Effects of Methanolic Leaf-extract of Costus pictus D. Don on Swiss Albino Mice with Hyperglycemia Associated Renal Complications

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Effects of Costus pictus leaf extract (CPLE) in ameliorating the adverse outcomes of hyperglycemia-associated renal injury is investigated in Swiss Albino Mice. To induce renal injury associated with hyperglycemia, adult mice were given intraperitoneal injections of Streptozotocin (STZ) dissolved in 0.5mol citrate buffer for five days. Animals were divided into five groups, namely CMC (vehicle control group, receiving only carboxy methyl cellulose), DC (Disease control group, receiving only STZ and without any treatment), GLD (positive control group, receiving standard drug glibenclamide), LD (experimental group treated with CPLE at 250 mg/kg bw) and HD (experimental group treated with CPLE at 500 mg/kg bw). After a rearing/treatment regimen of 4 weeks, animals were sacrificed to analyze various parameters. Oral glucose tolerance test (OGTT) based area under the curve of plasma glucose (AUCglucose), kidney index, lipid profiling, serum, urine parameters (creatinine, albumin, urea), antioxidant studies and kidney histopathology were carried out. It was found that harmful lipids like cholesterol, triglycerides (TG) and low-density lipoprotein-C (LDL-C) levels were significantly elevated in the DC group, whereas LD, HD and GLD showed a tendency towards normalcy. Urine and serum parameters also showed the same trend in treatments and the GLD group, whereas the DC group showed steep abnormalities. Antioxidant study also reveals that treatment with CPLE, balances antioxidant activity by mitigating the effects of reactive oxygen species (ROS). Histopathological investigations displayed significant disruptions in the microstructure of glomeruli of the DC group compared to both the treatments and GLD groups. These preliminary findings indicate that CPLE shows a trend to ameliorate hyperglycemia-associated renal complications and demands further detailed study.

Keywords: Alternative medicine; Hyperglycemia; Nephropathy; Nephroprotection; Renal Injury; Streptozotocin.

Diabetes mellitus (DM) has gained international attention as a remarkable health threat. Unusually prolonged hyperglycemia, a major hallmark of diabetic individuals, results in several pathophysiological problems often affecting vital organs like the eye, kidney, heart and nerves. There are two major forms of DM, one is type-I and other is type-II, which are also known as Insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), respectively. Type-I diabetes is an autoimmune disorder that causes a localized...
inflammatory response, in and around the islets of Langerhans, followed by the selective death of β-cells that secrete insulin. Type-II diabetes is characterized by reduced insulin secretion and peripheral insulin resistance. Diabetes prevalence is predicted to increase from 2.8% in 2000 to 4.4% in 2030 across all age groups and according to projections, there will be 366 million people with diabetes worldwide by 2030, up from 171 million in 2000. Diabetic nephropathy (DN) is the most prevalent and serious consequence of prolonged diabetes and has been identified as the leading cause of kidney damage and eventual renal failure, which ultimately necessitates regular dialysis or kidney transplantation. According to reports, DN or end-stage renal failure can develop in 30–40% of people worldwide sooner than later. The hallmarks of DN include thickening of the basement membrane, growth of mesangial cells and podocyte cell death, which result in morphological and functional abnormalities in the filtration barrier. In such nephropathic individuals, the impaired renal basement membrane causes an increase in the excretion of urine albumin, urea and creatinine which lowers the glomerular filtration rate (GFR).

One of the often used naturally occurring diabetes-inducing substances used on laboratory animals is streptozotocin (STZ), derived from Streptomyces achromogenes, which is chemically known as N (Methyl nitrosocarbamoyl)-D-glucosamine. According to studies, oral or intravenous administration of STZ to animals causes pancreatic beta cells to die, which causes the animals to develop diabetes. STZ enters the β cell via the GLUT-2 transporter and causes alkylation of DNA, thereby inducing the activation of polyADP ribosylation, leading to depletion of cellular NAD⁺ and ATP, which without medical intervention can lead to the condition of DN. STZ was intraperitoneally employed in our investigation to establish the DN mouse model.

In addition to conventional diabetic treatments, including insulin therapy and oral hypoglycemic medications, many diabetic patients adopt supplementary and alternative approaches, as it is believed to have fewer side effects and are cost-effective as well. Hence, adopting a plant-based therapeutic approach in context with the management of diabetes and associated complications is an essential strategy. It has also been reported that a related plant of C. pictus known as C. afer, originated in Africa having anti-nephrotoxicity effects. Moreover, the mixture of C. pictus with metformin and enalapril showed beneficial effects in the management of DN.

So, the current study investigated the effects of methanolic extract of C. pictus for attenuation of DN. A wide range of phytochemical substances, including alkaloids, glycosides, tannins, phenols, steroids, terpenoids, and flavonoids, were found in C. pictus. In particular, quercetin, a flavonoid available in C. pictus leaf extract has a strong antioxidant effect, which helps scavenge superoxide radicals and inhibit xanthine oxidase. Among all the parts of C. pictus, methanolic leaf extract demonstrated the best antioxidant activity. It has also been revealed that the leaf extract of C. pictus has anti-inflammatory properties. These extracts reduced the levels of pro-inflammatory cytokines such as tumor necrosis factor-α and C-reactive protein by inhibiting the molecules involved in the stress-sensitive signaling cascade. Additionally, it suppressed the expression of monocyte chemotactic protein and interleukin 6 (IL-6), the inflammatory cytokines often linked to insulin resistance, obesity, diabetes and metabolic syndrome.

The potent anti-inflammatory properties of the plant qualities could be attributed to the rich phenolic compounds present in C. pictus leaves. The biochemical pathways influenced by the bioactive components present in the methanolic extract of C. pictus leaves, leading to beneficial effects on diabetic kidney injury advocate for further molecular investigations.

**MATERIAL AND METHODS**

**Collection and Identification of C. pictus**

Naturally, growing C. pictus D. Don, also known as insulin plant or Sugar plant (Family: Costaceae), were collected in and around the area of Siliguri (26.7271°N, 88.3953°E), West Bengal, India during January-February in the year of 2020. The Taxonomy of Angiosperm and Biosystematics Laboratory, Department of Botany of the University of North Bengal authenticated the collected plant specimens. The voucher specimen was deposited in the herbarium of the Department of Botany, University of North Bengal, with the depository accession number (Accession no: 11783).
Preparation of methanolic extract of \textit{C. pictus} leaves

Collected \textit{C. pictus} leaves were shed and dried at room temperature for two weeks. Then the leaves were ground into fine powder and then stored in airtight containers at room temperature. For extraction, the powder was suspended in absolute methanol in a 5 g: 250 ml (w/v) ratio for 72 hours in the Soxhlet apparatus. The extract was concentrated using a Buchi-style rotary evaporator (Cole Parmer RV1010D596, India) at a lower pressure and temperature (45°C). Then, the concentrated semi-liquid component was taken into a Petri dish and dried at room temperature. Then, the final yield efficiency was calculated. The dried sample was collected into a glass vial and kept at -20°C for future use.

\textbf{Induction of renal injury}

For the induction of the renal injury associated with hyperglycemia, a batch of animals (n=6) were given intraperitoneal injections of STZ, dissolved in freshly prepared 0.5 mol citrate buffer (pH 8) at the fixed dosage of 30mg/kg body weight (bw) for five days with a gap of one day between 2 dosages. Animals that had fasting blood glucose levels above 215 mg/dL were selected for further study on the 10th day of the experiment\textsuperscript{23,24}. Animals were monitored for another seven days for any changes in blood glucose levels to ensure no reversal of the hyperglycemic state. On the 17th day of the experiment, animals were grouped for further experimentation.

\textbf{Animal Experimental Design}

The animals were divided into five groups: CMC (vehicle control group; animals that were given carboxy methyl cellulose along with food and water \textit{ad libitum} for 28 days duration), DC (Disease control group; renal injury-induced disease group that were subjected to only food and water \textit{ad libitum} without any specific treatment for 28 days duration), GLD (positive control group; renal injury induced group that were treated with glibenclamide dissolved in carboxy methyl cellulose along with food and water \textit{ad libitum} for 28 days duration), LD (Treatment groups with low dose; renal injury induced group that were treated with low dose of CPLE at 250 mg/kg bw dissolved in carboxy methyl cellulose along with food and water \textit{ad libitum} for 28 days duration) and HD (Treatment groups with high dose; renal injury induced group that were treated with a high dose of CPLE at 500 mg/kg bw dissolved in carboxy methyl cellulose along with food and water \textit{ad libitum} for 28 days duration)

\textbf{Determination of OGTT-based Area Under Curve of Plasma Glucose (AUC\textsubscript{glucose})}

OGTT-based AUC\textsubscript{glucose} was performed at the end of the experimental duration. The levels of blood glucose were measured by pricking the tail vein of 16 hours-fasted animals, aseptically with a sterile needle. For this study, the mice were administered a single oral dose of glucose (2 g/kg bw) and the reading was taken just before the glucose administration (0 min) and after 30, 60, 90, and 120-minute time intervals using a handheld glucometer (Dr. Morepen BG03, Morpen Laboratories Ltd., India). Then, using the values of the respective glucose levels at different time intervals, AUC\textsubscript{glucose} was measured\textsuperscript{25}.

\textbf{Kidney Index}

Before sacrificing, each mouse was weighed individually and after sacrifice, both kidneys were weighed\textsuperscript{23,24}. Finally, the kidney index was calculated by dividing the kidney weight by the bw and multiplying by 100.

\textbf{Biological samples collection}

Urine, blood and tissue samples were collected aseptically by following standard protocols. Specimens except blood were stored at -20°C until further evaluations. For the blood sample, serum was separated by centrifuging the clotted blood samples at 3000 rpm for 5 min and stored at -20°C until analysis.

\textbf{Serological Lipid Profiling}

Standard colorimetric kits were used (Coral Clinical System, India) to evaluate the lipid profile, which included serum total cholesterol (TC, Cat No. 1102040275), high-density lipoprotein-C (HDL-C, Cat No. 1102150040) and Triglyceride (TG, Cat No. 1102220075). The manufacturer’s instructions were followed for performing serum lipid profile testing, and LDL-C was calculated, following the Martin-Hopkin calculation\textsuperscript{26}.

\textbf{Serological and urological profiling of renal injury parameters}

Standard colorimetric kits from Coral Clinical System, India were used following the manufacturer’s instructions to estimate Albumin (Cat No. 1101021150), Creatinine (Cat No. 1101070275) and Urea (Cat No. 1102240075)
parameters of serum and urine as renal injury markers.

**In-vivo antioxidant study**

**Preparation of kidney homogenate**

After sacrifice, the kidneys were dissected and washed in phosphate buffer saline (PBS). The kidney tissue was homogenized to make 10% homogenate with phosphate buffer (PB) in a 50 mM concentration at 7.4 pH. The homogenate was centrifuged at 10000 g for 15 minutes at 4°C[27].

**Estimation of lipid peroxidation (MDA)**

Lipid peroxidation was estimated by thiobarbituric acid (TBA) method with slight modification[28]. In short, a reaction mixture was prepared with 1 ml of TBA (0.5%) and 1 ml of trichloroacetic acid (20%). In this mixture, 50 µl of tissue homogenate was mixed. The whole mixture was kept in a water bath at 95°C for 30 minutes. After that, to stop the reaction, it was ice-cooled for 5 minutes, followed by centrifugation at 2000 g for 12 minutes. Absorbance was taken at 532 and 600 nm, respectively.

**Estimation of superoxide dismutase (SOD)**

SOD was estimated by the nitroblue tetrazolium (NBT) method with slight modification[29]. A reaction mixture of 1 ml sodium carbonate (50 mM), 400 µl of NBT (25 mM) and 200 µl of hydroxylamine hydrochloride (0.1 mM) was made. On that mixture, 100 µl of tissue homogenate was mixed and absorbance was taken at 560 nm.

**Estimation of reduced glutathione (GSH)**

GSH was estimated by the Elman reagent method with slight modification[30]. At first, Elman reagent was prepared by mixing 19.8 mg of DTMB and it was dissolved in 100 ml of sodium nitrate (0.1%). On that reagent, 1 ml of tissue homogenate and 3 ml of phosphate buffer were added and absorbance was taken at 412 nm.

**Estimation of catalase activity**

Catalase activity was estimated by the hydrogen peroxide (H₂O₂) method with slight modification[31]. A reaction mixture of 500 µl of H₂O₂ (0.34 nM) and 2.5 ml distilled water was made. In that reaction mixture, 40 µl of tissue homogenate was added. After that, absorbance was taken at 240 nm.

**Histopathological Evaluation**

By following standard protocol, histological evaluations were carried out from the animal tissue. Surgically, the kidneys were removed and washed with chilled PBS and placed in Bouins fixative for fixation. After that, serial ethanol dilutions were used to dehydrate the tissues before embedding them in paraffin wax. Haematoxylin and eosin were used for staining and six µm thick sections were cut from a paraffin-embedded block[32]. Following that, using a light microscope, microscopic inspections were performed (Nikon Eclipse E200, Nikon, Tokyo, Japan) with a 40X magnification.

![Fig. 1. AUC_glucose values of the different experimental groups, where, *** p ≤ 0.001](image-url)
Fig. 2. Kidney index value of different experimental groups, where, $*p \leq 0.05$

Fig. 3. Serum lipid profile among experimental groups: A. Concentration of serum cholesterol B. Concentration of serum triglycerides C. Concentration of serum HDL-C and D. Concentration of serum LDL-C. Where, $***p \leq 0.001$ and $**p \leq 0.01$
Statistical analysis
With the aid of a statistical package program (KyPlot 6.0), Dunnett’s test was done to compare the groups statistically, and $p \leq 0.05$ was considered significant for all statistical analyses in this study.

RESULTS AND DISCUSSION

Plasma Glucose determined by OGTT-based Area Under Curve (AUC$_{\text{glucose}}$)
It has been found that the values of AUC$_{\text{glucose}}$ were significantly higher ($p \leq 0.05$) in the DC group (469.28 ± 87.93 mg h/dL) when compared with that of the CMC group (246.13 ± 7.30 mg h/dL); whereas, the values of GLD (252.76 ± 8.88 mg h/dL), LD (277.75 ± 24.26 mg h/dL) and HD (275.77 ± 11.19 mg h/dL) group were statistically non-significant with CMC group (Fig.1).

Kidney Index
Statistical analysis showed that the kidney index of the DC (1.24 ± 0.47 gm) group significantly ($p \leq 0.05$) elevated compared to the CMC (0.64 ± 0.25 gm) group. Whereas, LD (0.84 ± 0.25 gm), HD (0.84 ± 0.20 gm) and GLD (0.62± 0.27 gm) group shows insignificant results compared to the CMC group (Fig. 2).

Lipid Profile Analysis
Colorimetric kits were used to evaluate the lipid profile (serum TC, HDL-C and TG) of the experimental animals on the last day of the experiment. The HDL-C levels were significantly ($p \leq 0.05$) lower in the disease group (26.68±31 mg/dl) compared to the CMC (34.44±7.47 mg/dl), and GLD, LD, and HD group shows values are close to the CMC group. Whereas, serum total cholesterol was significantly higher in the disease group (156.27±4.50 mg/dl) compared to the CMC (136.66±12.20 mg/dl) group, the GLD,
LD, and HD parameters showed non-significant variation. The TG values in the disease group (141.37±2.57 mg/dl) and CMC group (122.55±7.04 mg/dl) also significantly (p<0.05) differed, but comparison with LD, HD with CMC showed non-significant differences. In the case of bad cholesterol like LDL-C, the value in disease control (101.67±5.31mg/dl) is considerably high compared to the CMC (77.66±13.51 mg/dl) group; the GLD, LD, and HD groups show non-significant variations (Fig. 3).

**Serum parameters of Albumin, Creatinine and Urea**

At the end of the experimental duration, it was found that the levels of serum albumin were significantly lower in the DC group (2.90 ± 0.18 g/dL) when compared with the CMC group (4.12 ± 0.59 g/dL). Whereas, the values of the same tests observed in the treated groups (HD and LD) and GLD were close to that of the CMC group. For the serum creatinine, the DC group showed significantly elevated (p≤0.05) levels (0.98 ± 0.25 mg/dL) when compared with the CMC group (0.77 ± 0.03 mg/dL). Other experimental groups LD, HD and GLD showed slight elevations in the serum creatinine levels (0.87 ± 0.03 mg/dL, 0.85 ± 0.03 and 0.82 ± 0.07 mg/dL respectively), but these are not significant (p<0.05) when compared with the CMC group. Serum urea parameter was also significantly (p<0.05) higher (42±1.67 mg/dL) in the DC group compared to the CMC group (29.66 ± 2.94 mg/dL), but the treatment groups (33.66 ± 2.87 mg/dL and 32.5 ± 3.61 mg/dL value of LD and HD respectively) and GLD (31.83 ± 3.06 mg/dL) group shows insignificant result compared with CMC group (Fig. 4).

**Urine parameters of Albumin, Creatinine, and Urea**

At the end of the experimental duration, it was found that the levels of urine albumin were non-significant variation. The TG values in the disease group (141.37±2.57 mg/dl) and CMC group (122.55±7.04 mg/dl) also significantly (p<0.05) differed, but comparison with LD, HD with CMC showed non-significant differences. In the case of bad cholesterol like LDL-C, the value in disease control (101.67±5.31mg/dl) is considerably high compared to the CMC (77.66±13.51 mg/dl) group; the GLD, LD, and HD groups show non-significant variations (Fig. 3).

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**Urine parameters of Albumin, Creatinine, and Urea**

At the end of the experimental duration, it was found that the levels of urine albumin were

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**Fig. 5.** Concentration of urine parameters among different groups; A. Concentration of urine albumin. B. Concentration of urine urea and C. Concentration of urine creatinine. Where, *p* ≤ 0.05 and ***p*** ≤ 0.001
significantly higher ($p<0.05$) in the DC group (3.83 ± 0.25 mg/dL) when compared to the CMC group (2.87 ± 0.22 mg/dL). Whereas, the levels of the same in the groups LD, HD (3.29 ± 0.28 mg/dl, 3.14 ± 0.43 mg/dL respectively) is a bit higher than in the CMC group but not significantly ($p<0.05$) differ. With regards to the urine creatinine, the DC group showed significantly elevated ($p<0.05$) levels (1.85 ± 0.49 g/L) when compared with the CMC group (1.26 ± 0.30 g/L). The groups LD, HD and GLD showed slight elevations in the levels (1.41 ± 0.35 g/L, 1.34 ± 0.32 and 1.32 ± 0.31 g/L respectively), but these are not significant ($p<0.05$) when compared with the CMC group. For the Urine urea parameter, it was also significantly higher levels (5.53± 0.53 mg/dL) in the DC group compared with the CMC group (3.22 ± 0.44 mg/dL), but the LD (3.95 ± 0.44 mg/dL), HD (3.55 ± 0.57) and GLD (3.08 ± 0.50 mg/dL) group shows insignificant result compared with CMC group. (Fig. 5).

**In-vivo antioxidant study**

The MDA level in the case of DC (0.08±0.01nM/mg tissue) was significantly higher ($p<0.05$) than other groups (0.048±0.015; 0.066±0.014; 0.066±0.011; 0.058±0.10 nM/mg tissue values of CMC, LD, HD and GLD respectively). The SOD level in the case of DC (0.079±0.023 Unit/mg tissue) was significantly lower ($p<0.05$), but the treatment group was able to increase the level of SOD (LD: 0.145±0.053; HD: 0.154±0.050; GLD: 0.151±0.11) which was close to CMC group (CMC: 0.149±0.058). The relative GSH concentration level in the case of DC (33.66±9.52) significantly ($p<0.05$) reduced than the CMC group (CMC: 87.5±4.13). The treatment group namely HD (77.83±9.70) and GLD (86.5±2.07) showed an increase in the GSH level toward normalcy. However, the LD (76.33±7.50) group GSH relative concentration value shows a bit significant difference compared to the CMC group. In terms of catalase levels, the

![Fig. 6. Kidney tissue antioxidant profile among experimental groups; A. Concentration of serum SOD B. Concentration of catalase C. Relative concentration of GSH and D. Concentration of MDA. Where, *** $p<0.001$ and * $p<0.05$](image-url)
group treated with DC (634.16±47.55 Unit/g tissue) displayed significantly ($p < 0.05$) lower levels of GSH compared to the CMC group (912.66±223.14 Unit/g tissue). All the HD (856.16±143.06 Unit/g tissue), LD (813.16±262.08 Unit/g tissue) and GLD (901.33±131.42 Unit/g tissue) groups demonstrated a trend towards normalization (Fig. 6).

**Histopathological evaluation**

In the histological study, we have found the normal architecture of the kidney and glomerular size in the CMC group. However, in the case of STZ-induced mice, it showed moderate to severe vascular degeneration of the glomerular tubule, increased glomerular and finally, atrophy in the glomerulus. At the same time, it is observed that among the treatment groups, the HD and the GLD groups showed a tendency toward normalcy of glomerulus architecture. However, LD also shows effects on restoration glomerulus architecture but is not as promising as HD (Fig. 7).

**DISCUSSION**

Nephropathy resulting from diabetes poses a substantial danger to human health due to its high morbidity and mortality rates. DN affects about 15–25% of type 1 diabetes patients and 30–40% of type 2 diabetes patients\(^3\). Despite the availability of therapeutics that slow the development of DN, there is increased interest in using herbal remedies to stop the development of this condition. When mice with STZ-induced hyperglycemia were administered ethanolic and methanolic extracts from *C. pictus* leaves, the hyperglycemic condition was significantly reduced\(^34,35\). *C. pictus* methanolic leaf extract is also reported to restore renal function, lower cholesterol levels and bring blood sugar levels back to normal in STZ-induced hyperglycemic mice\(^36\). In the initial and crucial stage of performing a study on phytomedicine, the choice of solvent holds immense importance. Opting for polar solvents such as methanol yields the greatest concentration of natural compounds\(^37\). Additionally, the methanolic extract boasts the highest levels of bioactive substances, further enhancing its extraction efficiency. Therefore, methanol is widely regarded as the optimal solvent for achieving elevated levels of phytochemical components\(^38\) and in our study, the yield efficiency of the methanolic leaf extract was 9.80%. However, our investigation observed that in the DC group, the glucose levels were elevated throughout the entire experimental duration (0 to 120 mins). However, the LD, HD, and GLD groups restored the glycemic profile just.

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**Fig. 7.** Kidney tissue histology at 40X magnification (25µm scale bar) of different experimental groups shows the effects of CPLE on glomerulus. A. Kidney histology of CMC group B. Kidney histology of DC group C. Kidney histology of LD group D. Kidney histology of HD group and E. Kidney histology of GLD group, where GS and G indicate glomerular space and glomerulus respectively.
like the CMC group. These findings indicated that the CPLE helps to restore metabolic stress along with glycemic parameters positively. Because of the hyperglycemia and hypoinsulinemia caused by STZ-induced diabetes, there is a considerable loss in bw and increased protein loss from tissues as well as muscle atrophy. The kidney index parameter of our study reveals that the bw of STZ-diabetic mice gradually decreased, while treatment with CPLE considerably increased the bw, indicating a reduction in the risk of hyperglycemia-induced damage to muscle tissue; it was also observed that in STZ-induced hyperglycemic mice, kidney weight (hypertrophy) increases proportionally to bw. Hypercholesterolemia and hypertriglyceridemia are brought on by diabetes mellitus. After receiving CPLE, these elevated levels returned to normal, suggesting the potential of CPLE to enhance lipid metabolism.

Albumin is by far the most abundant protein in nephrotic urine. The relationship between albuminuria and declining kidney function was shown by the fact that the serum albumin concentration was significantly lower, and urine albumin levels were higher in STZ-diabetic mice. Treatment with CPLE normalized these levels, demonstrating the drug’s protective effects against microalbuminuria. Reduced excretion of creatinine in the urine and elevated urea and serum creatinine levels are signs that DN is developing. These effects were reversed in STZ-diabetic Swiss albino mice treated with CPLE. Hyperglycemia also leads to an increase in reactive oxygen species (ROS), which disrupts normal tissue and cellular function, contributing to various diabetic complications, including DN. These elevated ROS levels impair cellular defences by interfering with the normal functioning of lipids, DNA and proteins. Glutathione (GSH) plays a crucial role in preserving the plasma’s normal state by scavenging free radicals. Superoxide dismutase (SOD) captures superoxide molecules, converting their activity into less reactive ROS. When catalase combines with hydrogen peroxide (H₂O₂), it neutralizes ROS. Malondialdehyde (MDA) is a byproduct of lipid oxidation and serves as an indicator of ROS. In our experiment, we observed that both high and low doses of CPLE effectively reduced MDA levels. Furthermore, GLD also demonstrated the ability to decrease MDA levels. However, we observed low levels of SOD, GSH and catalase, indicating a diminished antioxidant status in STZ-induced DN mice. Notably, the HD CPLE group exhibited a more significant tendency toward restoring normal GSH levels than the LD group. Additionally, both HD and LD groups displayed the capacity to normalize SOD and catalase levels. In conclusion, our findings suggest that CPLE can help maintain the balance between oxidants and antioxidants by mitigating the effects of ROS.

Kidney sections from STZ-diabetic mice underwent histopathological evaluation and the results revealed significant tubular vascular degeneration, increased glomerular space, thickening of the basement membrane and atrophy of the glomerulus in the DC group. Comparing the treatment groups and positive control group to the disease control group, the number of completely and partially malformed glomeruli is likewise deficient, which shows the restoration power of CPLE extract. Though the HD group’s restoration of glomerulus architecture proved to be more intense than that of the LD group, the LD group still shows the ability to restore glomerulus architecture compared to the DC group. It can be concluded, that treatment with CPLE significantly reduced the alterations of the glomerulus and restored other important parameters, like oxidative stress management. While compared with LD and HD, all the data shows promising results for managing renal complications except the value of GSH of the LD group, which showed a significant difference (<0.05) compared with the CMC group. Thus, our experiments demonstrate a protective role of CPLE in controlling the nephrological complications and glycemic parameters.

**CONCLUSION**

In the current study, STZ-induced diabetic Swiss albino mice that received CPLE treatment were found to have normalized blood sugar levels, cholesterol and triglyceride levels, improved serum and urine parameters of albumin, creatinine, urea and maintained the antioxidant level of the tissue. Histopathological investigations suggest that CPLE treatment helps repair the renal basement membrane, possibly through its anti-inflammatory and antioxidant activities. Although the mechanism of the nephroprotective action of the prepared leaf extract is yet to be established, the preliminary data...
suggest that CPLE possesses potent antioxidant, antihyperglycemic and antihyperlipidemic activity, thus exhibiting a protective action in STZ-induced DN. Further investigation may elucidate the detailed mechanism of action of CPLE at the cellular and molecular levels.

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Conflicts of interest

The authors declare no conflict of interest.

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