.001 vs. normal group;$^{***}P$<0.001, $^{**}P$<0.01, $^{*}P$<0.05 vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

3.2.3 Effect of ACRE on total cholesterol and triglyceride level

Table 3 illustrates the rise in blood sugar is accompanied by the increase in total cholesterol and triglyceride ($P < 0.001$) in diabetic rats. Further, post hoc test revealed that treatment with ACRE (300mg/kg; p.o.) statistical significant ($P<0.001$) reduction in these lipid level as compared with diabetic control rats. However, the standard drug glibenclamide exhibited reduction in lipid level on 21 days as compared to the diabetic control.

Table 3 Effect of ACRE treatment on total cholesterol and triglyceride level (mg/dl) in normal and diabetic rats.

Group	**Treatment**	**Triglyceride (mg/dl)**	**Total cholesterol (mg/dl)**
I	Normal	85.25 ± 4.54	64.36 ± 5.46
II	Diabetic Control	152.62 ± 3.36$^{\#}$	116.86 ± 2.54$^{\#}$
III	ACRE (100 mg/kg)	119.54 ± 3.25*	96.36 ± 5.38*
IV	ACRE (200 mg/kg)	110.32 ± 4.37**	91.52 ± 6.56*
V	ACRE (300 mg/kg)	101.34 ± 1.25***	89.67 ± 2.67**
VI	Glibenclamide (600µg/kg)	97.46 ± 4.23***	82.52 ± 6.46***

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at # $P<0.001$ vs. normal group; $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$ vs. diabetes control group respectively (One-way ANOVA followed by Tukey's post hoc test).

4. Discussion

Hyperglycemia is an important characteristics of diabetes mellitus; an endocrine disorder is one of the most common chronic diseases worldwide. Alloxan, a β-cytotoxin, induces diabetes mellitus by damaging the insulin secreting β-cells of the pancreas, resulting in decreased endogenous insulin release [19]. Alloxan-administered rats become hyperglycemic in a short period of time, followed by hepatic glucose overproduction [20]. Intraperitoneal administration of alloxan (120 mg/kg body weight) effectively induced diabetes mellitus in normal rats as reflected by blood glucose level and body weight loss compared with normal rats. Thus the quest for possible compounds to aid in the treatment of diabetes has intensified.

The results demonstrate that ethanolic extracts of *Alpinia calcarata* rhizomes (ACRE) have antidiabetic activity as evaluated reduced the blood glucose level in alloxan-induced diabetic rats. In our study, there was a significant elevation in blood glucose level in diabetic control group as compared with normal animals. The ACRE -treated group exhibited significant reduction of blood glucose levels as compared to the diabetic control group. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental basis of hyperglycemia in diabetes mellitus [21].

Dehydration and loss of body weight have been associated with diabetes mellitus [22].In diabetic rats, increased food consumption and decreased body weight were observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins [23].The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins [24].The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins [25, 26]. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidemia that characterize the diabetic state may therefore be regarded as

a consequence of the uninhibited actions of lipolytic hormones on the fat depots [27]. Excess of fatty acids in plasma produced by alloxan promotes the liver conversion of some fatty acids to phospholipids and cholesterol. These two substances, along with excess of TG formed in the liver, may be discharged into lipoproteins in the blood [28]. The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [29, 30]. A marked increase in total cholesterol and triglyceride level has been observed in diabetic control rats. Insulin deficiency results in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia [26, 29]. There was a significant control of the levels of serum lipids in ACRE -treated diabetic rats.

The results of the present investigation clearly indicate that the ACRE have a glucose lowering effect on alloxan-induced diabetic rats. Hence, the antihyperglycemic effect may be probably brought about by an extra pancreatic mechanism.

5. Conclusion

The antihyperglycemic effects of ethanolic extract *Alpinia calcarata* rhizomes (ACRE) is mediated through the peripheral mechanisms and the effects may be attributed to the components such as flavonoids, triterpenoids and reducing sugars principles present in the ACRE. It may be possible to develop safe and potent antihyperglycemic agents from *Alpinia calcarata* rhizomes.

Acknowledgement

The authors would like to thank Mr. R.R. Karsoliya Chairman, TIT group of institution, Bhopal, for funding the research under the head of Excellence in Research and Academics. We are grateful to Dr. A.Balasubrmanium, Director, TIT pharmacy for his valuable guidance.

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