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Annals of Phytomedicine 2(2): 57-62, 2013

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN 2278-9839

Comparative evaluation of *Costus pictus* D. Don leaf extracts against glucose challenged mice

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Received August 16, 2013: Revised September 30, 2013: Accepted October 10, 2013: Published online December 30, 2013

Abstract

We evaluated the glucose tolerance potential of *Costus pictus* D. Don leaf extracted with hexane (CPH), ethyl acetate (CPEA), methanol (CPM), water (CPW), 50% methanol (CPMW) and fresh juice (CPFJ) in Swiss albino mice. The extracts of *Costus pictus* were administered orally at two doses *viz.*, 200 and 500 mg/kg body weight and the glucose lowering effect was calculated in normal healthy mice by oral glucose tolerance test. The results showed that the tolerance of glucose was increased in all treated groups. The ascending order of glucose lowering effect of extracts was found as CPH-200 < CPW-200 < CPH-500 < CPFJ-500 < CPMW-200 < CPFJ-200 < CPEA-200 < CPW-500 < CPM-200 < CPMW-500 < CPEA-500 < CPM-500. The methanolic extract at a dose of 500 mg/kg body weight showed the highest percentage of glucose lowering (72.40%), followed by ethyl acetate (67.38%) and was comparable to standard drug metformin.

Key words: *Costus pictus* D. Don, OGTT, Glucose lowering effect, Metformin, Diabetes

Introduction

Diabetes is characterized by hyperglycemia and glucose intolerance associated with abnormalities in carbohydrate, protein and fat metabolism due to either total or partial insulin deficiency or to the impaired effectiveness of insulin action or a combination of both (Kevin, 2005; O'Brien and Granner, 1991). Heredity, ageing, unbalanced diet, obesity, sedentary lifestyle, stress, drugs, pancreatic dysfunction, hypertension, high serum lipid, lipoproteins, and less glucose utilization are some of the factors that promote the diabetes (Al-Taweel *et al.*, 2012). It is also associated with an increase in ischemic heart disease, stroke, hypertensive disease, renal failure, blindness and other debilitating diseases (Bradshaw *et al.*, 2007) and the prevalence has increased in epidemic proportions (Wild *et al.*, 2004). It is currently one of the most burdensome chronic diseases in the world (Anonymus, 2014) and World Health Organization (WHO) predicted that developing countries will bear the brunt of this epidemic in the coming decades. India has more than 50.8 million people with diabetes and projected to increase to 87 million by the year 2030 (Jawla *et al.*, 2012). The increment in type 2 diabetic patients in developing countries is a growing concern which might be due to the adoption of a western diet and lifestyle. The day-by-day growing number of diabetic patients has resulted in a renewed interest in the use of natural and traditional remedies for treating diabetes (Deutschländer *et al.*, 2012). Herbal drugs are now-a-days more popular because of natural origin and fewer side effects, hence explored for the discovery of potentially useful antihyperglycemic agents (Al-Aboudi and Afifi, 2011; Choi *et al.*, 2011; Rahmatullah *et al.*, 2009). Since, allopathic medicines in developing countries are expensive, not easily accessible and can have several adverse effects (Saxena and Vikram, 2004), WHO also recommends utilization of plants for the treatment of diabetes (Anonymus, 2011).

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Costus pictus D. Don (commonly known as insulin plant) is a perennial, rhizomatous herb belongs to Costaceae family. It is being used by traditional healers and herbalists in Kerala and Tamil Nadu states, in the treatment of diabetes, advising to chew a leaf daily and a phrase is popular about the plant "A leaf-a-day keeps diabetes away".

The various extracts of *C. pictus* leaves exhibited hypoglycemic effect in MIN6 β-cell line and isolated mouse and human islets (Al-Romaiyan *et al.*, 2011) and on albino rats (Sethumathi *et al.*, 2009; Antony, 2012; Isaac and Alphonse, 2011; Remya and Daniel, 2012; Suganya *et al.*, 2012). The carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase were also inhibited to some extent (Jayasri *et al.*, 2008). Although, glucose uptake was observed in 3T3-L1 preadipocytes using methanolic extract (Shilpa *et al.*, 2009) but no glucose uptake was reported in L6 myotubes using an ethanolic extract of the leaves (Pareek *et al.*, 2010). The high antiglycation activity of different extracts was also reported by Majumdar and Parihar (2012), but the exact mechanism of action still needs to be investigated. Based on the published literature and our prior report (Shiny *et al.*, 2013), the present study was designed to assess a comparative glucose lowering effect of *Costus pictus* D. Don leaf extracted with different solvents in glucose challenged Swiss albino mice.

Materials and Methods

Plant material

The leaves of *Costus pictus* D. Don were collected from the Kannur District of Kerala (India). The plant was authenticated by Dr. Santosh Nampy, Department of Botany, St. Joseph's College, Devagiri (University of Calicut, Kerala) and a voucher specimen no. SJC/BOT/RES-EXT/1/2012 was obtained. The plant leaves were dried in the shade, cut into pieces, powdered and stored in airtight container.

Preparation of leaf extracts

Dried powdered leaves (100g) were macerated with hexane (CPH), ethyl acetate (CPEA), methanol (CPM), water (CPW) and 50% methanol (CPMW) separately. The ratio of dried leaves to solvent was 1:5 (w/v) at 25°C with occasional shaking for 24 ± 1 h, then after the respective solvent extracts were decanted and filtered through Whatman filter paper no. 1. Further, a second equivalent amount of above solvents to the respective marc were added and allowed to extract for further 24 h. The filtrates were concentrated on the rotatory evaporator (Rotavapor R-220, Buchi India) at 40°C, separately and completely dried on a water bath at 45°C. Similarly, fresh juice of the leaves (CPFJ) was also filtered, concentrated and dried. All the dried extracts were stored in amber colored glass vials and kept in a refrigerator till experiments were performed. The yield of the dried extracts was found to be 4.98, 5.16, 16.91, 5.83 and 6.72% from hexane, ethyl acetate, methanol, water and 50% methanol, respectively.

Experimental animals

Swiss albino healthy male mice weighing 22 ± 2 g were used for this study. All the animals were acclimatized in groups of five in poly-propylene cages at controlled environment (22 ± 2°C, 55 ± 5% RH, 12 h dark and light cycle) with free access to standard rodent pellet diet (Dayal Industries, Lucknow) and water *ad libitum*. All experiments were conducted with ethical standards in accordance with Institutional Animal Ethics Committee (Registration. No. 400/01/AB/CPCSEA, AH-2012-08) constituted under CPCSEA guidelines.

Study design

Overnight fasted mice were divided into different groups of five animals each (n=5). The Group 1 received 0.4% w/v carboxymethylcellulose (CMC) (10 ml /kg^{-1} bw) only and served as a normal control. Group 2 was given metformin (250 mg kg^{-1} bw/10 ml) and served as positive control. Group 3-14 were administered with CPH, CPEA, CPM, CPW, CPMW and CPFJ at 200 and 500 mg kg^{-1} bw/10 ml, respectively and served as test control. After 30 min of administration of respective dose, glucose solution (2 g kg^{-1} bw/10 ml) was administered to each mouse. All the treatments were given orally. The blood samples were collected from the tail vein at fasting stage (prior to any treatment) and after 15, 30, 60, 90, and 120 min of glucose administration. The blood glucose level of individual animal was measured with a glucometer (Breeze-2®, Bayer India) using compatible and authentic strips (Gaur *et al.*, 2013; Yadav *et al.*, 2013).

Glucose lowering effect

Glucose lowering (%) was calculated using following equation (1).

$$\text{Glucose lowering } (\%) = \left(\frac{A-B}{B}\right)\times 100 \quad \text{..... Eq. (1)}$$

Where A = Initial blood glucose level and B = Final blood glucose level

Statistical analysis

All the values expressed are Mean ± Standard error of mean (S.E.M.). The data was statistically analyzed by one-way ANOVA following Dunnett's multiple comparison test using GraphPad PRISM® version 5.01 (GraphPad Software, Inc., USA) for comparing the treatment and vehicle groups. The differences were considered significant at $p < 0.05$ (Fakeye *et al.*, 2009).

Results and Discussion

Evaluation of plant products to treat diabetes is of growing interest as they contain many bioactive substances with therapeutic potential. In recent years, several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants, using experimental animals (Prabhakar and Doble, 2008; Grover *et al.*; 2002).

Table 1: Oral glucose tolerance test (OGTT) of *C. pictus* D. Don leaf extracts

Groups	Treatments	Blood Glucose Level (mg/dl)					
		Fasting	After 15 min	After 30 min	After 60 min	After 90 min	After 120 min
1	Normal control	74.5 ± 7.12	298.3 ± 14.90	197.2 ± 14.32	120.2 ± 9.62	109.7 ± 8.36	81.7 ± 9.72
2	Positive control	85.6 ± 5.82	220.0 ± 10.12*	109.6 ± 11.46***	59.7 ± 8.22***	48.7 ± 5.72***	40.7 ± 6.14***
3	CPH-200	72.7 ± 6.34	259.6 ± 12.88	185.6 ± 11.52	95.2 ± 7.24	89.8 ± 7.56	68.8 ± 9.14
4	CPH-500	79.6 ± 7.76	241.8 ± 21.34	181.8 ± 15.68	87.8 ± 8.64	68.8 ± 4.36*	59.8 ± 6.98
5	CPEA-200	72.4 ± 4.22	267.0 ± 14.67	154.0 ± 11.32	73.1 ± 6.32*	65.2 ± 2.48*	51.4 ± 5.63*
6	CPEA-500	78.0 ± 6.88	272.4 ± 17.46	137.0 ± 12.34*	66.8 ± 7.22**	56.0 ± 6.76**	46.6 ± 3.54**
7	CPM-200	71.4 ± 3.54	269.8 ± 13.82	135.2 ± 11.46*	74.2 ± 6.82*	68.4 ± 7.92*	49.6 ± 2.82**
8	CPM-500	76.2 ± 9.56	225.2 ± 8.46*	123.8 ± 15.12**	64.0 ± 8.22**	50.8 ± 5.44***	44.2 ± 3.42***
9	CPW-200	81.6 ± 8.10	283.4 ± 13.32	170.2 ± 22.52	97.4 ± 6.61	85.6 ± 7.34	72.0 ± 4.92
10	CPW-500	83.2 ± 6.92	273.6 ± 17.64	133.8 ± 11.26*	76.8 ± 7.12*	67.2 ± 7.98*	58.2 ± 7.28
11	CPMW-200	77.8 ± 5.56	267.2 ± 22.32	188.6 ± 14.72	91.4 ± 5.92	71.4 ± 3.26	56.8 ± 5.12
12	CPMW-500	80.2 ± 5.98	289.6 ± 14.38	129.0 ± 13.54*	89.6 ± 2.98	63.2 ± 2.28*	52.4 ± 2.20*
13	CPFJ-200	81.2 ± 7.90	288.8 ± 15.62	182.4 ± 8.58	101.4 ± 10.22	85.2 ± 5.66	58.6 ± 5.45
14	CPFJ-500	75.1 ± 6.28	230.8 ± 11.42	134.6 ± 6.72*	81.4 ± 5.54	65.2 ± 3.56*	55.4 ± 7.42

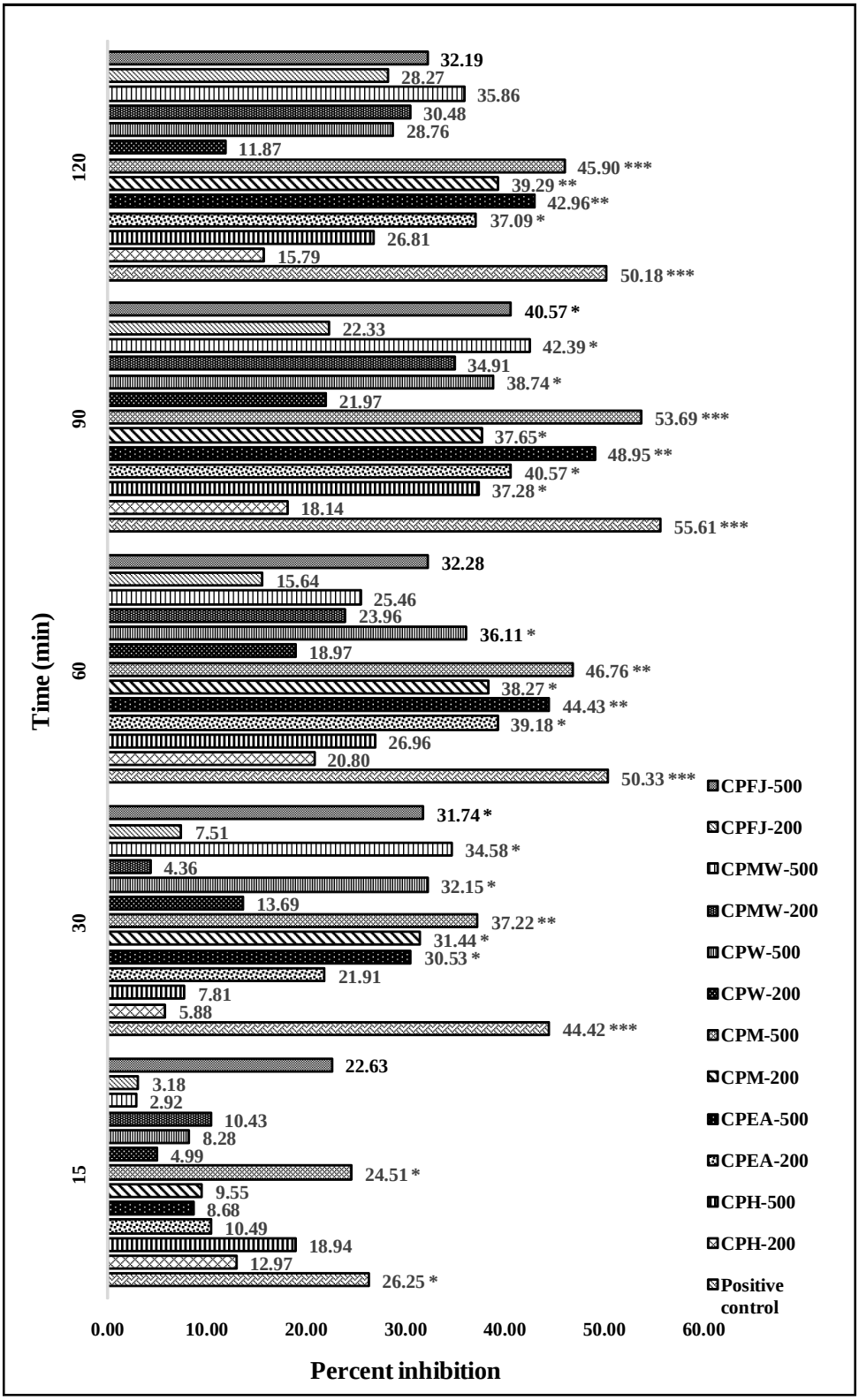
The data are expressed as Mean ± S.E.M., n=5. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to a normal control group. Positive control = Metformin; CPH = Hexane extract; CPEA = Ethyl acetate extract; CPM = Methanol extract; CPW = Water extract; CPMW = 50% Methanol extract; CPFJ = Fresh juice of leaves of *C. pictus*; The 200 and 500 is a numerical representation of the dose in mg/kg of body weight.

A variety of chemical constituents present in medicinal plants are thought to act on different targets by various modes and mechanisms. They have the potential to impart the therapeutic effect in diabetes and its complication (Narmadha and Devaki, 2013).

The oral glucose tolerance test (OGTT) is a test of immense value in favor of using fasting plasma glucose concentration alone (Urita *et al.*, 2006). The oral glucose tolerance test depends on giving a definite amount of D-glucose per kilogram body weight and on determining the blood sugar concentration before the test and at certain intervals thereafter. Normally the blood sugar rises to a maximum in half an hour period and returns to the normal level within 1 to 2 h (Du Vigneaud and Karr, 1925). GTT, a widely used procedure in the diagnosis of diabetes and intermediate stages of hyperglycemia, was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes. Apart from currently available therapeutic options for diabetes, like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes (Oyedemi *et al.*, 2012).

The OGTT results for the different groups before and during treatment are summarized in Table 1 and percent change in blood glucose levels has been depicted in Figure 1. It was observed that the percent change in blood glucose levels of the treatement groups were lower than the normal control group at all tested time points. The CPM-500 treatment was able to lower the blood glucose level (BGL) significantly ($p < 0.05$) just after 15 min of glucose administration. After 30 min of glucose loading, significant ($p < 0.05$) lowering in BGL was noticed in CPEA-500, CPM-200, CPM-500, CPW-500, CPMW-500 and CPFJ-500 treated groups. The ambient BGL were significantly ($p < 0.05$) lesser in CPEA-200, CPEA-500, CPM-200 and CPM-500 and CPW-500 treatment compared to the normal control group after 60 min of glucose loading during the OGTT experimentation. A marked ($p < 0.05$) modulation in the postprandial glucose profile (BGL) was noticed in most of the treatments (CPH-500, CPEA-200, CPEA-500, CPM-200, CPM-500, CPW-500, CPMW-500 and CPFJ-500) during the OGTT. This effect might be due to the slow absorption of the extracts. Even after 2 h of the glucose challenge, significant ($p < 0.05$) reduction was found in CPEA-200, CPEA-500, CPM-200, CPM-500 and CPMW-500 treated groups. The significant ($p < 0.05$) decrease in BGL was observed during whole time course after glucose administration in the positive control group.

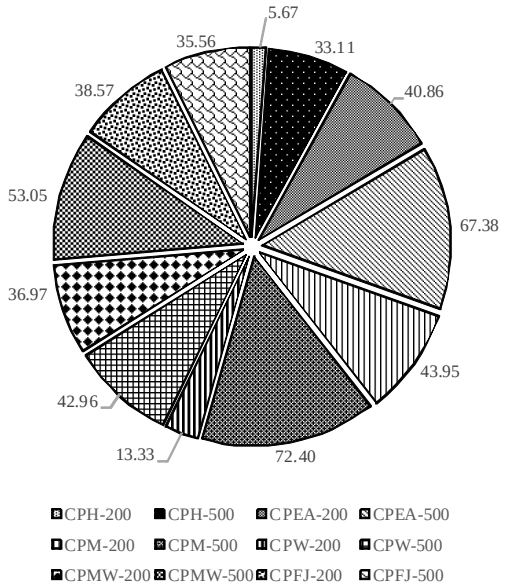
In diabetic patients, the hyperglycemia and associated complications are arisen either due to insulin resistance or reduced responsiveness of the pancreatic cells to glucose or both (Shen *et al.*, 2011). Figure 2 demonstrates the glucose lowering effect for variable doses of different leaf extracts



Positive control = Metformin; CPH = Hexane extract; CPEA = Ethyl acetate extract; CPM = Methanol extract; CPW = Water extract; CPMW = 50% Methanol extract; CPFJ = Fresh juice of leaves of *C. pictus*; The 200 and 500 is a numerical representation of the dose in mg/kg of body weight.

Figure 1: Percent change in blood glucose levels after administration of *C. pictus* D. Don leaf extracts

(namely CPH, CPEA, CPM, CPW, CPMW and CPFJ) of *C. pictus* in mice during OGTT studies. The range of glucose lowering effect was observed between 5.67-72.40% after 2 h of glucose administration with the doses of 200 and 500 mg/kg, respectively in test sample treated mice. The glucose lowering effect was 5.67, 13.33, 36.97, 38.57, 40.86 and 43.95%, respectively observed with CPH, CPW, CPMW, CPFJ, CPEA and CPM extracts at a dose of 200 mg/kg and 33.11, 35.56, 42.96, 53.05, 67.38 and 72.40% with CPH, CPFJ, CPW, CPMW, CPEA and CPM extract at a dose of 500 mg/kg. It was also confirmed that CPM @ 500 mg/kg had the highest and CPH @ 200 mg/kg had the lowest percentage of glucose lowering effect among all treatments. Furthermore, the ascending order of the glucose lowering effect of different extracts of *C. pictus* was observed as CPH-200 < CPW-200 < CPH-500 < CPFJ-500 < CPMW-200 < CPFJ-200 < CPEA-200 < CPW-500 < CPM-200 < CPMW-500 < CPEA-500 < CPM-500.



CPH = Hexane extract; CPEA = Ethyl acetate extract; CPM = Methanol extract; CPW = Water extract; CPMW = 50% Methanol extract; CPFJ = Fresh juice of leaves of *C. pictus*; The 200 and 500 is a numerical representation of the dose in mg/kg of body weight.

Figure 2 : Percent glucose lowering effect of *C. pictus* D. Don leaf extracts

Conclusion

OGTT measures the body ability to use glucose as a principal source of energy. In our study, it was observed that *C. pictus* D. Don leaf extracts show significant improvement in glucose tolerance in mice. Best results were shown by methanol and ethyl acetate leaf extracts at 500 mg/kg body weight with 72.40 and 67.38% glucose lowering effect, respectively. This might be attributed to the enhancement of glucose utilization in body tissues. The above study in part supports the use of *C. pictus* D. Don in the treatment and management of diabetes.

Acknowledgement

Authors are thankful to the Director, CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP) Lucknow, India for providing research facilities and financial support under the project OLP-08 to carry out the study.

Conflict of interest

We declare that we have no conflict of interest.

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