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Evaluation of antidiabetic activity of *Calamus erectus* in streptozotocin induced diabetic rats

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ABSTRACT

The present study was designed to evaluate the hypoglycemic, hypolipidemic and antioxidant activity of Calamus erectus (CE) fruit in streptozotocin (STZ) induced diabetic wistar rat. The fruit extracts of 100, 200, 300 and 400 mg/kg body weight (bw) were administrated orally to normal and STZ induced (55 mg/kg bw) diabetic (>200 mg/dl) rats. Glibenclamide (10 mg/kg) were used as a reference drug. Antioxidant effects were assayed in diabetic rats by estimating thiobarbituric acid reactive substances (TBARS), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels. Daily oral treatment with 400 mg/kg fruit extract for 14 days resulted in 73.68, 20.46, 36.6 and 43.9% reduction of blood glucose, serum cholesterol, triglycerides and LDL (low-density lipoprotein) respectively whereas HDL (high-density lipoprotein) cholesterol was found to be improved 12.7% when compared with STZ treated rats. GSH, SOD and CAT activity of liver homogenate was improved 33.46, 49.36 and 52.48% respectively while the TBARS decreased 36.18% with same treatment. Decreased levels of TBARS and increase of GSH, SOD and CAT activity indicated a reduction in free radical formation in liver of diabetic rats. The present study demonstrated that CE fruit extract possess good antidiabetic potential and could improve lipid profile and oxidative stress efficiently during diabetic condition.

Key Words: hypolipidemic, hypoglycemic, glibenclamide

INTRODUCTION

The term diabetes mellitus is the most prevalent metabolic disorder characterized with increased blood sugar level and improper primary metabolism [1]. It is the most common disease in the world affecting 25% of population and troubles 150 million people and is set to rise to 300 million by 2025 [2]. Diabetes also gives rise to various secondary problems such as retinopathy, peripheral vascular insufficiencies and neuropathy. These secondary problems take place due to the oxidative stress and DNA damage caused by the generation of free radicals in the cells [3]. Diabetes is still not completely curable by the present antidiabetic agents. Insulin therapy is the only satisfactory approach in diabetes mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment [4]. Diabetes mellitus is associated with increased oxidative stress. Free radicals are continuously produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. The level of lipid peroxidation in the cell is controlled by various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavenging systems during oxidative stress mediated propagation of reactive oxygen and nitrogen species (RONS), which is always related with diabetes [5,6]. But disturbances of innate antioxidant defense mechanism in prolonged diabetic condition showed alteration in antioxidant enzyme levels such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and

glutathione reductase (GR) along with impaired glutathione (GSH) metabolism [7]. Hence it is necessary to bring out the antidiabetic components which may also overcome the oxidative stress and hyperlipidemic problems. Ethno-botanical information indicates that more than 400 plants are used as traditional therapies for the treatments of diabetes [8]. The hypoglycemic activity of a large number of these plants have been evaluated and confirmed in different animal models [9, 10, 11, 12, 13, 14]. But unfortunately therapeutic potential of most of the established antidiabetic herbal products are lesser than standard synthetic drugs. Again the hypoglycemic plants which are non-edible are of limited use in therapeutics in ultra-high dose as intensive toxicity assessment for most of them are not yet completed. So the primary objective of herbal drug research should be to identify such kind of edible antidiabetic plant which has very less amount of toxicity and can be consumed in any required quantity for managing hyperglycemic condition.

The edible fruits of *Calamus erectus* Roxb. (CE) (Arecaceae) is traditionally used by the local people of Darjeeling Himalaya as antidiabetic medicine. The plant is a clump-forming, short, water-loving, fast growing, dioecious, non-climbing palm, growing in forest understory. It has spiny, green stems, 6 m (20 ft) tall. This plant is wildly grown in evergreen forest of Darjeeling Himalaya, Assam, Bangladesh, South-Central China, Laos, Myanmar and Thailand. The plant is commonly known as 'Bedgera' in Darjeeling Himalaya. The people of Darjeeling consume this fruit in unripe to ripe condition after removing the epicarp. Though ethno-medicinally well recognized, until now there is no published scientific reporting, where pharmacological investigations related to antidiabetic activity of this plant have shown. Hence in the present study, the CE fruit was investigated for its antidiabetic and hypo-lipidemic activity *in vivo* in steptozotocin induced diabetic rat model and to compare the same with glibenclamide, a standard hypoglycemic drug.

MATERIALS AND METHODS

5.1. Plant materials

Fruits of *Calamus erectus* (Bedgera) were collected (dated 16.10.09) from Takdah Basti, Darjeeling, West Bengal, India. Taxonomic position was authenticated by Professor A.P. Das, Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. A voucher specimen has been deposited in the 'NBU Herbarium' of the same department recorded against the accession number 9585 dated 28-05-10.

5.2. Animal materials

Male wistar albino rats having weights of 100-140 g were purchased from Ghosh Enterprise, Kolkata. For experimentation, the animals were grouped, housed in polypropylene cages and were kept in quarantine for 10 days under standard husbandry conditions (25 ± 2°C, relative humidity, 65 ± 10%) for 12 h dark and light cycle respectively. All animals were fed with standard laboratory diet (Hindustan Lever Ltd, Bangalore, India) and water *ad libitum*. The study was permitted by the Institutional Animal Ethical Committee.

5.3. Extraction procedure

Epicarps of the fruits were removed. The soft inner parts (2 kg) *i.e.* mesocarp and endocarp were taken and crushed. The pulp material of the fruit was defatted with petroleum ether (60-80ºC) in a Soxhlet extraction apparatus and further the same was extracted with methanol. The methanol extract of CE was selected for the present study. Methanol was evaporated through vacuum rotary evaporator, weighed for measuring for extractive values and the dried plant drug was kept in freezer for further use.

5.4. Experimental induction of diabetes

After fasting for 18 h, 35 rats were injected intra-peritonealy with a single dose of 55 mg/kg streptozotocin after dissolving it in freshly prepared ice cold 0.1 M citrate buffer (pH 4.5) [15]. After the injection they had given free access to feed and water and were given 5% glucose solution to drink over night to counter the hypoglycemic shock. The development of diabetes was confirmed after 48 h of the streptozotocin injection. The animals having fasting blood glucose levels more than 200 mg/dl were selected for the experimentation. Out of 35 animals three were died before grouping and two was omitted from the study because of mild hyperglycemia (below 150 mg/dl).

5.5. Experimental design

The 30 diabetic animals were divided into 6 groups each having 6 rats and extra 5 non-diabetic rats are taken for control group [16]. The standard used was glibenclamide and was given to one group. The remaining groups were

administrated orally with four different doses viz. 50, 100, 200 and 400 mg/kg body weight of powered drug of CE dissolved/suspended in water by tween80 (1% v/v).

- Group I Normal saline treated rats
- Group II STZ treated rats.
- Group III Diabetic rats given aqueous solution of glibenclamide 10 mg/kg per day for 14 days
- Group IV Diabetic rats given suspension of CE (50 mg/kg, per day p.o for 14 days)
- Group V Diabetic rats given suspension of CE (100 mg/kg, per day p.o for 14 days)
- Group VI Diabetic rats given suspension of CE (200 mg/kg, per day p.o for 14 days)
- Group VII Diabetic rats given suspension of CE (400 mg/kg, per day p.o for 14 days)

On day 14th, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar [17] was estimated. Serum was separated and analyzed for serum cholesterol, serum triglycerides, serum HDL and LDL. Total cholesterol, triacylglycerol, and HDL-C levels in plasma were determined by enzymatic kits that were procured from Bayer's Diagnostics Pvt. Ltd., Baroda, India. LDL-c levels were calculated by using Friedewald's equation.

The rat liver was weighted and 10% liver homogenate was prepared with 0.1 M phosphate buffer (pH 7.0) after centrifugation at 1000 rpm for 15 min. The supernatant was used to measure Lipid peroxidation (LPO), SOD and CAT.

5.6. Lipid peroxidation

Lipid peroxidation was estimated by the method of Ohkawa *et al*. [18]. Liver homogenate was mixed (1 ml) with 100 µl of 8.1% sodium dodecyl sulfate (SDS), and 600 µl of 20% acetic acid solution was kept for 2 min at room temperature, then 600 µl of 0.8% solution of thioburbituric acid (TBA) was added, heated at 95ºC for 60 min in water bath and cooled with ice cold water at 4ºC. The mixture of n-butanol and pyridine (15:1, v/v) were added, shaken vigorously and centrifuged at 10000 rpm for 5 min. The absorbance of the organic layer was measured at 532 nm.

5.7. Superoxide dismutase

The SOD was estimated by the method of Beauchamp and Fridvich [19] and Chidambara *et al*. [20], based on the reduction of NBT. 0.5 ml of liver homogenate, 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 µm NBT, and 0.2 ml of 0.1 mM EDTA were added. The reaction was started by adding 0.4 ml of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm followed by 5 min at 25º C. The control was determined without liver homogenate.

5.8. Catalase

Catalase was estimated by using the method of Sinha [21]. 0.1 ml of liver homogenate, 1 ml of 0.01 M phosphate buffer (pH 7.0) and 0.4 ml of 2 M hydrogen peroxide was mixed. The reaction was stopped by the addition of 2 ml dichromatic acetic acid reagent. The control was carried out without addition of hydrogen peroxide. Absorbance was measured at 620 nm.

5.9. Statistical analysis

Statistical analysis of the biochemical estimates were performed using SPSS 11.0 (SPSS Inc. Chicago, USA). The results were expressed as mean ± SEM. One-way ANOVA was employed for comparison among groups. Significant differences ($p < 0.05$) between means were determined using Duncan's multiple range test (DMRT) with the help of DSAASTAT ver. 1.022.

RESULTS

6.1. Effect of CE fruit extract on fasting blood glucose level in diabetic rats

A marked rise in fasting blood glucose level was observed in diabetic control group as compared with normal control rats. Methanolic extract of CE (at 50, 100, 200 and 500 mg/kg) exhibited a dose dependent significant antihyperglycemic activity on 0, 7th and 14th day post treatment. The antihyperglycemic effect of methanol extract was found to be more effective than the reference standard, glibenclamide which produced a significant reduction in blood glucose compared to diabetic control (Figure 1).

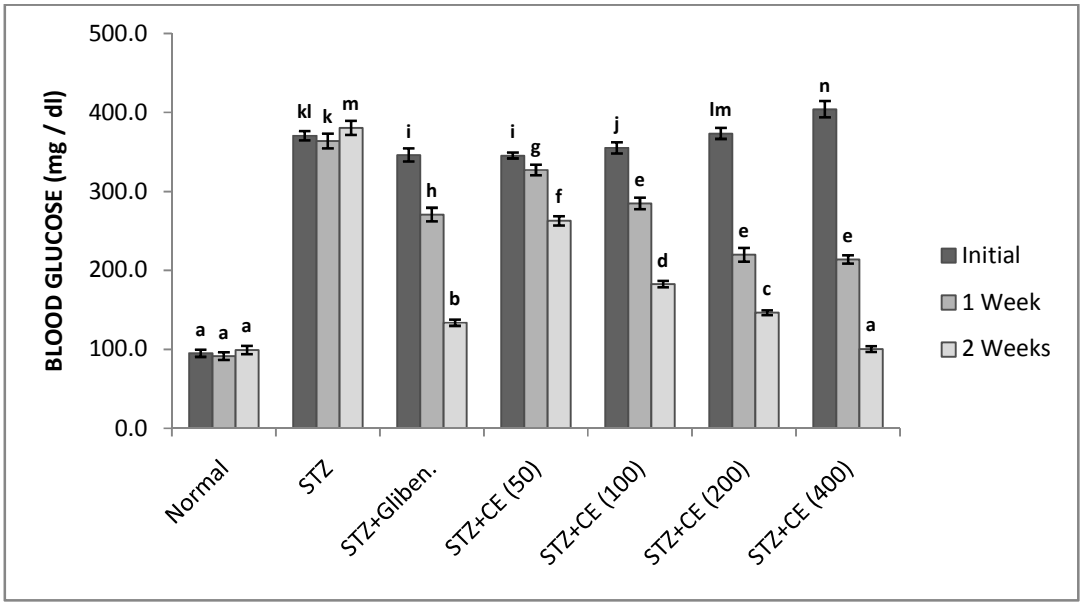
Table 1. Effect of extract on lipid profile in diabetic rats.

Groups/Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	HDL/LDL Ratio
I: Vehicle	64.67±1.45 ab	74.00±2.08 b	42.67±3.18 ab	14.33±1.45 a	0.34
II: STZ	86.33±1.76 c	92.00±2.14 c	41.11±4.91 ab	27.33±2.91 b	0.66
III: STZ+ Glibenclamide (10 mg/kg)	73.33±3.93 bc	61.67±3.18 a	44.67±4.91 ab	21.33±7.13 ab	0.48
IV: STZ+ CE (50 mg/kg)	76.33±3.76 bc	63.33±3.95 ab	38.33±2.60 b	22.67±3.52 ab	0.59
V: STZ+ CE (100 mg/kg)	70.28±4.91 ab	64.33±2.36 ab	40.33±1.76 ab	19.01±2.89 ab	0.47
VI: STZ+ CE (200 mg/kg)	64.72±3.56 ab	60.67±2.81 a	42.67±3.17 ab	17.67±2.60 ab	0.41
VII: STZ+ CE (400 mg/kg)	58.33±2.03 a	58.33±1.71 a	46.33±4.67 a	15.33±4.26 a	0.33

Table 2: Effect of CE on *in-vivo* antioxidant parameters from Liver Homogenate in STZ-induced diabetic rats.

Treatment Parameters	TBARS [mmol/100 g FWT]	GSH [mmol/100 g FWT]	SOD [unit/min/g tissue]	CAT [µmol H_2O_2/min/g tissue]
Vehicle	4.82 ± 0.060 a	60.82 ± 0.89 a	3.75 ± 0.018 a	16.48 ± 0.62 a
STZ	6.91 ± 0.011 c	41.11 ± 0.91 c	1.98 ± 0.009 b	7.57 ± 0.52 b
STZ + Glibenclamide	4.55 ± 0.049 a	57.29 ± 1.06 ab	3.62 ± 0.015 a	12.59 ± 0.46 ab
(100 mg / kg BW) CE + STZ	6.01 ± 0.038 b	49.81 ± 1.18 bc	2.04 ± 0.007 b	10.02 ± 1.05 ab
(200 mg / kg BW) CE + STZ	5.63 ± 0.091 b	52.92 ± 0.95 ab	2.88 ± 0.240 ab	13.11 ± 0.89 ab
(400 mg / kg BW) CE + STZ	4.41 ± 0.065 a	61.78 ± 0.29 a	3.91 ± 0.052 a	15.93 ± 0.21 a

Figure 1. Anti-diabetic effect of CE in STZ-induced diabetic rats.



6.2. Effect of CE fruit extract on body weight in diabetic rat

Normal control animals were found to be stable in their body weight but diabetic rats showed significant increase in body weight during 14 days. Streptozotocin mediated body weight incrassation was significantly reversed by the methanol extract in dose dependent fashion (at 200 and 400 mg/kg p.o). Results are shown in Figure 2.

6.3. Effect of CE fruit extract on hyper-cholesterol in diabetic rat

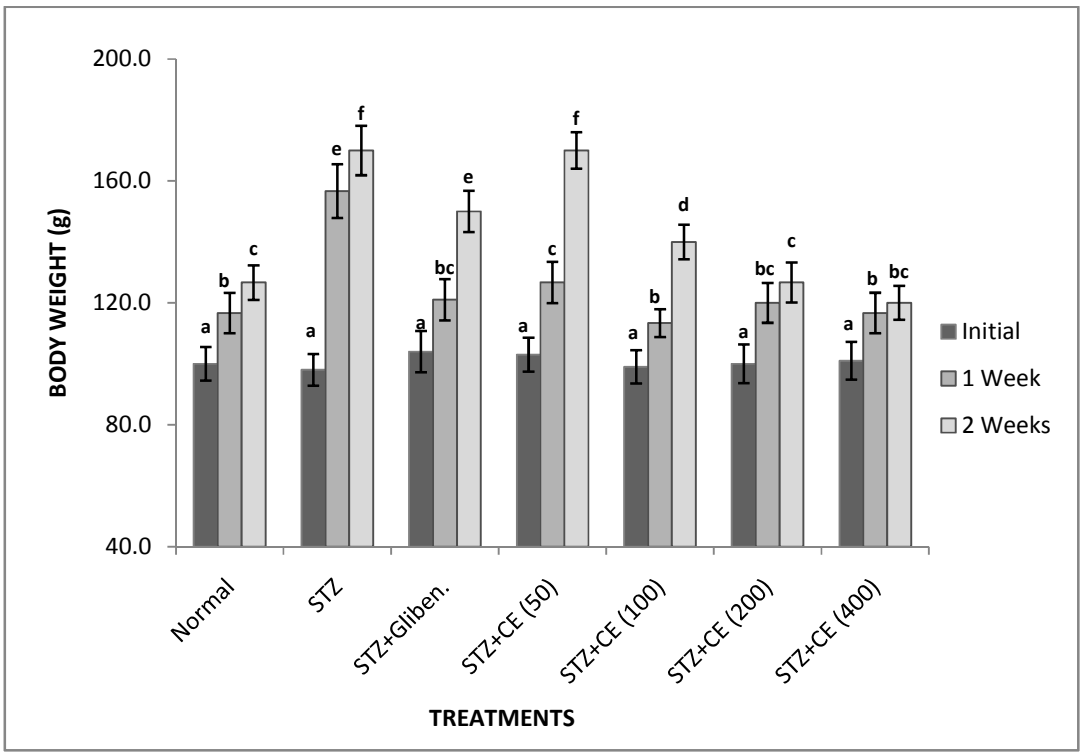
The change in the cholesterol, HDL-c and triglyceride level was measured and observed on potent reduction in serum cholesterol and triglycerides and effective elevation in HDL-c level over diabetic control when the rats were fed with aqueous reconstituted methanolic CE fruit extract. The level of serum cholesterol was lower in normal rats that were not treated with streptozotocin and enhancement of the same was found in diabetic control (Table 1) whereas maximum reduction was seen (58.33 ± 1.45 mg/dl) after the administration of 400 mg kg/body weight treatment of CE. When HDL-c was considered, it showed normal range of 42.67 ± 3.18 mg/dl in control rats but maximum elevation were recorded (46.33 ± 4.67 mg/dl) in CE treated group VII rats (Table 1). Remarkable

reduction of serum triglyceride was noted on feeding at 50, 100, 200, 400 mg/kg body weight extract (Table 1) over control. In the same way LDL level of CE treated groups was also significantly decreased from diabetic control on 14th day, whereas, HDL-LDL ratio was also increased after feeding of CE fruit drug, as shown in Table 1.

6.4. Effect on antioxidant enzymes

STZ induced diabetic rats were found to have decreased SOD, GSH and CAT enzyme level in liver as compared to control. Administration of CE fruit drug to the diabetic rats resulted in significant increase in the activities of SOD, GSH and CAT (Table 2). STZ diabetic rats were found to exhibit significant increase in TBARS level in lever as compared to control rats. Treatment with *CE* fruit extract produced significant decrease in TBARS (Table 2).

Figure 2. Effect of extract on body weights in diabetic rats.



DISCUSSION

Administration of STZ selectively destroys the β-cells of the islets of Langerhans [22]. The destruction of β-cells cause the marked decrease in insulin levels [23]. In this study the results indicate that fruit drug of CE can able to decrease the level of blood glucose in STZ induced diabetic rats. The hypoglycemic action of this drug in diabetic rats may be possible through the insulinomimetic action or by other mechanisms such as stimulation of glucose uptake by peripheral tissues, inhibition of endogenous glucose production, or activation of gluconeogenesis in liver and muscles, as similar mechanisms have been reported for plant drug with antidiabetic activity [24].

Consequently, the level of GSH, SOD and CAT were significantly improved by the application of this fruit extract on experimental diabetic rat. Earlier reports indicated that STZ-induced diabetic animals may exhibit most of the diabetic problem mediated through oxidative stress [25]. The GSH, SOD and CAT are the three effective scavenging enzymes that remove free radicals *in vivo* [26] as well as they play an important role in restoring antioxidant activities in the tissue of diabetic animals [27, 28, 29]. In our study, GSH, SOD and CAT enzymes were significantly decreased in STZ-induced diabetic control rats, may be due to inactivation caused by free radicals. The above observation may clearly suggest that increased level of these antioxidants enzymes with CE fruit extract may be due to free radical scavenging activity with that extract, which may exert a beneficial effect against pathological alterations caused by reactive oxygen species.

Lipid plays an important role in the pathogenesis of complications concerned with diabetes mellitus. The elevated level of serum cholesterol and reduced level of serum HDL cholesterol in diabetic condition may generate versatile factors for developing microvascular complication leading to atherosclerosis, which further culminates into different fatal cardiovascular disorders [30]. In the present study, there was a significant reduction in the levels of total cholesterol, triglycerides and LDL-c. Also the observation from our experiments determines that administration of these fruit extracts could enhance the level of HDL in blood through which the progression of atherosclerosis may be declined along with cardioprotection. The process of harmful cholesterol reduction in association with enhanced HDL/LDL ratio with herbal drug treatment may be due to activation of LDL receptors in hepatocytes which is responsible for taking up LDL into the liver [31].

CONCLUSION

In conclusion, the results of this investigation revealed that methanol extracts of CE fruits possesses significant antidiabetic activity in STZ-induced diabetic rats in a dose-dependent manner. Experimental results also showed that the extract is capable of improving the oxidative state associated with diabetes along with upgradation of bio-active cholesterol and lipid profile. Now research is continued to isolate the lead compound from this extract.

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