Anti-diabetic Activity of Methanol Leaf Extract of *Costus pictus* D.DON in Alloxan-induced Diabetic Rats

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The methanol extract of *Costus pictus* (*C. pictus*) D.DON (Family: Zingiberaceae) leaf was investigated for its anti-diabetic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of single doses of alloxan monohydrate (120 mg/kg, i.p.). The methanol extract of *C. pictus* (MECP) at a dose of 120 mg/kg, p.o. was administered as single dose per day to diabetes-induced rats for a period of 21 days. The effect of MECP leaf extract on blood glucose, plasma insulin, serum lipid profile [cholesterol, triglycerides, phospholipids, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)], serum enzymes [serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP)], total protein, and liver glycogen were measured in the diabetic rats. Histopathological studies of liver, pancreas and kidney were also carried out. MECP elicited significant ($p < 0.001$) reductions of blood glucose, lipid parameters except HDL, and serum enzymes and significantly ($p < 0.001$) increased HDL level. MECP also caused significant ($p < 0.001$) increases in plasma insulin levels in the diabetic rats. Furthermore, MECP significantly ($p < 0.05$), ($p < 0.001$) increased total protein and liver glycogen in diabetic rats. Histopathological observations revealed that leaf is non-toxic and regenerates the toxic effect of alloxan. From the above results, it is concluded that MECP possesses significant anti-diabetic effects in alloxan-induced diabetic rats.

Key words —— *Costus pictus*, anti-diabetic, alloxan

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1)] The presence of DM confers increased risk of many devastating complications such as cardiovascular diseases (CVD), peripheral vascular disease (PVD),[2)] complications such as coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy amputations, and blindness.[3)] Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas old and new are available for the treatment of diabetes. However, none of these medications is ideal due to toxic side effects and in some cases diminution of response after prolonged use.[4)] The main disadvantages of the currently available drugs are that they have to be given throughout the life and produce side effects.[5)] Medicinal plants and their bioactive constituents are used for the treatment of DM throughout the world, especially in countries where access to the conventional anti-DM agents is inadequate. Although several medicinal plants have gained importance for the treatment of DM, many remain to be scientifically investigated.[6)]

Many plants reported useful for the treatment of DM in the ayurvedic system of medicine have been tested for their hypoglycemic activity in experimental animals.[7)] The medicinal plant *Costus pictus* (*C. pictus*) is a very popular and fast-spreading ginger belonging to the family of zingiberaceae that has been used as an ornamental climbing plant and was found to have anti-diabetic properties.[8)] The present study was conducted to investigate the anti-diabetic activity of *C. pictus* in alloxan-induced diabetic rats.

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MATERIALS AND METHODS

Animals —— Male Albino rats of Wistar strain (150–200 g) were used for the study. The animals were maintained in an air-conditioned room controlled for temperature and humidity, where they were fed a standard rat pellets feed supplied by M/s Hindustan Lever Limited, Bangalore (India) and filtered water *ad libitum*. Animals described as fasted were deprived of food for ≥ 16 hr but allowed free access to water. Ethical clearance for the handling of experimental animals was obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose and the care of laboratory animals and taken as per the guidance of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India (CPCSEA No: 688/02/C–CPCSEA).

Plant Material —— *C. pictus* was collected from different areas of Kottayam District, Kerala, and identified and confirmed by the Taxonomist Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen (08903) is deposited in the herbarium of Tamilnadu Agricultural University, Coimbatore, India.

Preparation of Plant Extract —— The powered form of leaves of *C. pictus* was taken and subjected to successive solvent extraction. The extraction was carried out with the following solvents in increasing order of polarity: petroleum ether, chloroform, methanol, followed by water.[9, 10] The solvent was then distilled, evaporated, and vacuum dried. The successive methanolic extractive value of *C. pictus* was 28.5% w/v, which was done by prescribed monograph specified in I.P.1996.

Phytochemical Analysis —— The various extracts of leaves of *C. pictus* were subjected to the following test for the identification of its various active constituents by standard methods. Carbohydrates were identified by Molisch's test, proteins were identified by ninhydrin test, triterpenoids and steroids were identified by Libermann-Burchard test, alkaloids were identified by Draggendorf's test, tannins were identified by Braemer's test, glycosides were identified by Legal's test, saponins were identified by haemolysis test, flavonoids were identified by lead acetate test, and fixed oils were identified by the presence of oil stains on the filter paper.

Isolation and Identification of Active Compound —— Plant active constituents responsible for anti-diabetic properties were isolated by thin-layer chromatography (TLC). Acid hydrolysis was carried out on vacuum-dried concentrated methanol extract of *C. pictus* to liberate aglycones, if any glycosides were present. The concentrates were spotted on activated TLC plates of silica gel GF 254 (60–120 mesh) of 0.5 mm-thickness coating. The plates (20 cm × 5 cm) were developed with solvent system *n*-butanol-2 M ammonium hydroxide (1 : 1) to elute α- and β-amyrin.[11, 12] The developed plates were air-dried and detected by Carr-Price reagent, *i.e.*, 20% antimony chloride in chloroform was sprayed and dried in a chromatographic oven at 105°C for 30 min. The resolution bands were obtained and Retardation factor (R_f) values calculated. The β-amyrin found in the concentrate was identified by comparing the Rf value with earlier-reported study.[13] The fractions of similar TLC patterns were combined, concentrated, and rechromatographed repeatedly over silica gel GF 254 (100–200 mesh) columns of 60 cm × 3 cm to isolate active compound and confirmed by qualitative chemical analysis.[14]

Chemicals —— All chemicals and solvents used were of Analytical Grade. SD Fine Chem. Ltd., Mumbai, India, Ranbaxy Laboratories, New Delhi, Indea and Himedia Chemicals, Mumbai, India.

Induction of Diabetes —— DM was induced in the Albino rats by administering alloxan monohydrate. Animals were allowed to fast for 24 hr and were injected with freshly prepared alloxan monohydrate (120 mg/kg, i.p.) in sterile normal saline.[15, 16] The animals were maintained in the diabetic state over a period of 21 days. Serum glucose was measured by reported method.[17] Rats showing fasting serum glucose levels (> 250 mg/dl) were selected for the study.

Experimental Grouping of Animals —— The experimental rats were divided into four groups of six animals in each group. Group I, animals served as normal healthy controls, which received 0.5% w/v carboxymethylcellulose sodium (CMC). Group II, untreated diabetic control. Group III, diabetic rats given methanol leaf extract of *C. pictus* (120 mg/kg, p.o) at a single dose per day. The dose (120 mg/kg, p.o.) was selected on the basis of earlier-reported toxicity studies on methanol leaf extract of *C. pictus* D.DON.[18] Group IV, control rats given methanol leaf extract of *C. pictus* (120 mg/kg, p.o.) at a single dose per day. The extract was administered for a period of 21 days. Body weight of the animals was recorded every week.

Table 1. Qualitative Phytochemical Analysis in Different Extracts of Leaves of *C. pictus* Plant

Plant constituents	Extractive solvents of			
	Petroleum ether	Chloroform	Methanol	Water
Carbohydrate	–	–	+	+
Protein	–	–	+	+
Steroids	+	+	–	–
Alkaloids	–	+	+	–
Tannins	–	–	+	+
Glycosides	–	–	–	+
Saponins	–	–	+	+
Flavonoids	–	–	+	–
Fixed oils	+	–	–	–

+ve and –ve symbol indicates the presence and absence respectively of plant constituents with respect to extractive solvents in the increasing order of polarity.

Collection of Liver, Pancreas, Kidney, and Blood —— At the end of the treatment blood was collected by direct cardiac puncture and serum was separated by centrifugation at 2500 rpm. The rats were sacrificed by cervical dislocation and organs were excised immediately and thoroughly washed with ice cold physiological saline. The serum collected was used for biochemical estimations.

Estimation of Biochemical Parameters —— Serum glucose, plasma insulin (estimated by ELISA method using Boehinger Mannheim Gmbh kit, Werk Penzberg, Germany), liver glycogen, serum lipid profile, serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP), and serum protein content were determined standard procedures in an auto analyzer using Ecoline kits (E. Merck, Mumbai, India).

Histopathological Investigation —— Liver, kidney, and pancreas were washed in saline and a small portion of these organs was quickly fixed in 10% formalin. Then, the tissues were processed by standard histopathological technique (*i.e.* dehydration through graded isopropyl alcohol, cleaning through xylene and impregnated in paraffin wax for 2 hr). Wax blocks were made, sections were used for cutting microtome and stained by haematoxylin eosin method and photographed.

Statistical Evaluation —— All results are expressed as mean ± S.D. Statistical evaluation was done using one-way analysis of variance (ANOVA), followed by Student's *t*-test.

RESULTS AND DISCUSSION

Phytochemical Analysis

Compounds of different polarity from dried leaves of *C. pictus* were extracted by sequential extraction process using different solvents such as petroleum ether, chloroform, methanol, and water (Table 1). These sequential extracts were subjected to preliminary phytochemical screening for the presence of different chemical groups. Of all extracts tested, methanol extract was found to contain the highest number of phytochemicals such as carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, and flavonoids. The pentacyclic triterpenoids such as α- and β-amyrin and related compounds occur especially in waxy coatings of the leaves.[19,20] Mostly the terpenic compounds were successfully isolated from leaves.[21] From the results of earlier-reported studies, it is known that triterpene mixture possesses bioactive anti-diabetic properties.[22–26]

Body Weight

Body weight increased significantly ($p < 0.05$ and $p < 0.01$) in all groups except group II. All animals ingested normal amounts of food and water during the study period (Table 2). Our study results on body weight concur with earlier-reported toxicity studies of methanol extract of *C. pictus*.[18]

Biochemical Parameters

Serum glucose and liver glycogen levels in rats of different groups are shown in Table 3. The glucose level was significantly ($p < 0.001$) high in group II compared with group I. On the other hand, the level of serum glucose was significantly ($p < 0.001$) decreased in group III compared with group II. There was no significant difference between group I and group IV. It is evident from Table 3 that untreated diabetic rats had elevated serum glucose levels and that the methanol leaf extract was able to correct this methanol aberration signif-

Table 2. Effect of Methanol Leaf Extract of *C. pictus* on Body Weight of Animals

Groups	Base line	7th day	14th day	21st day
I [Control (0.5% w/v CMC)]	295 ± 18.3	298.8 ± 16.7	303 ± 12.9	308.1 ± 14.7
II [Alloxan (120 mg/kg, i.p.)]	298 ± 10.6[a]	297.12 ± 13.2[a]	292.7 ± 15.4[a]	286.1 ± 16.4[a]
III [Alloxan + MECP (120 mg/kg, p.o.)]	300.6 ± 14.9[b]	303 ± 10.2[b]	310.6 ± 19.2[b,*]	316 ± 13.9[b,*]
IV [MECP (120 mg/kg, p.o.)]	297.8 ± 13.9[c]	308.9 ± 12.1[c,*]	316 ± 14.5[c,**]	327.8 ± 17.1[c,**]

Values represent mean ± S.D. ($n = 6$); Comparisons between groups are as follows, a: Group I and II, b: Group II and III, c: Group I and IV; Statistical significance is as follows $^{*}p < 0.05$, $^{**}p < 0.01$.

Table 3. Effect of Methanol Leaf Extract of *C. pictus* on Serum Glucose, Liver Glycogen, and Lipid Profile

Group	Serum glucose (mg/dl)	Plasma insulin (μu/l)	Liver glycogen (mg/gm tissue)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
I [Control (0.5% w/v CMC)]	92.8 ± 6.7	15.30 ± 1.1	41.7 ± 3.6	136.3 ± 11.7	114.5 ± 10.4
II [Alloxan (120 mg/kg, i.p.)]	328.5 ± 27.1[a,***]	8.3 ± 0.6[a,***]	7.4 ± 0.4[a,***]	270.9 ± 21.2[a,***]	173.8 ± 14.5[a,***]
III [Alloxan + MECP(120 mg/kg, p.o.)]	99.6 ± 7.9[b,***]	13.21 ± 0.9[b,***]	50.3 ± 4.5[b,***]	142.5 ± 12.8[b,***]	117.3 ± 9.5[b,***]
IV [MECP (120 mg/kg, p.o.)]	92.9 ± 6.7[c,ns]	15.25 ± 2.0	42.6 ± 3.2[c,ns]	135.4 ± 10.6[c,ns]	112.5 ± 11.7[c,ns]
Group	Phospholipids (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	
I [Control (0.5% w/v CMC)]	147.3 ± 12.1	52.1 ± 3.7	22.9 ± 1.8	64.9 ± 4.9	
II [Alloxan (120 mg/kg, i.p.)]	286.6 ± 23.2[a,***]	37.3 ± 2.9[a,***]	34.7 ± 2.6[a,***]	203.5 ± 16.1[a,***]	
III [Alloxan + MECP(120 mg/kg, p.o.)]	152.6 ± 11.3[b,***]	51.0 ± 4.2[b,***]	25.4 ± 2.1[b,***]	74.9 ± 5.8[b,***]	
IV [MECP (120 mg/kg, p.o.)]	146.3 ± 9.1[c,ns]	51.3 ± 3.9[c,ns]	23.9 ± 2.1[c,ns]	62.8 ± 4.6[c,ns]	

Values represented as mean ± S.D. ($n = 6$); Comparisons between groups are as follows, a: Group I and II, b: Group II and III, c: Group I and IV; Statistical significance is as follows $^{***}p < 0.001$; ns: non significant.

icantly since there was no significant difference between group I and group IV, which clearly indicates that the extract has anti-diabetic activity but no hypoglycemic activity. Histological findings of liver and pancreas in the extract-administered group and control group were similar.[18] Thus it shows that high dose of the extract did not result in hypoglycaemia[27] unlike insulin and other common hypoglycaemic agents. The orally administered *C. pictus* to alloxan-induced diabetic rats elicited a significant anti-diabetic activity and significantly ($p < 0.001$) increased the plasma insulin levels. Methanol extract of *C. pictus* (MECP)-treated group III rats showed a significant ($p < 0.001$) increase in plasma insulin level when compared with group II. Since there was no significant difference in the plasma insulin levels between group I and group IV, the selected dose (120 mg/kg, p.o.) of MECP does not produce hyperinsulinemia. Alloxan, a β-cytotoxin, induces chemical diabetes in a wide variety of animal species by damaging the insulin-secreting β-cells of the pancreas. Alloxan causes time-and concentration-dependent degenerative lesions of the pancreatic β-cells.[28, 29] The mechanism of action of increase in plasma insulin concentration could be due to longer-lasting stimulant effect on β-cells of pancreatic islets or to pancreatic β-cell regeneration. The glycogen content of the liver was significantly ($p < 0.001$) decreased when compared with the control group in diabetic rats, and the level restored to normal after treatment. The prevention of depletion of glycogen in the liver tissue was possibly due to stimulation of insulin release from beta cells that activate the glycogen synthase system.[30, 31]

The lipid profiles in control and experimental rats are depicted in Table 3. In alloxan-induced diabetic rats, there was a significant ($p < 0.001$) increase of total cholesterol, triglycerides, phospholipids, and low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol and significant ($p < 0.001$) decrease in high-density lipoprotein (HDL) cholesterol in serum compared with normal control. The plant extracts used in the study significantly ($p < 0.001$) decreased the levels of cholesterol, triglycerides, phospholipids, and LDL and VLDL cholesterol and significantly ($p < 0.001$) increased HDL cholesterol. This indicates that the leaf extract had favourable effects, on lipid metabolism of diabetic rats. Derangement of glucose, fat, and protein metabolism in diabetes results in the development of hyperlipidemia.[32–34] Significant lowering of total cholesterol and rise in HDL cholesterol is a very desirable biochemical state for the prevention of atherosclerosis and is-

chemic conditions.[35)]

Serum enzymes (ALP, SGOT, and SGPT) and total protein levels are shown in Table 4. ALP, SGOT, and SGPT levels were increased significantly ($p < 0.001$) in alloxan-treated diabetic rats in comparison with normal animals. The extract significantly ($p < 0.001$) decreased the elevated ALP, SGOT, and SGPT levels in treated rats. Increased activity of transaminases, which are active in the absence of insulin because of increased availability of aminoacids in diabetes, are believed responsible for the increased gluoconeogenesis and ketogenesis observed in the disease.[36)] A significant ($p < 0.05$) decrease in total protein levels was observed in serum of alloxan-induced diabetic rats compared with control rats. There was no significant difference between group I and group IV in the level of total protein. Administration of methanol of *C. pictus* leaf extract restored the protein levels to near normal. Insulin deficiency leads to various metabolic aberrations in the animals such as decreased protein content. Insulin deficiency causes excessive catabolism of protein and the aminoacids released are used for gluconeogenesis.[37)]

Histopathological Investigation

Histopathology of the liver (Fig. 1) in control animals showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus, and central vein. In diabetic control, liver sections showed that the lobular architecture was maintained, but there was also severe fatty change, sinusoidal dilation and congestion, mild periportal inflammation, fibrosis, severe feathery degeneration, and necrosis. In diabetic rats treated with MECP, liver sections maintained lobular architecture and had mild fatty change, mild sinusoidal dilation and congestion, mild periportal inflammation, and mild feathery degeneration. In normal animals treated with MECP, liver sections showed normal hepatic cells

Table 4. Effect of Methanol Leaf Extracts of *C. pictus* on Serum Enzymes and Total Protein in Serum of Control and Experimental Rats

Groups	ALP (mg/dl)	SGOT (mg/dl)	SGPT (mg/dl)	Total protein (mg/dl)
I [Control (0.5% w/v CMC)]	12.9 ± 1.4	64.4 ± 4.9	45.3 ± 2.7	8.6 ± 2.4
II [Alloxan (120 mg/kg, i.p.)]	$23.6 \pm 2.1^{a,***}$	$126.6 \pm 8.2^{a,***}$	$52.3 \pm 4.2^{a,**}$	$4.9 \pm 2.8^{a,*}$
III [Alloxan + MECP (120 mg/kg, p.o.)]	$14.0 \pm 0.8^{b,***}$	$67.1 \pm 4.8^{b,***}$	$46.5 \pm 3.3^{b,**}$	$7.6 \pm 6.2^{b,*}$
IV [MECP (120 mg/kg, p.o.)]	$12.5 \pm 1.1^{c,ns}$	$64.2 \pm 5.3^{c,ns}$	$44.6 \pm 2.9^{c,ns}$	$8.49 \pm 5.8^{c,ns}$

Values represent mean ± S.D. ($n = 6$); Comparisons between groups are as follows, a: Group I and II, b: Group II and III, c: Group I and IV; Statistical significance is as follows $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; ns: non significant.

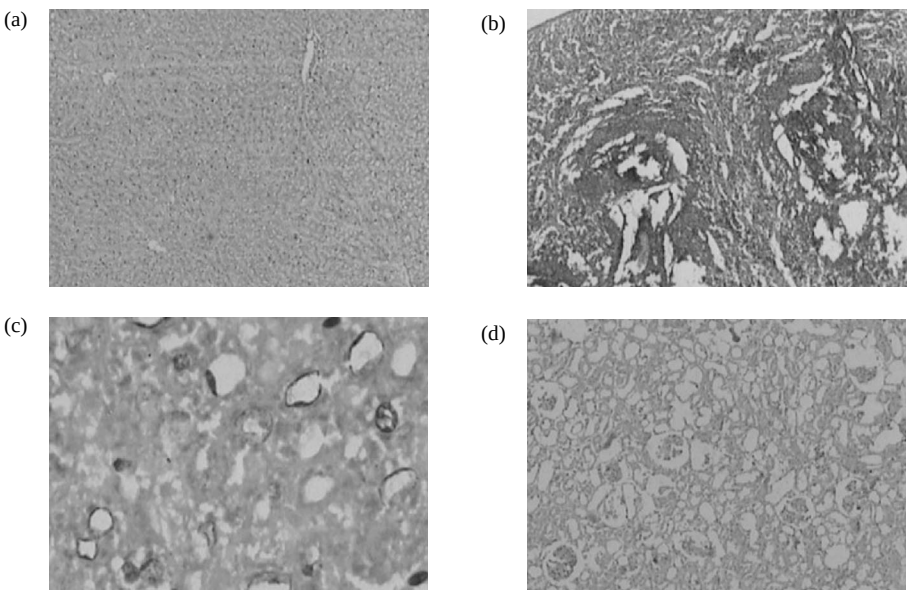

Fig. 1. Histopathology of the Liver

(a) Liver of control animal showing normal hepatic structure. (b) Liver of diabetic animal showing severe fatty changes, sinusoidal dilation, feathery degeneration, and necrosis. (c) Liver of diabetic animal treated with methanol leaf extract of *C. pictus* showing mild fatty change, mild sinusoidal dilation, and congestion. (d) Liver of normal animal treated with methanol leaf extract of *C. pictus* showing normal hepatic structure.

with well preserved cytoplasm, nucleus, nucleolus, and central vein, in which normal hepatic structure was maintained.

Histopathology of the pancreas (Fig. 2) in control animals showed normal pancreatic parenchymal cells and islet cells. In diabetic control, pancreas section showed moderate hyperplasia of islet cells, severe congestion in pancreatic parenchyma, and mild infiltration of inflammatory cells. In diabetic animals treated with MECP, pancreas section showed mild hyperplasia of islet cells and congestion of pancreatic parenchyma. In normal animals treated with MECP, the pancreas section showed normal pancreatic structure. Histopathology of the kidney (Fig. 3) in control animals revealed normal structure. In diabetic control, kidney sections showed severe tubular epithelial atrophy, mild mesangial proliferation, mild sclerotic changes in the glomerulus, and moderate congestion of capillaries. In diabetic animals treated with MECP, kid-

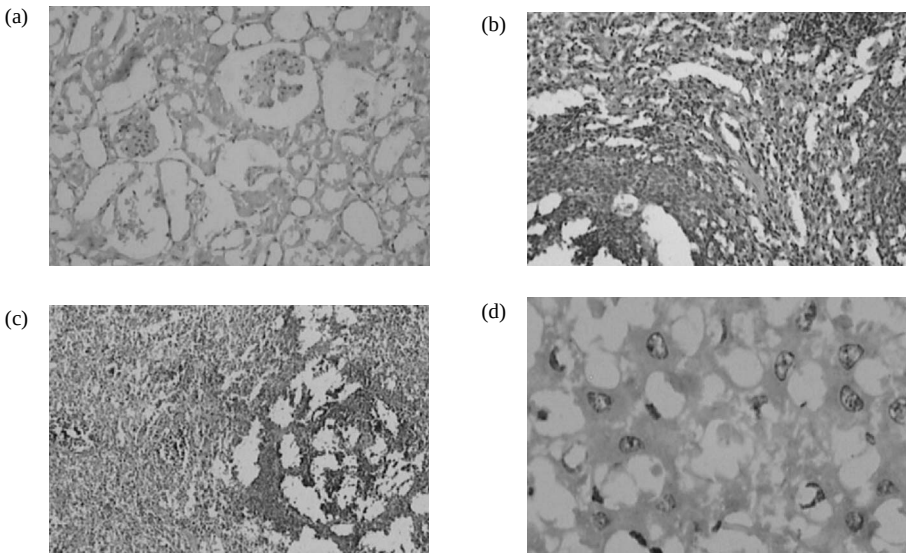

Fig. 2. Histopathology of the Pancreas

(a) Pancreas of control animal showing normal histology. (b) Pancreas of diabetic animal showing severe congestion of pancreatic parenchyma cells, infiltration of inflammatory cells and hyperplasia of islet cells. (c) Pancreas of diabetic animal treated with methanol leaf extract of *C. pictus* showing mild hyperplasia of islet cells and congestion of parenchyma. (d) Pancreas of normal animal treated with methanol leaf extract of *C. pictus* showing normal histology.

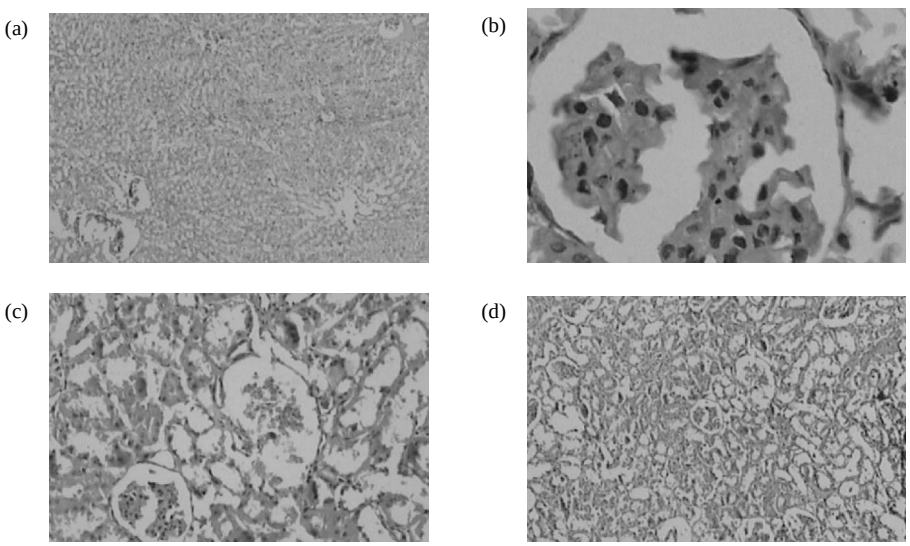

Fig. 3. Histopathology of the Kidney

(a) Kidney of control animal showing normal histology. (b) Kidney of diabetic animal showing severe tubular epithelial atrophy, mild mesangial proliferation, mild sclerotic changes in the glomerulus, and moderate congestion of capillaries. (c) Kidney of diabetic animal treated with methanol leaf extract of *C. pictus* showing mild tubular epithelial atrophy and congestion of capillaries. (d) Kidney of normal animal treated with methanol leaf extract of *C. pictus* showing normal histological structure.

ney sections showed mild tubular epithelial atrophy and congestion of capillaries. In animals treated with MECP, kidney sections showed normal histology. Morphological changes in liver (Table 5), kidney, and pancreas due to direct toxic effect of alloxan as well as diabetes (group II) were remarkably reduced in rats treated with MECP although unable to prevent them completely (group III). MECP on its own did not produce any morphological changes in the organs tested (group IV). Islet cell hyperplasia (possibly due to β-cell hyperplasia) due to alloxan-induced diabetes was less conspicuous in the rats treated with MECP (group III).

From the above observations it was revealed that diabetes mellitus is a major metabolic syndrome characterized by derangement in carbohydrate metabolism associated with defects in insulin secretion or action.[38] Different extracts of the *C. pictus* leaves were subjected to phytochemical screening, out of which methanol extract was found to contain the highest number of phytochemicals such as carbohydrates, triterpenoids, protein, alkaloids, tannins, saponins, and flavonoids. Pentacyclic triterpene compounds in general exhibit a wide range of pharmacological activities that include anti-oxidant, anti-allergic, anti-inflammatory, anti-tumor, anti-bacterial, gastroprotective, and hepatoprotective effects.[39–45] Hence the present study was done with methanol leaf extract of *C. pictus* in animal models to investigate its anti-diabetic activity. Furthermore, TLC separation was carried out to identify the active constituents responsible for anti-diabetic activity by using specific solvent system.

In the present study, an increase in serum glucose and liver glycogen was noted in diabetic rats, after the administration of the methanol leaf extract there was a significant decrease of serum glucose level and increase in liver glycogen, which may be due to enhancement of glycogen synthase by the leaf extract. Also, increase in plasma insulin level was observed, possibly due to longer-lasting stimulant effect of β-cells of pancreatic islets or to pancreatic β-cell regeneration. Decreased protein level and improved lipid profile (cholesterol, triglyceride, HDL, LPL, VLDL, and phospholipids) were noted in diabetic rats and HDL was decreased. This may be due to excessive catabolism of protein and aminoacids that are released and used for glu-

Table 5. Histopathological Investigation of Liver, Kidney, and Pancreas of Control and Experimental Rats

Microscopic Study in Comparison:					
	Microscopic Features	Liver Groups			
		Group I	Group II	Group III	Group IV
1	Lobular architecture	Maintained	Maintained	Maintained	Maintained
2	Fatty change	–	++++	+	–
3	Sinusoidal dilatation and Congestion	–	++	+	–
4	Periportal inflammation and fibrosis	–	+	+	–
5	Feathery degeneration	–	+++	+	–
6	Necrosis	–	+	–	–
	Microscopic Features	Pancreas Groups			
		Group I	Group II	Group III	Group IV
1	Islet cell hyperplasia	–	++	+	–
2	Congestion in pancreatic parenchyma	–	+++	+	–
3	Inflammatory cell infiltrate in pancreas	–	+	–	–
	Microscopic Features	Kidney Groups			
		Group I	Group II	Group III	Group IV
1	Tubular epithelial atrophy	–	+++	+	–
2	Mesangial proliferation	–	+	–	–
3	Sclerotic changes in the Glomerulus	–	+	–	–
4	Congestion of capillaries	–	++	+	–

Symbol +++, ++, + and – indicates the presence of severe degree, moderate degree, mild degree and no changes respectively, with respect to microscopic features of liver, pancreas and kidney of group I, II, III and IV.

coneogenesis. This also stimulates lipolysis in adipose tissue which gives rise to hyperlipidemia. The level of liver marker enzymes was also increased. The restoration of transaminases to their normal levels after the treatment also indicates revival of insulin secretion. Histopathological studies of tissues (liver, kidney, and pancreas) were undertaken and it was found that the leaf was non-toxic and regenerated the toxic effect of alloxan.

It has been well documented that methanol leaf extract of *C. pictus* exhibits strong glucose-lowering activity. We presume that pentacyclic triterpene compounds such as β-amyrin might be the active principle contributing to the anti-diabetic actions. This was supported by TLC separation technique and histopathological observations of different tissues such as liver, pancreas and kidney. Thus in conclusion, the methanol leaf extract of *C. pictus* had anti-diabetic effects and improved hyperlipidemia consequent upon diabetes.

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