Antinephrotoxic and antihyperlipidaemic activities of *Cucurbita maxima* leaf supplemented diets in STZ-induced diabetic rats

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**Author contributions**
Job Itanyi Onuche, designed the work, funded the research; carried out the lab work; Michael Sunday Abu prepared the manuscript, Arowora Kayode Adebisi, supervised the work; Joseph Ikwebe, provided technical support

**Competing interests**
The authors declare no conflicts of interest.

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**Abstract**
**Background:** *Cucurbita maxima* plant materials are frequently used to manage a number of disorders, according to previous studies. **Objective:** The goal of the present report was to look into further possible effects of this plant on kidney disorder in rats brought on by diabetes as well as the related abnormalities in lipid metabolism. **Methods:** To assess the ameliorative effect of the plant, streptozotocin (STZ) (45 mg/kg/day) was given as a single dosage to cause type 1 diabetes. After then, diabetic rats received supplemented diets of *Cucurbita maxima* for four weeks at 5%, 10%, 15% and 20% *ad libitum*. However, in another experiment to evaluate the preventive capacity of *Cucurbita maxima*, the STZ-induction came at the end of four weeks supplementation. Blood and kidney tissues were obtained at the end of the treatments and investigated. Consequently, the analysis of kidney function and lipid profile was made using serum obtained from the blood sample. Histological change in kidney was also observed using haematoxylin and eosin stain. **Results:** Our findings demonstrated that *Cucurbita maxima* drastically (*P < 0.05*) decreased the elevated kidney indicators such as urea and creatinine in the blood, and restored the electrolytes and lipid profiles anomalies as compared to the normal control. However, the alteration of biochemical parameters in the STZ-induced diabetic control remained unchanged throughout as compared to the normal control. The above mentioned biochemical changes that took place in kidney tissues were further corroborated by histological alterations. **Conclusion:** The results suggest that *Cucurbita maxima* leaves supplemented diets reversed STZ-induced renal disorder and abnormal lipid metabolism, and that these effects may be mediated by interacting with multiple receptors to raise the levels of antioxidant enzymes in the system.

**Keywords:** antinephrotoxic; antihyperlipidaemic; *Cucurbita maxima*; supplementation; STZ-induced; diabetes
Introduction

The primary symptoms of diabetes mellitus, a complicated disease, include an imbalance in blood glucose homeostasis that results in hyperglycemia (high blood sugar) and a number of secondary problems brought on by a complete or partial shortage of insulin. One of the most frequent consequences of diabetes mellitus, which affects 40% of people with the disease, is abnormalities in lipid profiles [1]. According to Soltani et al. [2], diabetes induction results in an increase in cholesterol, triglycerides, LDL, and VLDL. Diabetes mellitus typically results in higher serum lipid levels, which is a risk factor for coronary heart disease [3].

Idiot cell carcinoma and malignant carcinoid tumours are mostly treated by streptozotocin (STZ), a nitrosourea molecule that typically shares a fate of disposal with other nitrosoureas [4]. In addition to causing stomach ulceration, it is also diabetogenic, hepatotoxic, and nephrotoxic [5]. STZ causes significant pancreatic insulitls with the eventual loss of insulin-secreting beta cells and diabetes mellitus when administered intravenously or intraperitoneally to experimental mice. Streptozotocin, administered intraperitoneally at a dose of 45 mg/kg body weight of rats, successfully caused hyperglycemia in an experimental investigation [6]. In a different rat investigation, STZ was successfully used to induce hyperglycemia and stomach mucosal ulcerations at a dose of 65 mg/kg body weight [5]. From one to six weeks after therapy, the incidence and severity of lesions caused by STZ in the pancreas, liver, kidney, and GIT gradually increased [5]. Studies have linked certain diabetes problems to changes in liver enzyme levels [7], but there is no information on whether diabetic nephropathy and kidney enzyme changes are related [8].

Cucurbita maxima commonly known as pumpkin, is an annual herb that belongs to the family cucurbitaceae with prickly or hairy stem and axillary tendrils, large flowers, fleshy fruits and unisexual [9, 10]. It is largely cultivated in parts of Nigeria, India and in several warm regions of the globe, where its fruits and aerial parts are used as vegetable as well as medicine. This plant has a long history of usage as an anti-diabetic, anti-tumor, anti-hypertensive, anti-inflammatory, immuno-modulatory, and antibacterial agent in various nations, including India, China, Brazil, Yugoslavia, and America. [9, 11, 12]. The researcher’s interest in this plant was sparked by the prevalence of pumpkin in several traditional systems of medicine for a variety of diseases.

Methanolic extract of the leaf of the plant have been reported to possess’s antioxidant and α amylase inhibition activity [13]. There is also evidence that the leaves have anti-anaemic characteristic by its effect on the PVR, HB, and RBC count of albino rats [14]. Onuche and Abu [15] reported the anti-cancer effect of the leaf of Cucurbita maxima on colon carcinogenesis with significant improvement in the Malondialdehyde (MDA) values of induced colon carcinogenesis in male albino rats. Different parts of the plant contain certain bioactive components that influence the metabolic processes of the body, therefore considering its reported health benefits, Cucurbita maxima has been enlisted among the other medicinal plants. The purpose of the current study is to assess the antinephroprotective and antihyperlipidemic effects of Cucurbita maxima leaf in streptozotocin-induced diabetic rats.

Materials and methods

Sample collection and preparation

Fresh leaves of Cucurbita maxima will be collected from Ejule in odu LGA of kogi State and taken to a standard Herbarium, for identification, authentication and a voucher will be deposited, it will then be rinsed in clean water, dried at room temperature and ground to powder form, which will then be kept in an air tight container away from sunlight till needed

Experimental diet

Grower mash feed produced by UAC Company was procured from Jos, Plateau State. The feed served as the standard diet and was also used to mix the powdered leaves of Cucurbita maxima to formulate dietary inclusion of (5% and 10%, 15% and 20% w/w) for the experimental group as described by [15].

Quantitative assessment of some phytochemical constituents of Cucurbita maxima

Evaluation of Phenol Content. The test tubes contained 1 mL aliquots of the sample, 10 g/mL, 20 g/mL, 60 g/mL, 80 g/mL, and 100 g/mL of gallic acid standard, 5 mL of distilled water, and 0.5 mL of Folin Ciocalteu’s reagent. After five minutes of incubation, 10 mL of distilled water and 1.5 mL of 20% sodium carbonate were added, and the mixture was incubated again for two hours at room temperature. The absorbance of the sample was measured at 750 nm using UV-visible Jasco V-630 equipment upon incubation. There were three separate runs of the analysis. A solvent-containing reagent blank was used for comparison. We used gallic acid to create the calibration curve. The total phenolic content of Cucurbita maxima leaves was determined to be mg/100 g of dry mass, expressed as gallic acid equivalent (GAE) [16].

Tannin concentration analysis. Tannins were evaluated quantitatively using a modified version of the method described by Mjueeb et al. [17]. The leaves of Cucurbita maxima were finely powdered and added to 20 mL of 50% methanol in a beaker, which was then covered with paraffin and heated in a water bath at 80 °C for an hour while being stirred constantly. Whatman with two layers To filter the extract, we soaked 1 sheet of filter paper in 50% methanol. After waiting 20 minutes, 1 mL of the extract was mixed with 20 mL of distilled water, 2.5 mL of the Folin-Denis reagent, and 10 mL of 17% Na2CO3. This produced a bluish-green coloration. Tanning concentration was determined by plotting the measured absorbance at 760 nm against a standard curve covering a concentration range of 0–10 ppm.

Saponin concentration analysis. Mjueeb et al’s [17] method was used to calculate the saponin concentration. One gram of finely powdered leaves and one hundred millilitres of isobutyl alcohol were mixed together and swirled for five hours. The solution was filtered after adding 20 mL of a 40% saturated magnesium carbonate solution. To achieve the blood-red colour, 1 mL of the solution was added to 50 mL of distilled water along with 2 mL of the 5% FeCl3 solution, and the mixture was let to stand for 30 minutes. The absorbance was determined to be 380 nm. For concentrations of saponin between 0 and 10 ppm, a typical curve was constructed.

Total flavonoid concentration. Total flavonoid concentration was determined by adding 1 mL aliquots of the sample, 100, 200, 400, 600, 800, and 1000 g/mL of standard quercetin solutions, 4 mL of distilled water, and 0.3 mL of a solution containing 5% sodium nitrite to a test tube. After five minutes of incubation, 0.3 mL of 10% aluminium chloride and 2 mL of 1 M sodium hydroxide were added. Once the desired volume was reached, 10 mL of distilled water was added and well incorporated to turn the solution an orange-yellow tint. 510 nm is a visible-to-the-human-eye wavelength for ultraviolet The absorbance was measured with a Jasco V-630 spectrophotometer. The void was filled with pure water. There were three separate sets of analyses performed. Standard quercetin was used to create the calibration curve. The total flavonoid content of the leaf powder was expressed as quercetin equivalents per gramme of dry mass [18].

Classification of experimental animals

Sixty Wistar albino male rats weighing between 80 and 100 g were bought from the National Veterinary Research Institute (NVRI) in Vom, Plateau state, Nigeria. All animals were housed in a typical laboratory environment (24 °C, 12/12 h light-dark cycle), fed a regular pelleted diet, and had free access to water during the 28-day study period. Each animal was given two weeks of conditioning in the lab before the trial began. After the adaptation period, the animals were weighed and divided into six groups of five (n = 5) for studies of preventative and ameliorative treatments.

Diabetes induction and therapy
To induce diabetes, we followed Sunil et al.'s [19] protocol. Six sets of five mice were used in the ameliorative experiment. Streptozotocin (STZ; 45 mg/kg b.w.) was injected intraperitoneally once into animals after an overnight fast, in 0.1 M citrate buffer at pH 4.5. Hyperglycemia and glycosuria are adverse effects of streptozotocin, a nitrosourea-glucosamine derivative that destroys pancreatic islet cells. After the diabetic rats experienced the first stage of drug-induced hypoglycemic mortality, they were given access to a 5% (w/v) glucose solution to drink all night long. After 48 hours, the rats' blood sugar was checked using a glucometer (Glucocard-01 Mini, Bengaluru) using the tail-tilt method. If the fasting blood glucose level was over 250 mg/dL, the rat was diagnosed with diabetes. Rats receiving STZ had free access to food and water for the duration of the experiment, while control rats received an injection of vehicle (0.1 M citrate buffer at pH 4.5) at the time of induction. In the protective study, rats were given diets containing Cucurbita maxima leaves for a period of four weeks. On day 28, we diagnosed diabetes and assessed fasting blood glucose levels. At the beginning of the study on day 0 and again on days 7, 14, 21, and 28 weekly afterwards, the rats' body weights and blood sugar levels were recorded. On day 28, blood and kidney samples were obtained from the rats and frozen for subsequent examination while they were under chloroform anaesthesia.

Group-1: Normal control rats received an equal volume of vehicle orally (P. O.) + normal feed (unformulated feed).
Group-2: Diabetic group received STZ 45 mg/kg b.w. L.P. + standard feed (unformulated feed).
Group-3: Diabetic rats received 5% (w/w) Cucurbita maxima leaf formulated diet and water ad libitum.
Group-4: Diabetic rats received 10% (w/w) Cucurbita maxima leaf formulated diet and water ad libitum.
Group-5: Diabetic rats received 15% (w/w) Cucurbita maxima leaf formulated diet and water ad libitum.
Group-6: Diabetic rats received 20% (w/w) Cucurbita maxima leaf formulated diet and water ad libitum.

**Evaluation of renal function via measurement of serum urea concentration**

This was evaluated using Fawcett and Scout's [20] technique. The breakdown of urea by urease yields ammonia and carbon dioxide. In the presence of sodium nitroprusside, dicarboxyindophenol is synthesised from ammonia, hypochlorite, and salicylate in an alkaline medium. The spectrophotometric measurement of the amount of colour produced is done at a wavelength of 578 nm.

\[ \text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_3 + 2\text{CO}_2 + \text{salicylate} \rightarrow \text{dicarboxyindophenol (blue compound).} \]

The procedure involved placing 0.01 mL of sample, 0.01 mL of the standard reagent, and 0.01 mL of distilled water in three separate clean test tubes. Afterwards, sodium nitroprusside and urease were added to the mixture in a reagent (1 mL). After 10 minutes of standing at room temperature (25-30 °C), the contents of each test tube were mixed. At 578 nm, the absorbance of both the sample under test and the reference standard was determined and compared to a blank reagent.

The concentration of urea in the serum was determined using the following formula;

**Concentration of Urea in the Blood (mg/dL) = Test Absorption x Reference Value/Reference Value**

The formula for BUN concentration is as follows: BUN (mg/dL) = 0.467 x Urea (mg/dL).

**Evaluation of renal function by serum creatinine measurement**

Serum creatinine concentration was measured using the colorimetric method described by Bertels and Bohmer [21]. The principle is that when alkaline picrate is added to serum containing creatinine, a colourful combination is formed. The generation rate of colour complexes is proportional to the amount of creatinine present. The rate of reaction, expressed as the intensity of the orange colour produced, is measured colorimetrically at 510 nm and compared to a reference.

Creatinine + Picric acid + NaOH Creatinine picrate (510 nm) (Yellow) (Alkaline medium) (Orange)

The procedure is dividing a 1 mL working reagent containing picric acid and sodium hydroxide between two clean test tubes labelled sample test and standard and filling them with 0.1 mL of test sample and 0.1 mL of standard solution, respectively. After 20 seconds of agitation, we measured the absorbance of the standard (ST1) and test sample (TS1) at 510 nm and 80 seconds later, we did the same for the standard and sample (ST2).

TS2 – TS1/ST2 – ST1 X Standard conc. (mg/dL)

**Renal function evaluation using estimation of serum sodium, potassium, and chloride ions**

Instrumentation Laboratory, Inc. of Lexington, Massachusetts, USA, supplied both the flame photometer Model 143 and the automated diluter Model 144 (dilution ratio of 200:1). Distilled water and a standard containing 140 mequiv./l of Na+ and 5 mequiv./l of K+ were used to calibrate the flame photometer. Following each sample measurement, the reference solution was used to evaluate the instrument’s stability.

**Lipid profile analysis**

Total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein were all tested using an auto-chemistry analyzer (Cobas C111, Germany). The principal method used by the Cobas C111 to determine the absorbance of a fluid is called absorption photometry. Absorbance is used to determine the concentration of a solution. The Cobas C111 features a touchscreen, on-screen keyboard, and four global action buttons. When it is safe to add or withdraw samples, chemicals, or other fluids, LEDs and audible indications alert you.

**Histological study kidney**

A portion of each animal’s kidney was fixed in 10% formalin (Ljille, 1965). Haematoxylin and eosin was used to prepare and stain the paraffin sections. A high-resolution (X100) microscope with a photographic capacity was used to view the thin slices of liver and kidney on permanent slides, and photomicrographs were obtained.

**Statistical analysis**

ANOVA was used to examine the data using the SPSS programme (version 20 SPSS Inc., Chicago, IL, USA). The Bonferroni multiple comparison test (post-hoc test) was used to examine the parameter differences across the various animal groups. Data was reported in mean ± standard deviation. Significant data was defined as those with a P value < 0.05. Utilising Microsoft Word and Excel, the results were shown in tables, charts, and graphs.

**Results**

**Quantitative phytochemical analysis of Cucurbita maxima leaves**

The phenol concentration of Cucurbita maxima leaves was determined using the Folin-Ciocalteu reagent technique and compared to the gallic acid standard curve using the linear regression equation \( y = 0.0745x + 18.348, R^2 = 0.8976 \). The quercetin standard curve was used to quantify the total flavonoid concentration using the aluminium chloride technique (\( y = 0.0499x + 6.7813, R^2 = 0.864 \)). The levels of tannins and saponins in the leaves of Cucurbita maxima were calculated similarly. The total phenol, total flavonoid, tannin, and saponin contents are shown in Figure 1.

**Body weight change in STZ-induced diabetic rats supplemented with Cucurbita maxima diet**

The percentage body weight changes following administration of C. maxima fortified diet to rats in preventive and ameliorative treatment is shown in Figure 2 and Figure 3 respectively. There was weight loss across the various percentages inclusion in both preventive and ameliorative treatments. However, the weight loss in groups placed on C. maxima fortified diet was significantly (\( P < 0.05 \) lower compared
to STZ control group. The normal control group shows weight gain in preventive and ameliorative treatments.

**Cucurbita maxima** leaf supplementation in the diet reduced and reversed renal marker elevations in rats with STZ-induced diabetes

Table 1 shows that after 28 days of supplementation with *Cucurbita maxima* leaves, the levels of creatinine, urea, and electrolytes were significantly \( P < 0.05 \) higher in the STZ control group than in normal control rats and the supplemented groups in the preventive treatments. In this student, the supplemented groups showed a significant \( P < 0.05 \) decrease in renal function metrics compared to the normal control group.

Serum urea, creatinine, and electrolytes (sodium, chloride) all return to near-normal levels after receiving ameliorative treatments, while they remain significantly \( P < 0.05 \) elevated in the STZ control group compared to the normal group (Table 2).

*Cucurbita maxima* leaf supplementation in the diet decreased lipid levels in rats forced to develop diabetes by streptozotocin

Table 3 shows that after 28 days of supplementation, the STZ control group had significantly higher total cholesterol, triglyceride, and LDL levels compared to the normal control group and the supplemented group due to diabetes induction. Total cholesterol (TCHOL), triglyceride (TGL), and low-density-lipoprotein (LDL) levels were all significantly reduced \( P < 0.05 \) by *Cucurbita maxima* supplementation, whereas high-density-lipoprotein (HDL) levels were enhanced across the board.

The *Cucurbita maxima* supplementation considerably \( P < 0.05 \) improved the lipid profile abnormality caused by STZ. Table 4 shows that the supplemented groups had higher levels of high-density-lipoprotein (HDL) and lower levels of low-density-lipoprotein (LDL), total cholesterol (TCHOL), and triglycerides (TGL) than the STZ control group. The supplemented groups had results similar to the normal group regarding the correction of the lipid profile abnormality.
Table 1 Preventive effect of *Cucurbita maxima* leaves supplemented diet on kidney function of STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Sodium (mmol/L)</th>
<th>Chloride (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.05 ± 0.00^a</td>
<td>0.23 ± 0.06^a</td>
<td>3.87 ± 0.49^a</td>
<td>1.71 ± 0.01^b</td>
</tr>
<tr>
<td>STZC</td>
<td>1.02 ± 0.00^a</td>
<td>2.57 ± 0.85^b</td>
<td>9.72 ± 1.09^b</td>
<td>2.46 ± 0.03^c</td>
</tr>
<tr>
<td>PV5%</td>
<td>0.43 ± 0.03^a</td>
<td>0.69 ± 0.09^a</td>
<td>5.25 ± 0.25^a</td>
<td>1.49 ± 0.04^b</td>
</tr>
<tr>
<td>PV10%</td>
<td>0.13 ± 0.00^b</td>
<td>0.58 ± 0.08^a</td>
<td>4.88 ± 0.79^a</td>
<td>1.38 ± 0.10^c</td>
</tr>
<tr>
<td>PV15%</td>
<td>0.08 ± 0.01^a</td>
<td>0.44 ± 0.03^a</td>
<td>4.32 ± 0.23^a</td>
<td>1.46 ± 0.05^c</td>
</tr>
<tr>
<td>PV20%</td>
<td>0.07 ± 0.01^a</td>
<td>0.43 ± 0.10^a</td>
<td>4.13 ± 0.09^a</td>
<td>1.62 ± 0.04^b</td>
</tr>
</tbody>
</table>

n = 5; Results are in mean ± standard deviation; values with different superscript down the column are significantly different at (P < 0.05); NC = normal control, STZ = STZ control, PV = Preventive treatment.

Table 2 Ameliorative effect of *Cucurbita maxima* leaves Supplemented diet on kidney function of STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Sodium (mmol/L)</th>
<th>Chloride (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.05 ± 0.008^a</td>
<td>0.23 ± 0.06^a</td>
<td>3.87 ± 0.49^a</td>
<td>1.71 ± 0.01^b</td>
</tr>
<tr>
<td>STZC</td>
<td>1.02 ± 0.00^b</td>
<td>2.57 ± 0.85^b</td>
<td>9.72 ± 1.09^b</td>
<td>2.46 ± 0.03^c</td>
</tr>
<tr>
<td>AM5%</td>
<td>0.06 ± 0.01^a</td>
<td>0.70 ± 0.07^a</td>
<td>7.83 ± 1.34^a</td>
<td>1.47 ± 0.01^b</td>
</tr>
<tr>
<td>AM10%</td>
<td>0.06 ± 0.01^a</td>
<td>0.42 ± 0.06^a</td>
<td>4.08 ± 0.07^a</td>
<td>1.41 ± 0.02^c</td>
</tr>
<tr>
<td>AM15%</td>
<td>0.04 ± 0.01^a</td>
<td>0.32 ± 0.01^a</td>
<td>4.19 ± 0.41^a</td>
<td>1.49 ± 0.12^c</td>
</tr>
<tr>
<td>AM20%</td>
<td>0.05 ± 0.02^a</td>
<td>0.35 ± 0.03^a</td>
<td>4.30 ± 0.52^a</td>
<td>1.54 ± 0.04^b</td>
</tr>
</tbody>
</table>

n = 5; Results are in mean ± standard deviation; values with different superscript down the column are significantly different at (P < 0.05); NC = normal control, STZ = STZ control, AM = ameliorative treatment.

Table 3 Preventive effect of *Cucurbita maxima* leaves supplemented diet on lipid profile of STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCHOL (mmol/L)</th>
<th>TGL (mmol/L)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>237.84 ± 27.22^a</td>
<td>0.37 ± 0.05^a</td>
<td>3.07 ± 0.05^a</td>
<td>0.14 ± 0.01^a</td>
</tr>
<tr>
<td>STZC</td>
<td>550.09 ± 44.12^b</td>
<td>2.81 ± 0.40^b</td>
<td>0.45 ± 0.11^a</td>
<td>0.85 ± 0.09^c</td>
</tr>
<tr>
<td>PV5%</td>
<td>485.54 ± 6.48^b</td>
<td>1.22 ± 0.53^b</td>
<td>1.08 ± 0.05^b</td>
<td>0.55 ± 0.09^b</td>
</tr>
<tr>
<td>PV10%</td>
<td>380.63 ± 13.00^b</td>
<td>0.65 ± 0.31^a</td>
<td>1.33 ± 0.17^b</td>
<td>0.43 ± 0.04^b</td>
</tr>
<tr>
<td>PV15%</td>
<td>361.99 ± 44.31^b</td>
<td>0.55 ± 0.03^a</td>
<td>1.59 ± 0.06^b</td>
<td>0.41 ± 0.05^b</td>
</tr>
<tr>
<td>PV20%</td>
<td>289.28 ± 34.21^a</td>
<td>0.49 ± 0.09^b</td>
<td>2.44 ± 0.24^a</td>
<td>0.24 ± 0.05^b</td>
</tr>
</tbody>
</table>

n = 5; Results are in mean ± standard deviation; values with different superscript down the column are significantly different at (P < 0.05); NC = normal control, STZ = STZ control, PV = Preventive treatment.

Table 4 Ameliorative effect of *Cucurbita maxima* leaves supplemented diet on lipid profile of STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCHOL (mmol/L)</th>
<th>TGL (mmol/L)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>237.84 ± 27.22^a</td>
<td>0.37 ± 0.05^a</td>
<td>3.07 ± 0.05^a</td>
<td>0.14 ± 0.01^a</td>
</tr>
<tr>
<td>PC</td>
<td>550.09 ± 44.12^b</td>
<td>2.81 ± 0.40^b</td>
<td>0.45 ± 0.11^a</td>
<td>0.85 ± 0.09^c</td>
</tr>
<tr>
<td>AM5%</td>
<td>524.91 ± 22.43^b</td>
<td>0.88 ± 0.04^a</td>
<td>1.21 ± 0.13^b</td>
<td>0.35 ± 0.02^b</td>
</tr>
<tr>
<td>AM10%</td>
<td>342.29 ± 19.85^b</td>
<td>0.61 ± 0.07^a</td>
<td>1.57 ± 0.06^b</td>
<td>0.32 ± 0.08^b</td>
</tr>
<tr>
<td>AM15%</td>
<td>384.51 ± 19.91^b</td>
<td>0.48 ± 0.24^a</td>
<td>1.71 ± 0.06^b</td>
<td>0.24 ± 0.04^a</td>
</tr>
<tr>
<td>AM20%</td>
<td>293.51 ± 18.45^a</td>
<td>0.47 ± 0.02^a</td>
<td>2.75 ± 0.22^a</td>
<td>0.22 ± 0.02^a</td>
</tr>
</tbody>
</table>

n = 5; Results are in mean ± standard deviation; values with different superscript down the column are significantly different at (P < 0.05); NC = normal control, STZ = STZ control, AM = ameliorative treatment.
Histopathological examination

Figure 4A and 5A show a section of the renal cortex from a person without diabetes, revealing the typical arrangement of the kidney's proximal tubules (PT), distal tubules (DT), Bowman's capsule, and glomerulus (G). Significant vacuolation in renal tubular cells and loss of nuclei from karyolysis were observed in the kidneys of untreated diabetic rats (Figure 4B and 5B). When the kidneys of diabetic rats were pre-treated with Cucurbita maxima (10, 15, and 20%), the harmful effects of STZ on the glomeruli and renal tubules were reversed (Figure 4D, 4E, and 4F). Figure 5F shows that the renal cortex of diabetic rats treated with Cucurbita maxima (5, 10, 15, and 20%) was somewhat protected.

![Figure 4A](https://www.tmrjournals.com/atr)

**Figure 4** Representative photomicrograph of kidney of albino rats supplemented with Cucurbita maxima leaves diet. (A) Normal control showing normal tubules and glomerulus. (B) Negative control showing degenerated and total collapse of cell walls with no distinct cell lining. (C) PV5% group with degeneration of cells and also, nephritis noticed in the glomerulus. (D) PV10% group with normal cell arrangement with intact podocyte in the glomerulus. No nephritis noticed. (E) PV15% group with normal cell structure. No glomerular nephritis but mid community of cells (inflammation) as circled. (F) PV20% group congested glomerulus and dark pyknosis noticed, showing restoration from necrotic state.

GM, Glomerulus; CT, convoluted tubules; PCT, proximate convoluted tubules; DCT, distal convoluted tubules; BS, Bowman's space; BC, Bowman's capsule; PD, podocytes. Scale: 50µm. (H&E STAIN X100)

![Figure 5A](https://www.tmrjournals.com/atr)

**Figure 5** Representative photomicrograph of kidney of Albino rats supplemented with Cucurbita maxima leaves diet. (A) Normal control showing normal tubules and glomerulus. (B) Negative control showing degenerated and total collapse of cell walls with no distinct cell lining. (C) AM5% group with glomerular nephritis and no distinct cyto-architecture of cells and cell walls. (D) AM10% group with dark pyknotic nuclei in and outside the glomerulus and necrosis of cells noticed. (E) AM15% group with nephritis of the glomerulus. (F) AM20% group with restoration status, community of podocytes in the glomerulus (nephritis), and cells not too clear but with a good cell arrangement.

GM, Glomerulus; CT, convoluted tubules; PCT, proximate convoluted tubules; DCT, distal convoluted tubules; BS, Bowman's space; BC, Bowman's capsule; PD, podocytes. Scale: 50µm. (H&E STAIN X100)
**Discussion**

This study showed that daily administration of *Cucurbita maxima* leaves to rats might lessen the development of diabetic problems brought on by streptozotocin (STZ). In order to assess the impact of novel antidiabetic diet in rats, the current study utilised an experimentally produced diabetic model after a single STZ injection. From our study, rats that consumed *Cucurbita maxima* leaves fortified diet on a daily basis displayed potential positive effects that were visible in the restoration of the kidney's histological architecture and its biochemical parameters, as well as in the normalisation of lipid metabolism as determined by the lipid profile analysis. It is generally known that diabetes mellitus causes issues that impair the metabolic processes of important organs including the liver and kidneys [22].

According to several studies, phytochemicals are the bioactive components of plants that provide a fresh approach to halting the start and progression of oxidative stress, chronic illnesses, and ageing. Nutraceuticals of plant origin, often known as phytochemicals, have been suggested by Cicero and Colletti [23] as a potential treatment for metabolic syndrome. The phytochemicals and their effects on diabetes and associated consequences were also included in the paper. In order to evaluate the effects of flavonoids, phenols, tannins, and saponins in the solvent extracts of *Cucurbita maxima* leaves, a preliminary phytochemical study was conducted while keeping in mind the significance of phytochemicals in the reduction of various diseases. The most significant phytochemicals are phenols and flavonoids, which have antibacterial, anti-inflammatory, antidiabetic, anti-cancer, and antioxidant activities [24]. As a result, in vitro measurements of the total phenols and total flavonoids in *Cucurbita maxima* leaves were made. By lowering the risk of metabolic syndrome and the associated consequences of type 2 diabetes, phenolic substances, such as phenolic acids and flavonoids, may offer health advantages. There is still a need for more research since different phenolic chemical groups have diverse biological properties and little is known about the processes through which they can help prevent illness [25].

The percentage body weight loss was significantly (P < 0.05) lower in the *C. maxima* supplemented groups than STZ control group. Reduction in body weight of diabetic animals was previously reported by Oyedemi et al. [26]. Degradation of structural proteins and muscle atrophy may be to blame for the loss in body weight in STZ-induced diabetic rats [27]. When compared to normal and treated rats, STZ-induced diabetic control rats displayed higher polyuria, polyphagia, and polydipsia, indicating that the rats' excessive tiredness finally led to weight loss. The reduction in body weight percentage seen in the treated rats may be due to *Cucurbita maxima's* ability to stabilise blood sugar, which consequently inhibits weight loss [28].

The by-product of muscle metabolism, creatinine, must be filtered and eliminated by the kidneys. Increased blood levels of creatinine are the outcome of impaired renal function. The rise in serum urea and creatinine levels is caused by an increase in tissue proteolysis, a decrease in protein synthesis, and a negative nitrogen balance [29]. When compared to the diabetic control rat group in this study, the levels of urea, creatinine, and electrolytes in the STZ-induced diabetic rats supplemented with *Cucurbita maxima* leaves were considerably (P < 0.05) reduced in both the preventative and ameliorative treatments. These findings point to a plant's leaves having a protective effect against diabetes caused by STZ that is nephrotoxic. The restoration of the kidney's structural integrity from the histological analysis and the normalisation of its biochemical parameters in both treatments point to the protective and ameliorative effects of the leaves' bioactive substances, which may have displayed modulatory activity against the oxidative stress that might have been brought on by the STZ's toxicity.

Typically, plant extracts rich in antioxidant phytochemicals including alkaloids, phenolic compounds, and flavonoids [30] are able to offset harmful effects of chemicals in the body. Because their hydroxyl groups have the potential to scavenge free radicals, phenolic substances found in plants limit the oxidative destruction of phospholipids found in cell membranes thereby conserving the integrity of the cell [31]. Additionally, the authors found that the plant's current concentrations of flavon, flavonoid, tannins, and saponins play a significant influence in biological activities including antibacterial, antioxidant, and anticancer, as well as the plant's current modulatory impact on renal and lipid profiles. The results of this investigation are consistent with the findings of Yazdi et al. [32], who reported that STZ-induced diabetes mellitus was linked to a considerable deterioration in renal functions and that a plant source (D. viscose) daily therapy improved this relationship.

The mobilisation of free fatty acids from fat deposits increases during lipolysis in conjunction with hyperglycemia, which leads to an imbalance in the metabolism of lipoproteins. In diabetic rats, there were significant changes (P < 0.05) in the lipid metabolism. There is overproduction of TG from the liver as a result of low insulin levels or insulin resistance that inhibits lipoprotein lipase activity thereby raises blood levels of LDL. A rise in TG stimulates the release of tiny, dense LDL molecules while simultaneously lowering HDL cholesterol levels [33]. Numerous studies showed that the first line of defence against the alterations in lipid metabolism by raising insulin levels was glycemic management. The *Cucurbita maxima* leaves feed supplements enhanced the lipid profile when used for diabetes prevention and treatment; it was clearly seen in the current study. The simultaneous improvement in HDL levels, drop in TCHOL, LDL and TGL levels were detected substantially at P < 0.05, together with the raised level of blood insulin and downregulated glucose values.

The pathophysiological changes brought on by diabetes are linked, either directly or indirectly, to insulin resistance or insufficiency. As a result, the modulation of other serum biochemical parameters, such as glucose, TCHOL, HDL, LDL and TGL levels, may be mediated via the upregulation of insulin levels by *Cucurbita maxima* leaves bioactive components. Apinya et al. [34] also showed that lipid profiles, including total cholesterol (TCHOL), triglycerides (TGs), and low-density lipoprotein (LDL), dramatically rose while the high-density lipoprotein (HDL) significantly dropped in diabetes controls. In line with the present findings, diabetic rats fed with *Cucurbita maxima* leaf fortified diet had significantly lower TCHOL, TGL and LDL levels but higher HDL levels after receiving it once daily for four weeks.

Overall, our research has demonstrated that *Cucurbita maxima* has healing properties for diabetic kidney injury and preventive advantages against the pathogenesis of altered lipid metabolism brought on by STZ-induced diabetes. However, this study did not evaluate the actual biological mechanisms by which the improvements noticed in the various parameters were achieved. Therefore, further research needs to be conducted to ascertain the mechanistic pattern of this formulated diet on diabetes.

**Conclusion**

According to these results, *Cucurbita maxima* leaves may protect against STZ-induced hyperglycemia and nephrotoxicity. These benefits may be mediated by *Cucurbita maxima* leaves' interactions with a variety of cellular receptors, which raise the levels of antioxidant enzymes in the body and lessen the severity of organ damage caused by STZ toxicity. More study is needed to determine its mechanism of action and safety for usage as therapy against different metabolic issues brought on by diabetes.

**References**


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