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Anti-diabetic effect of *Costus pictus* leaves in normal and streptozotocin-induced diabetic rats

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Abstract

Costus pictus D. Don, commonly known as 'insulin plant' is a member of Zingiberacea family and is used as a munching dietary supplement for the treatment of diabetes in Southern India. The present study was carried out to evaluate the antidiabetic effect of *Costus pictus* leaves in normal and streptozotocin-induced diabetic rats. The oral feeding of aqueous leaf solution of this plant in diabetic rats for 28 days at a dosage of 2gm/kg body weight exhibited a significant ($p<0.001$) reduction in fasting blood glucose level and a remarkable increase in serum insulin level. There was a significant reduction ($p<0.001$) in serum parameters like SGOT, SGPT, lipids, triglycerides, total cholesterol, urea, TBARS, and albumin in diabetic rats treated with leaf solution. The body weight of diabetic rats was restored to normal state when treated with the *C. pictus*. Morphometric analysis of *C. pictus*-treated rat pancreatic islets showed a significant ($p<0.001$) increase in the number and area of islets when compared with normal and diabetic control rats. Histopathology studies in liver and kidney of diabetic rats treated with aqueous solution did not show any marked difference from normal which revealed the non-toxic effect of this plant. Estimation of trace elements using particle induced X-ray emission analysis in the leaf was also determined to find the antidiabetic potential elements in this plant. Based on the above results it is evident that the leaves of *C. pictus* have antidiabetic effect and must be considered as a potential candidate for future studies on diabetes mellitus.

Key words: *Costus pictus D.Don, Insulin plant, Diabetes, Morphometric analysis, PIXE*

Introduction

Diabetes mellitus is a known metabolic disorder of varied etiology characterized by chronic hyperglycemia due to relative deficiency of insulin or its resistance. Diabetes is associated with disturbances of carbohydrate, fat and protein metabolism. Since oral hypoglycemic agents cause side effects, there is a growing interest in herbal remedies for the treatment of diabetes mellitus.[1] Many plant preparations are used in folk medicine to manage diabetes mellitus. New oral hypoglycemic compounds from medicinal plants may provide a useful source for development of pharmaceutical entities or as a dietary adjunct to existing therapies.[2,3] Herbal drugs are considered to be less toxic and more free from side-effects compared to synthetic drugs.[4] Wide arrays of plant-derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of NIDDM.[5] Recently, a search for appropriate anti-hyperglycemic agents has focused on plants used in traditional medicine because natural products may be a better option than currently used drugs.[6] The ethno-botanical information reports that about 800 plants possess anti-diabetic potential.[7]

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Plants have always been an exemplary source of drugs and many of the currently available drugs were derived directly or indirectly from them.[8]

With this background, the present study was undertaken to examine the anti-diabetic activity of the fresh leaves of *C. pictus* in streptozotocin-induced diabetic rats. *C. pictus* D.Don commonly known as 'spiral ginger' 'step ladder' or 'insulin plant' is a member of Zingiberacea family and is a newly introduced plant in India; originated probably in Mexico. In India it is grown in gardens especially in the state of Kerala where the fresh raw leaves are eaten by diabetic people. It is used as a munching supplementary food for the treatment of diabetes. Toxicity studies and antidiabetic activity of methanolic extract of this plant has been reported previously.[9,10] The aim of the present study was to evaluate the antidiabetic potential of fresh solution of *C. pictus* leaves in normal and streptozotocin-induced diabetic rats.

Materials and Methods

Plant material

Fresh *C. pictus leaves* were collected from the herbal garden of VIT University, Vellore, Tamilnadu. 5gm of *C. pictus* leaves were grounded with 10 ml of water and the resulting fresh aqueous solution was used to feed the rats orally.

Chemicals

Streptozotocin (STZ) was purchased from Sigma (St Loius, MO, USA), glucose oxidase/peroxidase (GOD/POD)

reagent (glucose kits were obtained from Randox Laboratories Ltd, UK). Serum analysis was done using kit by Biocrest Systems. All other chemicals used were of analytical grade.

Animals

Albino Wistar rats with body weights of (150 -200 gm) were obtained from the Animal house, Department of Physiology & Diabetes Research Centre, Christian Medical College, Bagayam, Vellore, India. Animals were maintained in the Animal house at an ambient temperature of 25-30˚ C at 12 h dark and light cycle. Animals were fed with pellet diet (Saidurga Agencies, Bangalore) and water *ad libitum*.

Induction of Diabetes

The rats were injected with streptozotocin (STZ) dissolved in citrate buffer (pH 4.4) at a dose of 35 mg/kg body weight intraperitoneally. Diabetic state was confirmed on the third day and rats whose fasting plasma glucose (FPG) levels > 200 mg/dl were considered to be diabetic.

Experimental design

18 rats were divided into 3 equal groups as follows: (i) Normal rats with a dose of 2 gm/kg body weight of aqueous solution of *C. pictus* (2 gm/kg body weight per rat) orally for 28 days. (ii) Diabetic control rats: Rats were made diabetic by a single intraperitonial injection of streptozotocin at a dose of 35 mg /kg body weight and with normal water and diet for 28 days. (iii) *C. pictus* treated rats: Rats were made diabetic by a single intraperitonial injection of streptozotocin at a dose of 35 mg /kg body weight and fed with aqueous solution of *C. pictus* (2 gm/kg body weight per rat) orally for 28 days.

Biochemical assays

On the 28th day of the experiment all animals were sacrificed under sodium pentathione anesthesia. The ethical committee guideline of the institute (Christian Medical College) was strictly followed. The rats were sacrificed and blood was collected immediately for serum separation and relevant organs like liver, kidney and pancreas were dissected out and stored in 10% formalin for histopathological studies and rest stored at -20˚C for biochemical enzyme assays. Plasma glucose level was estimated by GOD-POD method of Trinder.[11]

Serum SGOT, SGPT, protein, urea, albumin, triglycerides, cholesterol, ALP was estimated using approximate kits by Biocrest Systems. Total lipids in serum, liver and kidney were estimated using the method of Frings *et al* [12]. Liver and kidney TBARS were estimated by the method of Okhawa *et al*. [13] Liver and kidney cholesterol, triglycerides estimated using kit by Biocrest systems. Serum insulin levels were estimated using a radioimmunoassay kit (Mercodia, Germany).

Histology studies

After taking blood for the biochemical analysis, the animals were sacrificed, and small slices of liver, pancreas and kidney were taken and fixed in 10% buffered formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 5 μm in thickness were prepared and stained with haematoxylin and eosin then examined under microscopy.

Morphometry studies

Morphometric studies on rat pancreatic islets were done using image processing software (Image Analysis). Area and diameter of pancreatic islets was measured and results represented in micrometer and compared with control for statistical significance.

Statistical analysis

Data were statistically evaluated using one way ANOVA and expressed as mean ± SD. Kruskall Wallis test and Mann Whitney U test using 11.0 version of SPSS Software were used when applicable. $p \leq 0.05$ was considered to be significant.

Phytochemical and elemental analysis

Preliminary phytochemical screening of the *C. pictus* leaf was carried out as described earlier.[14] *C. pictus* leaf contains various phytochemicals like alkaloids, glycosides, carbohydrates, saponins, proteins and phenols. Trace elemental analysis was carried out using PIXE (Particle Induced X-ray Emission) technique, as it is one of the most promising analytical techniques used in trace elemental analysis.

Trace elements play a very important role in the formation of active chemical constituents present in medicinal plants and are, in part, responsible for their medicinal properties.[15] The present experiment in trace elemental analysis of *C. pictus* leaf was carried out at the Institute of Physics, Bhubaneswar with a 3MV pelletron accelerator. A quantity of 150 mg of powdered sample was mixed with high purity graphite

Table 1: Effect of oral feeding of *C. pictus* on body weight of normal and diabetic rats

Treatment	Body weight before Inducing diabetes	3 days after	Days after treatment			
			Day 7	Day 14	Day 21	Day 28
Normal+ *C. pictus*	156.17 ±14.21	-	155.83±14.63	156.50 ±14.19	156.5±10.93	160 .17 ±12.61
Diabetic Control	147.33* ± 9.63	141.17*±8.01	136.67* ±8.55	136.00* ±11.31	133.83*±10.40	132.67* ±11.22
Diabetic *C. pictus*	155.83* ±15.94	153.17 * ±15.51	153.33*±14.02	150.00* ±8.94	153.67* ± 15.70	156.17* ±13.12

Data represented as mean ± SD values of 6 animals each. * Significant values when compared with diabetic control p<0.001(ANOVA).

Table 2: Effect of oral feeding of *C. pictus* on fasting plasma glucose level of normal and diabetic in rats

Treatment	FPG level before inducing diabetes	3 days after	Days after treatment			
			Day 7	Day 14	Day 21	Day 28
Normal+ *C.pictus*	118.67 ± 5.16	-	117 ±3.52	113.33 ± 3.93	107 ± 6.03	98.33 ±1.50
Diabetic Control	120* ± 6.32	226.33* ± 22.25	229.3* ±16.23	230.67* ± 14.89	231.33*± 6.66	231.33* ± 17.22
Diabetic *C.pictus*	118.33* ± 3.88	261.33* ± 37.66	189* ± 2.58	165 * ±16.28	148.3* ±15.72	129* ±7.67

Data represented as mean± S.D values of 6 animals each. * Significant values when compared with diabetic control p<0.001(ANOVA)

Table 3: Effect of oral feeding of *C. pictus* on serum parameters of normal and diabetic of rats

Groups	ALP	Protein	Urea	Albumin
Normal+ *C.pictus*	96.67 ±10.42	7.02 ±0.44	29.40 ±4.72	4.23 ± 0.29
Diabetic control	149.17* ±17.55	4.64 * ±0.56	77.58 * ±12.54	2.79 * ±0.51
Diabetic+ *C.pictus*	96.55* ±11.10	7.10 * ±0.31	34.56 * ±4.50	4.84 * ±0.26

Data represented as mean ± S.D values of 6 animals each. Significant values when compared with diabetic control p<0.001 (ANOVA).

Table 4: Effect of oral feeding of *C. pictus* on serum parameters of normal and diabetic rats

Groups	SGPT	SGOT	TC	TG	Lipids
Normal+ *C.pictus*	21.00 ±5.58	26.67 ±6.53	101.06 ± 12.15	79.75 ±10.43	51.87 ± .10.02
Diabetic control	38.17* ±4.49	34.67 *±5.16	165.92* ±18.59	141.92 * ±11.83	168.67* ±.25.81
Diabetic+ *C.pictus*	21.00* ±7.32	22.00* ±6.69	65.92* ±21.96	87.62*±9.39	47.93* ±.8.73

Data represented as mean ± S.D values of 6 animals each. Significant values when compared with diabetic control p<0.001 (ANOVA). TC-Total cholesterol,TG-Triglycerides, SGPT- Serum Glutamate Pyruvate Transaminase, SGOT- Serum Glutamate Oxidase Transaminase

Table 5: Effect of oral feeding *C. pictus* on liver TBARS, Lipids, TC and TG of normal and diabetic rats

Groups	TBARS	Lipids	TC	TG
Normal+*C.pictus*	12.99 ±3.27	75.99 ±19.37	41.90 ±15.67	91.29 ±24.45
Diabetic control	37.90* ±6.98	124.68* ±15.99	106.42* ±15.47	142.71* ±9.26
Diabetic+ *C.pictus*	11.17* ±2.34	97.46* ±6.76	49.70* ±7.90	94.32* ±7.26

Data represented as mean ± S.D values of 6 animals each. *Significant values when compared with diabetic control p<0.001 (ANOVA). TBARS –Thiobarbituric acid reactive substances,TC-Total cholesterol, TG –Triglycerides

Table 6: Effect of oral feeding of *C. pictus* on kidney TBARS, Lipids, TC, and TG of normal and diabetic rats

Groups	TBARS	Lipids	TC	TG
Normal+*C.pictus*	8.45 ±1.97	116.49 ±10.45	54.48 ±19.77	108.00 ±11.57
Diabetic Control	15.17* ±3.83	145.48* ±13.45	125.17* ±19.52	131.35* ±8.84
Diabetic +*C.pictus*	14.41* ±3.61	95.53* ±9.61	44.95* ±8.69	101.07* ±10.54

Data represented as mean ± S.D values of 6 animals each. *Significant values when compared with diabetic control p<0.001 (ANOVA). TBARS-Thiobarbituric acid reactive substances, TC-Total cholesterol, TG -Triglycerides

powder in a 3:2 ratio. These pellets were then used as targets for the PIXE experiment.[16] From the PIXE spectra, different elements and their concentrations were estimated using GUPIX Software (Ennetturgi, Switzerland.

Results

Changes in body weight

Table 1 shows the body weight changes in the normal and experimental animals in each group. The mean body weight of the diabetic rats decreased compared to extract-treated rats. There was a significant reduction in body weight of the diabetic rats compared with normal and extract-treated diabetic rats. After aqueous extract of *C. pictus* supplementation for 28 days there was a significant increase in the body weight of diabetic rats (p<0.001).

Biochemical studies

Effect of *C. pictus* on FPG level of experimental rats is shown in Table 2. Oral administration of *C. pictus* at a dose of 2g/kg body wt produced a significant reduction in the fasting blood plasma level in diabetic rats (p<0.001). There was a reduction of about 44.48% when compared with diabetic control. Significant reduction in serum parameters of protein, urea, albumin, lipids, total cholesterol, triglycerides SGOT and SGPT was also observed in *C. pictus* treated rats (Table 3, 4, 5) when compared with the diabetic control (p<0.001).

Table 7: Trace element content of *C. pictus* leaves as analyzed using PIXE Technique

K (%)	0.40 ± 0.12
Ca (%)	1.02 ± 0.10
Ti	1.2 ± 0.3
V	1.0 ± 0.1
Mn	25.7 ± 3.2
Fe	85.7 ± 4.9
Cu	2.5 ± 0.4
Zn	3.6 ± 0.3
As	0.11 ± 0.05
Se	0.03 ± 0.01
Sr	6.2 ± 0.4
Pb	1.1 ± 0.2

Mean concentration and standard deviation (in ppm) of elements present in *C. pictus* leaves

Serum insulin assay in treated group showed a maximum of 0.3µg/ml when compared with that of normal (0.23µg/ml) and diabetic control (0.11 µg/ml).

Morphometric studies

Morphometric studies in rat islet pancreatic islets (n=7) showed an increase in area and diameter in *C. pictus* treated diabetic rats when compared with diabetic control which was then compared statistically using Mann Whitney U test & Kruskal Wallis test ($p<0.005$).

Trace elemental & phytochemical analysis

In the present study, estimation of trace elements was performed to determine the content of elements in this plant. Our results show that the analyzed leaves and rhizomes of *C. pictus* contain appreciable amounts of the elements K, Ca, Cr, Mn, Cu, and Zn, which may be responsible for potentiating insulin action (Table 7). Among the various trace elements in the leaf, the concentration of Fe (85.7 ppm) and Mn (25.7 ppm). Supplementation of manganese reverses the impaired glucose utilization.[17] Diabetes mellitus is a disease of metabolic disorder, elements may play important role in the management of diabetes. The various elements present in these anti-diabetic medicinal plants have either direct or indirect role in the control and management of diabetes mellitus since diabetes is associated with marked alterations in the concentrations of trace elements. Regulation of trace elemental concentrations has therefore been proposed as a potential preventive and treatment strategy for this disease. The results of the present study support the usage of these medicinal plants in the treatment of diabetes since they are found to contain appreciable amounts of the elements such as K, Ca, Cr, Mn, Cu, and Zn, which may be responsible for potentiating insulin action.

Discussion

The present study investigates the antidiabetic effect of *C. pictus* on streptozotocin-induced diabetic rats. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues.[18] Twenty-eight days administration of aqueous solution of *C. pictus* resulted in significant reduction in the fasting blood glucose level compared to diabetic rats. The difference observed between the initial and final fasting plasma glucose levels of different groups revealed a significant elevation in blood glucose in diabetic control group compared to normal. It is evident from these investigations that the aqueous extract is effective in maintaining the blood glucose levels in normal and STZ-nicotinamide-induced diabetic rats. There was a significant decrease in blood glucose levels and an increase in serum insulin level upon the administration of aqueous extract of *C. pictus* to diabetic rats.

No significant alteration was found in FPG level of control rats which justifies the antidiabetic activity of this plant. The extract did not show much reduction in the FPG level of normal rats. During the 28-day experimental period the body weight is reduced in diabetic rats, whereas there was a significant gain of body weight in treated rats. The failure of STZ-induced diabetic rat to gain weight has already been reported.[19] The administration of *C. pictus* restored these levels significantly (P <0.001) towards normal. The ability of the aqueous extract to restore body weight seems to be a result of its ability to reduce hyperglycemia.[20] Diabetic rats treated with the aqueous extract showed an increase in body weight compared to diabetic control. This may also be due to the protective effect of the extract in controlling muscle wasting i.e. reversal of gluconeogenesis. Oxidative stress has been shown to play a role in the pathogenesis of diabetes as such, antioxidants may have a role in the alleviation of diabetes.[21] STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood sugar seen in animals.[22] In our study, the aqueous extract was found to have strong antioxidant activity and to lower the TBARS levels in liver and kidney of treated rats. This may be due to the presence of phenols and flavonoids, which may have a major role in reducing oxidative stress associated with diabetes. Other mechanisms may involve improved glucose homeostasis such as, increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes, inhibition of intestinal glucose absorption, and reduction of glycaemic index of carbohydrates.[23] Medicinal plants could be considered as potential sources for providing a reasonable amount of the required elements other than diet to the patients of diabetes mellitus. Several controlled clinical trails of trace element supplements for glycemic control revealed the beneficial role for supplementation for the control and management of diabetes.[24,25,26] In diabetic rats there was a significant increase in lipids, total cholesterol, triglycerides (p<0.001). In *C. pictus*-treated rats, there was a reduction in cholesterol, triglycerides, lipids, which shows the hypolipidemic effect of this plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis.[27] In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing

hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after *C. pictus* treatment may be directly attributed to improvements in insulin levels.

C. pictus lowered serum SGPT, SGOT levels which shows the protective effect and normal functioning of liver in reversing the organ damage due to diabetes which is clearly observed by high levels of SGOT and SGPT in diabetic control.[28] Histopathology studies in liver and kidney of *C. pictus*-fed rats did not show any significant difference from normal rats, which suggest that this plant is not having any toxic effect. There was no significant difference between normal and *C.pictus* fed rats in protein level, whereas there was a significant reduction in the diabetic control and the protein level was restored in *C. pictus* fed rats ($p<0.001$)

Serum urea level in untreated diabetic rats was very high when compared with normal and treated rats, which shows renal dysfunction is associated with diabetes.[29] It is concluded that aqueous extract of *C. pictus* has antidiabetic activity. Analysis of trace elemental may be helpful in the formulation of new ayurvedic drugs which can be used for the control of diabetes. Our results show that the analyzed plant can be considered as a potential source of required elements other than diet for patients with chronic diabetes..

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