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Original Article

Antihyperglycemic and Antihyperlipidemic Potential of *Colocasia esculenta* Corms - in vivo

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ABSTRACT

Aims: *Colocasia esculenta* Linn has been used regularly in folk medicines to treat various diseases including diabetes mellitus but the antidiabetic effect of its corms has not been validated scientifically so far. The present study is an attempt to evaluate antidiabetic and antihyperlipidemic potential of aqueous extract of *C. esculenta* corms *in vivo*.

Methods: Various doses of C. esculenta corms were given orally to normal and streptozotocin induced mild diabetic rats to identify the most effective dose for lowering the elevated blood glucose level (BGL). The most effective dose of 200 mg kg^{-1} was given to severely diabetic rats for 28 days to assess the antihyperglycemic and antihyperlipidemic potential of the extract.

Results: The results reveal the maximum decrease of 34% in the BGL of normal rats at 6 h during Fasting Blood Glucose (FBG) studies and 43.1% at 3 h during Glucose Tolerance Test (GTT). Whereas, in case of mild diabetic rats, the maximum reduction observed in BGL during GTT was only 9.6%. In case of severely diabetic rats a noteworthy reduction of 41.6, 31.1, 34.8 and 46.2% was observed in FBG, Post Prandial Glucose, serum Total Cholesterol and Triglycerides levels respectively. A significant increase of 26% in High Density Lipoprotein level of severely diabetic rats was also observed.

Conclusions: Hence, C. esculenta corms could be exposed as antidiabetic agent being significantly effective not only as hypoglycemic and antihyperglycemic agent but also as antihyperlipidemic. Thus, it could be developed as a new source for diabetes treatment.

1. Introduction

Diabetes is a metabolic disorder identified as persistent hyperglycemia, associated with disturbed metabolism of carbohydrate, fat and protein, caused either by defects in insulin secretion or insulin action, or both [1]. Diabetes being one of the five principal causes of death in the world, affecting more than 382 million people, is rightly recognized as a global public health issue [2,3]. Several hypoglycemic agents such as sulfonylureas, biguanides and thiazolidinediones are in use for the treatment of diabetes in addition to insulin. However, the various side effects associated with these treatments as well as pain associated with daily intravenous injections of insulin, signify that discovery of more effective and less toxic novel oral hypoglycemic agents are urgently needed [4,5]. In recent time, medicinal plants are in trend because of minimal or no side effects. From traditional system of medication, numerous medicinal plants are renowned for their use in diabetes treatment. But, not many plants have been investigated methodically for antidiabetic assessment [6-10].

Colocasia esculenta Linn, herbaceous perennial plant, belongs to the Araceae family and commonly known as Taro in English. It is a starchy root crop with wide edible leaves [11]. Its corms supply easily digestible starch and protein, vitamin C, B1, B2, B3 and fiber [12]. The in vitro antioxidant and antibacterial activities of its corms had already been explored by our research group and were found to be of high impact. Preliminary Phytochemical screening of *C. esculenta* corms reveals the presence of saponins, steroids, carbohydrates, glycosides, tannins, flavonoids and proteins in the same extract [13,14].

The aim of the present study was to evaluate the antihyperglycemic and antihyperlipidemic effect of *C. esculenta* corms extract (CECE) on blood glucose and serum lipid profile of streptozotocin (STZ) induced diabetic rats in order to develop a novel oral antidiabetic agent to treat as well as manage diabetes.

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MATERIAL AND METHODS

Plant material

Corms (1 Kg) of *C. esculenta* were purchased from Allahabad, U.P., India and identified by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been submitted to the University herbarium (No. MRL/CE/02).

Preparation of extract

The corms of *C. esculenta* were first peeled off, washed well and shade dried. Dried Corms were boiled in distilled water for 2 days. The extract of corms was filtered and filtrate was concentrated and dried in lyophilizer. The finally prepared *C. esculenta* corms extract powder was a dark brown solid material (9.7g) which was used for evaluating its antihyperglycemic and antihyperlipidemic activities.

Chemicals

STZ was purchased from Sigma-Aldrich, Seelze, Germany. Diagnostic kits were purchased from Erba Diagnostics, Mumbai, India and blood glucose meter was purchased from SD codefree Biosensor, Republic of Korea.

Experimental animals

Albino Wistar rats (body weight 150-200g) were selected for the experiments and obtained from the National Institute of Communicable Disease (NICD), New Delhi, India. All the rats were placed in polypropylene cages with 12 h each dark and light cycle at the temperature of 25-30°C and 45-55% relative humidity. Animals were fed pellet diet (Pashu Aahar Kendra, Varanasi, India) and water ad lib. The study was permitted by the Institutional Ethical Committee (Registration No. 839/a/04/CPCSEA).

Induction of diabetes

Diabetes was induced to overnight fasted rats by an intraperitonial injection of STZ 50 mg/kg body weight in 0.1 M citrate buffer (pH = 4.5). After 3 days of STZ administration, rats with noticeable hyperglycemia were selected for the study [15] and divided into two different categories:

· Mild diabetic animals (MD) - FBG 150 - 200 mg/dl and PPG > 250 mg/dl

· Severe diabetic animals (SD) - FBG > 250 mg/dl and PPG > 350 mg/dl

Estimations

Blood glucose level (BGL) was estimated by SD Biosensor CodeFree glucose strips. Total Cholesterol (TC), Triglycerides (TG) and High Density Lipoprotein (HDL) levels in serum were measured spectrophotometrically by prescribed method of the manufacturer [16,17] using standard kit from Erba Diagnostics India Ltd. All the parameters were measured before as well as after the treatment.

Experimental design

For hypoglycemic activity the extract was screened with a series of variable doses by FBG and GTT studies in normal healthy rats. The antidiabetic effect was assessed in mild diabetic rats with the same range of doses by GTT studies. The most effective dose identified was then evaluated for its antihyperglycemic and antihyperlipidemic potential in severely diabetic animals on long term treatment of 28 days.

Hypoglycemic studies in normal healthy rats

Overnight fasted normal rats were used in the experiment for FBG and GTT studies. The normal healthy rats were divided into six groups of six rats each. Group I served as control treated with distilled water only, whereas the animals of groups II, III, IV, V and VI were treated with CECE at doses 50, 100, 150, 200 and 250 mg kg^{-1}, respectively. FBG levels of all the groups were checked at 2, 4 and 6 h after treatment. For GTT studies the CECE was given orally to overnight fasted normal healthy rats and the BGL was checked at 1.5 h and treated as 0 h value. The animals were then given 2 g kg^{-1} of glucose orally and the glucose tolerance was studied for next 3 h at regular intervals of 1 h each.

Antihyperglycemic studies in mild diabetic rats

The antidiabetic effect of CECE in mild diabetic rats was also assessed by improvement in glucose tolerance. The rats were divided into seven groups of six rats each. Group I control, received vehicle (distilled water) only, whereas variable doses of 50, 100,150, 200 and 250 mg kg^{-1} of CECE extract were given orally to groups II, III, IV, V and VI, respectively. BGLs were first checked after 90 min of FBG, considered as 0 h value, and animals were then given 2 g kg^{-1} of glucose orally and the glucose tolerance was studied for next 3 h at regular intervals of 1 h each. The results were compared with group VII rats, treated with 2.5 mg kg^{-1} of glibenclamide, a reference drug.

Antihyperglycemic and Antihyperlipidemic studies in severely diabetic rats

Four groups of six rats each were used in the experiment. Group I and II served as normal and severely diabetic (SD) controls respectively received distilled water only, group III received CECE at a dose of 200 mg kg-1 and group IV served as positive control received glipizide at a dose of 2.5 mg kg-1 as a reference drug. All the groups were treated once a day up to 28 days. Blood samples were collected at the beginning and then weekly up to 28 days and levels of FBG, PPG, TC, TG and HDL were assessed.

LD_{50} experiment

The toxic effect of the CECE was also studied by a LD_{50} experiment. Two groups of rats of both sexes (six animals per group, three females and three males), weighing about 180-200g, were orally treated with a single dose of 10 and 15 times of the CECE. Rats were observed for toxic effects, neurologic, autonomic and gross behavioural. Food consumption, faeces and urine were also examined.

Statistical analysis

Statistical analysis was performed using two-way analysis of variance (ANOVA), using statistical package PRISM 3.0 version. The significance of difference between and within various groups was determined. Differences were considered to be significant when $p<0.001$.

RESULTS

Hypoglycemic effect - Normal healthy rats - FBG and GTT Results

Table 1 depicts the hypoglycemic effect of an oral treatment of variable doses of CECE in normal healthy rats during FBG studies. Treated rats showed a regular fall of 17.0, 10.0, 24.7 and 34.0% from the doses of 50, 100, 150 and 200 mg kg-1, respectively, after 6 h during FBG studies. However, a lesser fall of only 25.4% was observed with an increased dose of 250 mg kg-1 after the same interval of time.

Table 2 reveals the hypoglycemic effect of CECE in normal healthy rats during GTT studies. Similar set of doses of CECE was given orally to overnight fasted rats and BGL was checked after 1.5 h considered as 0 h value before giving 2 g kg^{-1} of glucose. After glucose administration the fall was observed up to 3 h at 1 h intervals and the results unveil that the percentage fall in BGLs was regular up to the dose of 200 mg kg^{-1} and reached its maximum at 43.1%. Whereas, the fall of only 14.7, 16.6, 30.3 and 22.5% was observed with the dose of 50, 100, 150 and 250 mg kg-1 at the same interval of time.

Antihyperglycemic effect - Mild diabetic rats - GTT Results

Figure 1 illustrates the antidiabetic effect of variable doses of CECE in mild diabetic rats. Fall of 3.4, 1.92, 4.2, 9.6 and 3.0% in BGLs of mild diabetic animals was observed after 3 h of glucose administration with doses of 50, 100, 150, 200 and 250 mg kg^{-1}, respectively. However, the dose of 2.5 mg kg^{-1} of standard drug, glibenclamide reduced BGL by 25%.

Antihyperglycemic Effect - Severely diabetic rats - FBG and PPG Results

Table 3 shows the impact of the most effective dose identified as 200 mg kg^{-1} on BGL of severely diabetic rate after long term treatment of 28 days. A significant reduction of 41.6% and 31.1% was observed in their FBG and PPG levels at the end of the treatment. Whereas, in case of positive control, rats treated with 2.5 mg kg^{-1} of glipizide, FBG level has shown fall of 42.9% and PPG level has shown fall of 44.1% after 28 days. Moreover, BGL of normal control was maintained throughout the treatment while BGL of severely diabetic control was continuously enhancing.

Antihyperlipidemic effect - Severely diabetic rats - TC, TG and HDL Results

Table 4 shows the antihyperlipidemic effect of the CECE on lipid profile of severely diabetic rats. After the induction of diabetes and subsequent treatment with CECE, significant decrease was observed in serum total cholesterol and triglycerides. The results showed that administration of CECE decreased the levels of total cholesterol and triglycerides with a fall of 34.8 and 46.2% respectively after 28 days treatment. Glipizide treated rats showed fall of 27.0 and 33.8% in TC and TG respectively. HDL cholesterol level was also significantly increased by 26% in severely diabetic rats after 28 days treatment. Moreover, the untreated diabetic rats continued to show enhanced levels of TC and TG cholesterol in addition to a slight percentage fall in HDL cholesterol level.

LD_{50} Studies

The experiment was carried out on normal healthy rats. The behaviour of the treated rats appeared normal. No toxic effect was reported at doses up to 10 times and 15 times of the effective dose of the CECE and there was no death in any of these groups.

Table 1: Effect of CECE on BGL of normal healthy rats during FBG studies (mean ± SD).

Groups	Treatment	Pre-treatment FBG	Blood Glucose levels (mg/dL)		
			2h	4h	6h
I	Control	95–1.15	95–2.30	85–2.08	72–2.64
II	50 mg kg^{-1}	88–1.00	83–2.51	79–2.30	73–1.52
III	100 mg kg^{-1}	90–2.30	95–2.64	85–2.51	81–2.51
IV	150 mg kg^{-1}	89–2.08	91–1.52	75–2.99	67–2.00
V	200 mg kg^{-1}	100–1.52	90–1.73	80–2.64	66–2.08**
VI	250 mg kg^{-1}	106–3.05	93–1.15	94–2.30	79–1.15

**p < 0.01, *p < 0.5 as compared with control

Table 2: Effect of CECE on BGL of Normal healthy rat during GTT studies (mean ± SD).

Groups	Treatmen	Pre-treatmen FBG	Blood Glucose levels (mg/d			
			0h	1h	2h	3h
I	Control	79–0.57	78–2.08	135–3.05	110–3.21	102–151
II	50 mg kg^{-1}	84–2.30	80–1.52	131–2.51	81–2.64	87–1.00
III	100 mg kg^{-1}	77–1.52	93–2.51	113–2.08	80–1.73	85–2.00
IV	150 mg kg^{-1}	73–3.21	67–2.08	144–2.64	82–0.57	71–1.52
V	200 mg kg^{-1}	84–1.15	88–1.00	105–3.51	89–1.15**	58–2.51*
VI	250 mg kg^{-1}	82–1.00	76–2.64	91–3.60	79–2.30	79–1.52

***p < 0.001, *p < 0.5 as compared with control

Table 3: Effect of long term treatment of CECE on BGL of severely diabetic rats (mean ± SD).

Groups	Treatment	Doses	Pre-treatment level (0 day)	Post treatment levels (days)			
				7	14	21	28
			FBG (mg/dl)				
I	Normal Control	DW	80–4.9	82–3.2	78–5.2	79–5.3	80–47
II	SD Control	DW	452–3.8	477–5.1	512–4.6	538–4.2	548–58
III	SD Treated (*C. esculenta*)	200 mg kg^{-1}	360–2.6	316–4.1	287–4.1	249–2.0	210–1.5* **41.6% Fall**
IV	SD Treated (Glipizide)	2.5 mg kg^{-1}	312–5.9	301–7.4	288–7.4	205–7.3	178–7.2 **42.9% Fall**
			PPG (mg/dl)				
I	Normal Control	DW	172–5.4	171–4.6	171–5.7	172–4.8	170–4.8
II	SD Control	DW	480–2.5	500–4.9	525–5.1	550–5.2	550–38
III	SD Treated (*C. esculenta*)	200 mg kg^{-1}	408–2.5	398–2.0	346–3.2	320–3.6	281–2.6 **31.1% Fall**
IV	SD Treated (Glipizide)	2.5 mg kg^{-1}	412–7.0	376–8.3	309–8.1	270–7.6	230–7.3 **44.1% Fall**

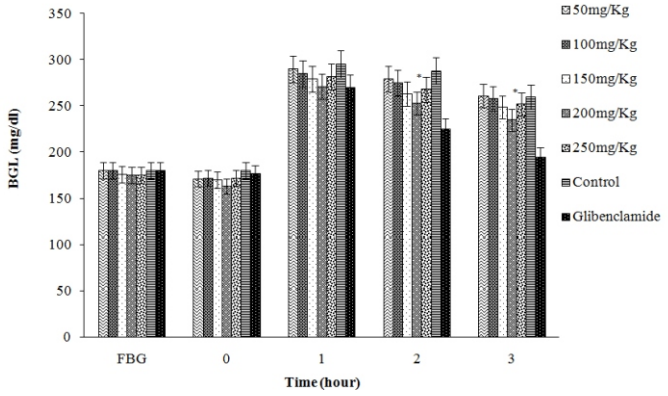
***p < 0.001, **p < 0.01 as compared with control

Table 4: Effect of long term treatment of CECE on TC, TG and HDL of severely diabetic rats (mean ± SD).

Groups	Treatment	Doses	Pre-treatment level (0 day)	Post treatment levels (days)			
				7	14	21	28
			TC(mg/dl)				
I	Normal Control	DW	142.7–4.9	143.8–6.1	144.3–5.1	141.4–5.6	140.2–4.4
II	SD Control	DW	159.5–4.9	160.6–4.1	161.2–4.6	163.7–4.3	162.5–4.7
III	SD Treated (*C. esculenta*)	200 mg kg^{-1}	148.9–0.6	139.1–1.5	130.8–1.0	119.1–0.9	97.0–2.0** **34.8% Fall**
IV	SD Treated (Glipizide)	2.5 mg kg^{-1}	132.4–4.5	130.6–4.3	124.3–4.7	112.5–4.6	96.6–4.9 **27.0% Fall**
			TG(mg/dl)				
I	Normal Control	DW	64.5–3.7	64.9–4.6	64.9–3.9	64.7–3.5	64.2–3.2
II	SD Control	DW	143.9–5.4	145.2–5.1	149.0–6.3	180–7.5	182.4–6.9
III	SD Treated (*C. esculenta*)	200 mg kg^{-1}	165.9–1.4	139.7–4.0	120.5–2.0	101.2–3.7	89.1–3.9** **46.2% Fall**
IV	SD Treated (Glipizide)	2.5 mg kg^{-1}	183.6–5.2	181.3–4.9	170–5.2	165.2–4.3	121.6–4.7 **33.8% Fall**
			HDL (mg/dl)				
I	Normal Control	DW	27.2–3.8	28.5–4.2	27.9–3.1	28.4–3.6	27.1–3.1
II	SD Control	DW	22.7–3.3	21.8–2.8	21.1–3.2	20.0–3.8	20.1–3.5
III	SD Treated (*C. esculenta*)	200 mg kg^{-1}	23.6–0.8	25.7–1.4	26.2–1.9	28.3–2.0	31.9–1.9** **26.0% Rise**
IV	SD Treated (Glipizide)	2.5 mg kg^{-1}	20.6–2.4	22.8–2.5	24.9–2.8	26.4–2.7	27.1–3.2 **31.5% Rise**

***p < 0.001, **p < 0.01as compared with control

Fig. 1: Effect of CECE on BGL of mild diabetic rats during GTT. *p < 0.5 as compared with control.



DISCUSSION

Although a large number of synthetic hypoglycemic agents are available, but several side effects associated with them have limited their clinical utility and hence the search for novel pharmacotherapy from medicinal plants to cure diabetes have gained considerable importance. The present study was designed to explore the effect of CECE on levels of blood glucose and serum lipid in various experimental models viz. normal, STZ-induced mild and severely diabetic rats. The observed difference between initial and final BGLs of rats on treatment with CECE revealed a significant reduction in BGL of treated groups as compared with the control. In case of normal rats, maximum hypoglycemic effect was showed at 6 h and maximum glucose tolerance was observed at 3 h. Maximum antihyperglycemic effect was also observed at 3 h in case of mild diabetic rats based on their GTT studies. It is interesting to note that the maximum hypoglycemic effects as well as maximum antihyperglycemic effect, both were associated with the dose of 200 mg kg^{-1}. The result of GTT studies of mild diabetic models validated the results observed in normal models confirming thereby, that the dose of 200 mg kg^{-1} is the most effective dose of CECE.

This identified most effective dose of 200 mg kg-1 of CECE also showed the maximum reduction in BGL of severely diabetic rats on its long term treatment of 28 days. Though, the effectiveness of the CECE in severely diabetic rats was almost at par as compared with the synthetic drug, glipizide during FBG studies. Moreover, during PPG studies the fall produced by CECE was found little lesser as compared to glipizide. Since, glipizide has been used to treat diabetes, by stimulating insulin secretion from pancreatic beta cells, therefore results of CECE which are comparable with those of glipizide suggest that the mechanism of action of this plant is somewhat similar to the reference drug. Thus, the plausible mechanism by which CECE decrease blood sugar level may be by increasing the pancreatic secretion of insulin from beta cells of islets of langerhans.

Since, lipid abnormality is related with the premature atherosclerosis which is the major cause of cardiovascular diseases in diabetic patients, therefore the ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile [18]. High level of HDL protects against cardiovascular diseases whereas, low level increases the risk [19]. So, HDL cholesterol is recognized as good cholesterol which removes cholesterol from arteries and transports it back to the liver for reutilization. Most of the synthetic drugs that decrease TC also decrease HDL. In the present study, the most effective dose of 200 mg kg^{-1} of CECE, not only lowered the TC and TG levels but also enhanced the cardio protective lipid HDL. It is interesting to note that the maximum antihyperlipidemic effect of the dose of 200 mg kg^{-1} of CECE was found to be even more effective than the dose of 2.5 mg kg^{-1} of glipizide in case of TC and TG levels. Whereas, in case of HDL level, the rise associated with the dose of 200 mg kg^{-1} of CECE was in close proximity with the dose of 2.5 mg kg^{-1} of glipizide in severely diabetic rats after 28 days of treatment. Hence, protective effect of CECE on cardiovascular disease is of value addition.

CONCLUSIONS

It may therefore be concluded that the CECE could be explored further for developing it as a novel, antidiabetic agent with great margin of safety for treating diabetes and managing its complications, as it can reverse dyslipidemia associated with diabetes, and prevent cardio vascular complications which are extensively prevalent in diabetic patients. The study has clinical implications as well.

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