Evaluation of antidiabetic potential of different fractions of methanolic leaf extract of *Costus pictus* D. Don in Swiss albino mice

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Abstract

The aim of the present study was to determine the most effective hypoglycemic fraction of methanolic extract from *Costus pictus* (CP) in streptozotocin (STZ)-induced diabetic mice. Methanolic extract of leaves of *Costus pictus* (CPME) was fractioned with different solvents in order of increased polarity. The glucose tolerance of each fraction was tested in normal mice orally and, further, assessed for antidiabetic activity in STZ-induced diabetic mice. The blood glucose levels and body weight were measured periodically. Serum biochemical parameters were also evaluated and it was observed that the treatment with methanol fraction CPMF (500 mg/kg body weight) was found to be most effective as significant ($p<0.05$) changes were perceived in random blood glucose levels, transaminase activities, alkaline phosphatase, total bilirubin, creatinine and triglycerides levels.

Key words: *Costus pictus* D. Don, Oral Glucose Tolerance, Streptozotocin, Antidiabetic, Blood glucose, Alkalin phosphatase, Creatinine, Triglycerides

Introduction

It is now well known that Diabetes mellitus (DM) is the most common endocrine disorder and in the coming decade, it is going to be a life style disease, affecting majority of people across the globe. Now-a-days, due to the complications of available antidiabetic drugs, people are inclined towards natural remedies for the prevention of diabetes. A number of plant species are known to have antidiabetic effects (Sabu and Kuttan, 2002; Reyes *et al.*, 2006; Hong *et al.*, 2007) and *Costus pictus* D. Don (CP) is the plant that has recently been introduced to India from Mexico. This exotic plant is a perennial, rhizomatous herb and has large green leaves on red spiral stem, yellow with red streaked flowers on the spike at the tip of the mature stem. Since its introduction to Kerala (India), the people of the state used to consume the leaves of CP to control diabetes and the plant is popularly known as 'insulin plant'.

Its miraculous effect in the lowering of blood glucose has been studied (Merina, 2007) and various extracts of the plant have been proved as diuretic (Camargo *et al.,* 2006), anti-inflammatory (Anaga *et al.*, 2004), antidiabetic and insulin secretory (Merina, 2007; Jothivel *et al.,* 2007), enhancing glucose uptake (Shilpa *et al.*, 2009; Gireesh *et al.,* 2009; Romaiyan *et al.,* 2010), antioxidant (Devi and Urooj, 2008; Jaysree *et al.*, 2009), antibacterial (Beena and Reddy, 2010) , ameliorative on mitochondrial enzymes (Maruthappan and Sakthisree, 2010) and anticancer (Nadumane *et al.*, 2011) agent.

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Although, a number of reports on various extracts of leaves of CP are available, no efforts have been made to investigate the most active extract(s) or fraction(s) regarding its antidiabetic effect. Therefore, this study was focused to evaluate the antidiabetic potential of different fractions of methanolic extract of CP leaves.

Materials and Methods

Chemicals and reagents

Streptozotocin (Product code: 1001422342, CAS: 18883-66-4) and 1,1-Dimethylbiguanide hydrochloride (Metformin; Product Code: 101188574, CAS: 1115-70-4) was procured from Sigma-Aldrich, New Delhi, India. Biochemical kits were obtained from Randox (Randox Laboratories Ltd. Co., Antrium, UK). All other chemicals used, were of analytical grade and were purchased either from HiMedia India Ltd. or Merck India Ltd., Mumbai, India.

Plant material

Fresh leaves of *C. pictus* were collected during October, 2011 from the herbal garden of Sacred Heart College, Sitapur, Uttar Pradesh (India) and were authenticated by Professor Santhosh Nampy, Department of Botany, St. Joseph's College, Devagiri, University of Calicut, Kerala (India) and a voucher specimen (No. SJC/BOT/RES-EXT/1/2012) of the herbarium was deposited for future reference.

Preparation of methanolic extract and its fractions

The fresh leaves were cut into pieces and shade dried at room temperature. The dried leaves were subjected to size reduction to a coarse powder, using a grinder. The powdered leaves (250g) were packed into soxhlet apparatus and extracted with 95% methanol for six hours. The extract was dried at 40°C in a rotary evaporator (CPME; yield 6.0%).

The CPME (10g) was then partitioned with hexane (CPHF), ethyl acetate (CPEAF) and water (CPWF), respectively (3 x 100 ml, each) and the last dried residue was considered as methanol fraction (CPMF). All the fractions were concentrated to dryness and stored at 4°C in well labeled airtight containers (yield 1.0%, 1.2%, 1.3% and 2.5%, respectively). Fractionation scheme is shown in Figure 1.

Preparation of dose (s)

The glucose solution was prepared by dissolving the dextrose sugar (2g/kg body weight/10ml) in distilled water at least 12h before the oral administration to animals. The required doses of the respective fractions were suspended in 0.4% w/v carboxy methyl cellulose (CMC) in order to get 200 and 500mg/kg body weight/10ml concentrations. Metformin was dissolved in distilled water to get 250mg/kg body weight/10ml dose.
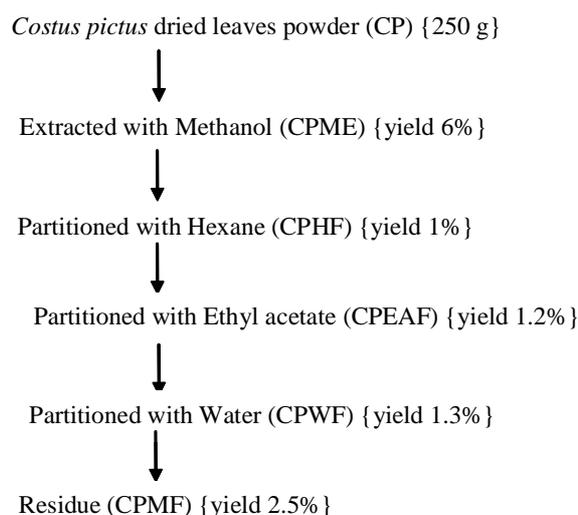
Costus pictus dried leaves powder (CP) {250 g}

↓

Extracted with Methanol (CPME) {yield 6%}

↓

Partitioned with Hexane (CPHF) {yield 1%}

↓

Partitioned with Ethyl acetate (CPEAF) {yield 1.2%}

↓

Partitioned with Water (CPWF) {yield 1.3%}

↓

Residue (CPMF) {yield 2.5%}

Figure 1: Schematic presentation of fractionation procedure

Animals

Swiss albino male mice (outbred strain) of 32-40 g were used for the study. The mice were fed with standard diet (Dayal Industries, Lucknow, India) freely and water *ad libitum*. The mice were acclimatized for a period of 7 days under standard environmental conditions (temperature 25 ± 2 °C; RH $50 \pm 5\%$; 12h light/dark cycle) before commencing the experiment. Institutional Animal Ethics Committee duly approved the experimental protocol (Reg. No. 400/01/AB/CPCSEA, AH-2012-08) and the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) recommendations were followed for animal care.

Oral glucose tolerance test in mice

After overnight fasting, mice were divided into twelve groups of five animals each (n=5). The Group I received 0.4% w/v carboxy methyl cellulose (CMC)/10ml body weight and was kept as a normal control. Groups II was given metformin (250 mg/kg body weight/10ml). Group III-XII were administered CPME, CPHF, CPEAF, CPWF, CPMF at 200 and 500mg/kg body weight/10ml, respectively. Thirty minutes later, glucose solution (2g/kg body weight/10ml) was administered to each mouse. All the treatments were given orally. Blood samples were collected from the tail vein at – 30 min (fasting; prior to any treatment) and at 15, 30, 60, 90, and 120 min after glucose administration. The blood glucose level of individual animal was measured with a glucometer using compatible and authentic strips (Elite, Bayer India).

Antidiabetic activity in streptozotocin-induced diabetic mice

After an overnight fasting, seventy mice were injected intraperitoneally freshly prepared streptozotocin (STZ) @ 100 mg/kg dissolved in 0.01 M citrate buffer (pH 4.5). Hyperglycemia was confirmed by the elevated blood glucose levels (>200mg/

dl) after 4 days of STZ treatment. After 7 days of STZ injection, mice with blood glucose levels ≥ 250 mg/dl were included in the study and respective treatment was started.

All the mice were randomly divided into the seven groups with ten animals in each group (n=10). Group I served as normal control (non-diabetic + vehicle). Group II was named as diabetic control (diabetic + vehicle). Group III served as positive control (diabetic + metformin 250mg/kg body weight). Group CPEAF-200 (diabetic + CPEAF 200mg/kg body weight), group CPEAF-500 (diabetic + CPEAF500 mg/kg body weight), group CPMF-200 (diabetic + CPMF 200mg/kg body weight) and group CPMF-500 (diabetic + CPMF 500mg/kg body weight) served as test groups, respectively.

Random blood glucose level and body weight were measured on initial day (0), 7th, 14th and 21st day of the study. On day 21st, blood was collected from each overnight fasted mouse by cardiac puncture under deep ether anesthesia. The serum was separated from the blood and analyzed for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatinine (CRTN), total bilirubin (TBIL), triglycerides (TG), and total protein (TP) using commercially available kits.

Statistical analysis

The results are expressed as mean ± S.E.M. and the data were analyzed with the help of GraphPad Prism 5.01 (GraphPad Software Inc., USA) using one-way-ANOVA, followed by Tukey's Post Hoc Test. Differences between groups were considered significant at $p<0.05$ or $p<0.01$ or $p<0.001$.

Results and Discussion

The prevalence of diabetes is increasing rapidly and it is one of the leading cause of mortality worldwide (Qi et al., 2008). Hyperglycemia is the common characteristic of diabetes and the decreased utilization of glucose by the tissues results into severe and late complications. Lowering blood glucose to basal level is one of the basic approaches to treat all diabetic patients (Shirwaikar et al., 2005). The reduction in blood glucose level in hyperglycemic normal mice during OGTT may occur due to a reduction in intestinal glucose absorption or induction of glycogenic process along with reduction in glycogenolysis and glyconeogenesis (Porchezhian et al., 2000). During OGTT, the oral administration of CPME-500, CPEAF-500 and CPMF-500, blood glucose level was decreased significantly ($p<0.05$) at each time interval (15, 30, 60, 90, and 120 min). The CPMF-200 also displayed significant ($p<0.05$) change after 15, 60, 90 and 120 min of glucose administration. However, CPEAF-200 exhibited significant ($p<0.05$) antihyperglycemic effect after 60 min and 90 min of an oral glucose load but CPME-200 showed significant ($p<0.05$) changes after 120 min only. While both CPHF and CPWF were failed to pose any change in oral glucose tolerance at the tested doses. The results of this preliminary study clearly indicated that CPEAF and CPMF are the active fractions of CPME so we selected both fractions for the further study (Table 1).

Streptozotocin (STZ), commonly used for induction of experimental diabetes causes selective necrosis of the pancreatic β-cells via generation of superoxide dismutase (SOD) anions in mitochondria resulting in elevated blood glucose level (Papaccio et al., 2000; Szkudelski, 2001). The random blood glucose level (RBGL) was significantly reduced to ~48% ($p<0.05$), 53% ($p<0.01$) and 53% ($p<0.001$) on day 7, 14 and 21 respectively by CPMF-500 as compared to diabetic control. Oral administration of CPMF-200 also significantly decreased RBGL to ~44% ($p<0.01$) and 43% ($p<0.001$) on day 14 and 21 respectively. The CPEAF-500 reduced RBGL to ~44% ($p<0.05$), 49% ($p<0.01$) and 47% ($p<0.001$) on day 7, 14 and 2, respectively. However, CPEAF-200 showed a significant effect (~33%; $p<0.05$) on RBGL at day 21 compared to diabetic control group. The result of the above investigation clearly depicts the effectiveness of CP for controlling hyperglycemia (Table 2).

In diabetes mellitus, breakdown of muscle and tissue proteins increases during glycogenolysis cycle i.e., catabolism of fats and proteins which directly leads to loss in body weight (Shirwaikar et al., 2006; Shirwaikar et. al., 2004). On day 21, significant improvement in body weight was observed as ~49% ($p<0.01$) and ~41% ($p<0.05$) after CPMF-500 and CPEAF-500 treatment, respectively compared to STZ treated mice. CPMF-200 and CPEAF-200 were unable to produce significant change in the body weight, indicating the fact that CP has low protective effect in controlling fat and/or muscles waste i.e. slow reversal of gluconeogenesis (Figure 2).

The rise in serum level of ALP, AST, ALT, TBIL, TP have been attributed to the damaged structural integrity of the liver (Asayama et al., 1994) which are considered as a sensitive marker of liver injury (Yadav et al., 2008). These manifestations are a consequence of a metabolic alteration with an increase of glyconeogenesis, ketogenesis and/or of hepatic lesions that occur in diabetic animals. The significant decrease in liver enzyme levels namely AST (~52%; $p<0.01$), ALT (~42%; $p<0.05$), ALP (~83%; $p<0.05$) and TBIL (~52%; $p<0.01$) were noticed after oral administration of CPMF-500 while CPMF-200 was able to reduce AST (~42%; $p<0.01$), ALP (~78%; $p<0.05$) and TB (~45%; $p<0.01$) as compared to diabetic animals. The CPEAF-500 also brought down the levels of AST (~44%; $p<0.01$) and ALP (~77%; $p<0.05$) towards basal level indicating the hepatoprotective action of CP.

Table 1: OGTT of *Costus pictus* leaves methanolic extract and its fractions

	Blood glucose level (mg/dl)					
		After glucose administration				
Groups	Fasting	15 min	30 min	60 min	90 min	120 min
Normal	76.0±9.63	301.0±10.19	208.2±7.96	124.6±15.44	115.6±12.10	83.4±8.65
Metformin	88.8±5.96	231.0±12.13*	110.2±7.48***	52.4±7.10***	40.2±6.19***	46.2±7.05*
CPME-200	55.4±6.38	239.0±10.39	161.0±10.18	81.8±5.36	71.4±6.38	46.0±6.07*
CPME-500	56.8±5.89	227.6±13.96*	127.4±9.22**	73.2±7.02*	64.2±7.02*	44.6±8.25*
CPHF-200	62.6±7.75	285.6±18.11	237.6±9.54	101.6±8.89	92.8±7.64	74.2±7.21
CPHF-500	62.6±7.75	282.8±15.31	232.8±11.65	93.8±5.44	80.6±10.25	72.2±7.16
CPEAF-200	41.4±3.39	236.0±12.19	189.0±7.42	75.2±7.00*	70.6±4.93*	54.6±4.91
CPEAF-500	51.0±10.83	231.0±16.80*	123.0±9.40**	61.6±13.76**	56.6±7.14**	45.2±7.42*
CPWF-200	54.6±8.41	270.8±14.04	157.2±18.60	83.2±8.60	109.8±16.11	59.2±5.91
CPWF-500	51.0±6.33	251.6±15.78	157.2±18.60	81.0±6.67	75.8±11.42	51.2±6.79
CPMF-200	47.6±4.80	229.6±10.54*	180.4±26.13	68.2±11.93**	60.2±6.37**	44.0±4.79*
CPMF-500	51.0±5.81	217.6±12.49**	125.2±17.14**	59.6±10.09***	49.2±7.47***	49.0±8.13*

"Values are expressed as Mean ± S.E.M., n = 5" *$p<0.05$, **$p<0.01$, ***$p<0.001$ when compared to normal group

Table 2: Random blood glucose level (RBGL) of experimental mice

|	Blood glucose level (mg/dl)				
Groups	Fasting	Day 0	Day 7	Day 14	Day 21
Normal	66.4±2.54	136.2±8.16	144.0±13.42	125.4±10.12	124.2±6.75
Diabetic	63.6±6.93	339.2±20.54##	394.4±11.70###	456.8±15.35###	477.6±19.12###
Metformin	60.0±5.69	353.8±13.02###	242.0±11.78**	243.2±16.92***	305.2±10.85###,***
CPEAF-200	59.8±3.46	341.7±27.84###	339.4±23.30###	353.3±29.66###	361.9±20.46###,*
CPEAF-500	63.4±3.86	335.3±27.25###	283.7±21.52##,*	294.6±22.46##,**	313.0±21.85###,***
CPMF-200	65.2±7.86	337.1±29.20###	294.0±17.86##	310.4±26.60##,*	325.9±25.95###,***
CPMF-500	71.3±5.05	324.3±27.92##	272.4±32.78#,*	280.9±34.16#,**	289.4±18.16###,***

"Values are expressed as Mean ± S.E.M., n = 10" #$p<0.05$, ##$p<0.01$, ###$p<0.001$ when compared to normal group; *$p<0.05$, **$p<0.01$, ***$p<0.001$ when compared to diabetic group

Table 3: Biochemical parameters of experimental mice

Groups	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	T BIL (mg%)	TP (mg%)	CRTN (mg%)	TG (mg%)
Normal	151.79±10.46	61.06±5.29	112.49±9.64	0.52±0.05	4.96±0.36	0.65±0.04	113.06±7.81
Diabetic	238.36±14.91##	191.26±12.79###	244.86±10.24###	2.02±0.17###	2.55±0.27	1.51±0.06###	196.16±0.17###
Metformin	161.99±12.10**	99.88±8.99***	147.40±19.08***	1.09±0.10***	4.59±0.56	0.87±0.07***	134.28±8.69**
CPEAF-200	189.23±12.13	161.60±7.46	204.10±4.76	1.61±0.06	3.12±1.06	1.15±0.08	151.90±11.58*
CPEAF-500	171.85±17.20*	157.09±17.14	186.13±7.60**	1.43±0.22	4.38±0.24	1.08±0.08*	144.12±7.02*
CPMF-200	170.51±13.81*	142.40±6.94	189.80±8.48*	1.34±0.11*	3.95±0.80	1.16±0.12	149.06±12.20*
CPMF-500	166.79±10.72*	136.88±12.63*	176.10±7.69**	1.23±0.09**	4.03±0.18	1.09±0.08*	142.04±7.68**

"Values are expressed as Mean ± S.E.M., n = 10" ##$p<0.01$, ###$p<0.001$ when compared to normal group; *$p<0.05$, **$p<0.01$, ***$p<0.001$ when compared to diabetic group
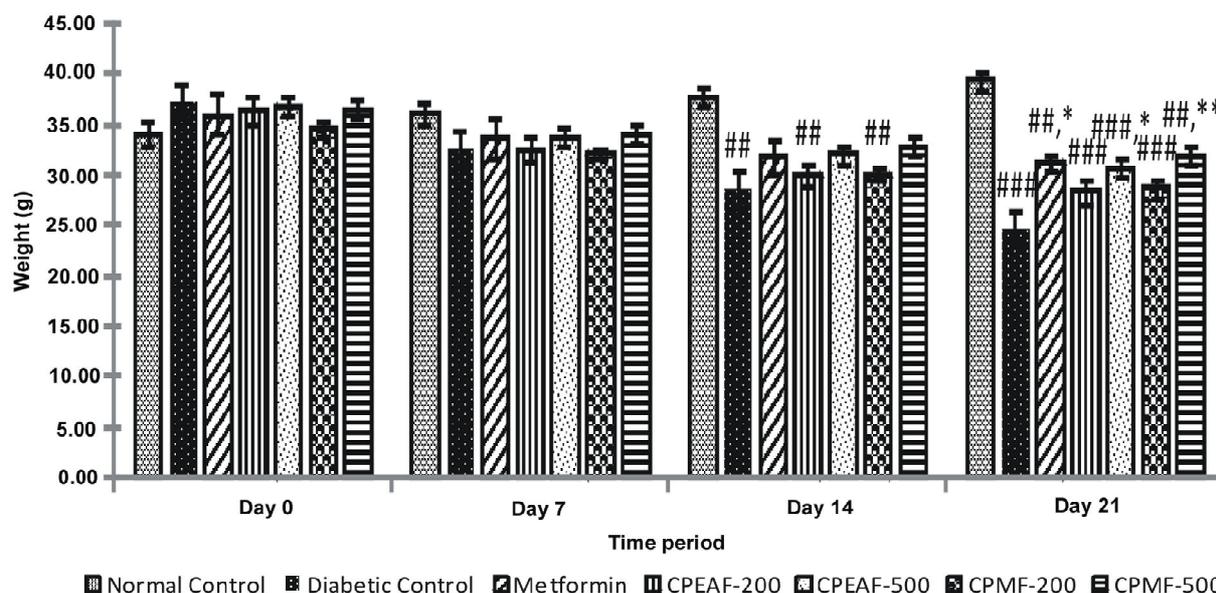
Figure 2: Effect on body weight (g) of experimental mice. Data is presented as Mean ± S.E.M., n = 10, # p<0.01, ### p<0.001 when compared to normal control group ; *p<0.05, **p<0.01when compared to diabetic group

Insulin deficiency in diabetic condition fails to activate lipoprotein lipase, thereby, causing hypertriglyceridemia (Shirwaikar *et al*., 2005). The CPMF-500, CPMF-200, CPEAF-500 and CPEAF-200 significantly affected the serum TG levels by ~65 (*p*<0.01), 57 (*p*<0.05), 63 (*p*<0.05) and 53% (*p*<0.05) %, respectively as compared to STZ-treated animals. Higher dose of streptozotocin has cytotoxic effects on kidney leading to renal toxicity (Valentovic *et al*., 2006; Tay *et al*., 2005) and elevated serum urea and creatinine level are the significant markers for renal dysfunction (Almdal *et al*., 1988). The CPMF-500 and CPEAF-500 significantly (*p*<0.05) reduced creatinine level to ~49 and ~51 %, respectively compared to diabetic animals, suggesting its renal protective function (Table 3).

Our data suggests that CP leaves have antihyperglycemic effect and the administration of methanol and ethyl acetate fractions reduced the complications associated with diabetes, by decreasing elevated blood glucose level, preserving body weight and protecting serum biochemical parameters. It is evident that the CPEAF and CPMF were effective in maintaining the blood glucose near to normal value in streptozotocin-induced diabetic mice. However, the effect of the methanol fraction (CPMF) with respect to the magnitude of studied parameters appeared very promising compared to its ethyl acetate fraction (CPEAF). However, the mechanism is not clearly understood but the antihyperglycemic effect may be probably due to an extrapancreatic mechanism and/or the regeneration of pancreatic β-cells.

Conclusion

In conclusion, our results clearly indicate that methanol fraction of CP leaves (CPMF) was more effective to that of ethyl acetate fraction (CPEAF) in STZ-induced diabetic condition. This may be due to difference in bioactive component (s) of CP responsible for the activity. Further research is recommended to elucidate the bioactive compound (s) and its mechanism of action.

Acknowledgement

Conflict of interest

The authors declare no conflict of interest.

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