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Preventive and Ameliorative Effects of Diet Supplemented with *Cucurbita maxima* Leaf on Hyperglycemia and Hepatotoxicity in STZ-Induced Diabetic Rats

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ABSTRACT

Background and Objective: Plant materials rich in antioxidant phytochemicals are able to offset the harmful effects of chemicals in the body. The antidiabetic and anti-hepatotoxic effects were investigated in STZ-induced diabetic rats fed a diet supplemented with *Cucurbita maxima* leaves. **Materials and Methods:** Sixty rats were randomly assigned into 6 groups of five animals each (n = 5) in preventive and ameliorative trials. Rats were supplemented with 5, 10, 15 and 20% *Cucurbita maxima* leaf for four weeks. The ANOVA in SPSS version 20 was used to examine the data followed by the Bonferroni multiple comparison (*post hoc*) Test. **Results:** Preliminary analysis of *Cucurbita maxima* leaf powder revealed antioxidant potential, with an IC_{50} for DPPH scavenging activity of 3.1 µg mg^{-1}. The glucose levels and liver function parameters of the groups whose diets were supplemented with *Cucurbita maxima* leaf did not show signification ($p > 0.05$) variations as compared to the normal control group in both preventive and ameliorative treatments. **Conclusion:** In both preventive and ameliorative trials, it can be concluded that the *Cucurbita maxima* leaves exhibit antihyperglycemic and anti-hepatotoxic effects.

KEYWORDS

Diabetes, *Cucurbita maxima,* antihyperglycemic, anti-hepatotoxic, ameliorative effects, anti-diabetic effects

INTRODUCTION

The prevalence of diabetes mellitus (DM) worldwide continues to increase, making it a major challenge for global healthcare services[1]. Diabetes mellitus is connected to several critical issues that affect most bodily systems due to hyperglycemia and glucose intolerance, including hepatotoxicity, neuropathy, cardiovascular illnesses and renal disorders[2]. Insufficient intracellular glucose during DM harms and necrotizes hepatic cells, which promotes the apoptotic pathway. Pro-inflammatory responses have been observed in hepatic cells during DM[3]. Reactive oxygen species (ROS) generation and oxidative stress are two major factors in the pathogenesis of diabetic hepatotoxic damage[4]. Lipid peroxidation and oxidative necrosis occur when the intracellular production of free radicals exceeds the hepatocytes' antioxidant system's capacity. Due to hepatocyte injury, neutrophil infiltration and Kupffer cell activation produce inflammatory cytokines and apoptosis[5].

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Hyperglycemia is common in diabetic patients following trauma or a severe illness. According to Mahmoodpoor *et al*.[6], most critically ill patients have insulin resistance, so blood sugar levels are difficult to control. In particular, the liver and skeletal muscles have a role in glucose metabolism, as do stress mediators such as stress hormones, cytokines and the central nervous system[7]. Systemic catecholamine release, cytokine release following systemic inflammation and direct systemic stimulation can all promote hepatic glycogenolysis and, eventually, hyperglycemia[8].

Several medicinal plants with active compounds such as flavonoids, tannins, phenolics and alkaloids have hypoglycemic effects that help manage diabetes[9]. They also include antioxidant minerals like vitamin C, lutein and lycopene and, in some cases, substantial levels of crude fibre[10]. For instance, tannin enhances pancreatic beta-cell function and boosts insulin release. The antioxidant quercetin prevents lipid peroxidation and metal ion chelation through several mechanisms associated with eliminating oxygen radicals[11]. According to Hegazy *et al*.[12], hypoglycemic plants raise insulin secretion, increase glucose absorption by muscle and fat tissues, limit glucose uptake from the intestine and inhibit glucose production by liver cells.

Due to its hypoglycemic activity, *Cucurbita maxima* has been used extensively in China and Mexico for diabetic patients[8]. Additionally, this herb is traditionally used for managing diabetes in Northwest Iran[13]. The *C. maxima* belongs to the family *Cucurbitaceae* and genus *Cucurbita*. The squash of *C. maxima* resembles a squash of *Cucurbita mixta, Cucurbita pepo* and *Cucurbita moschota*[14]. According to Mohaammed *et al*.[14] and Onuche and Abu[15], *C. maxima* is a giant pumpkin (English), Echi in Igala, Kabewa in Hausa, Anya in Igbo and Isi in Yoruba. It is a traditional crop cultivated in Nigeria for food and traditional medicine sources[16] . Its leaves, fruits and seeds were boiled, roasted or baked. The leaf methanol extract possesses antioxidant activity and amylase inhibition[17]. Its leaf also has anti-anemic properties that could affect rats' PVC, Hb and RBC counts[16]. A study by Onuche and Abu[15] showed that the leaf of *Cucurbita maxima* affected colon carcinogenesis by significantly improving malondialdehyde (MDA) values of induced colon carcinogenesis in male albino rats. This study was conducted to determine the protective and ameliorative effects of *C. maxima* leaf supplementation in the standard feed of diabetic albino rats.

MATERIALS AND METHODS

Study area: The *Cucurbita maxima* leaf was bought from Ejule in Ofu LGA of Kogi State, Nigeria. The animal breeding, intoxication, treatments and biochemical analysis took place at the Biochemistry Laboratory of the Federal University of Agriculture Makurdi Benue State, Nigeria, whereas the data analysis and manuscript preparation were carried out at Federal University Wukari Taraba State, Nigeria. The study took six months to complete (March-August, 2023).

Plant collection and identification: Fresh *Cucurbita maxima* leaves (1 kg) were procured from Ejule in Ofu LGA of Kogi State and transported to a standard herbarium for identification, authentication and voucher issuance.

Plant preparation: Fresh *Cucurbita maxima* leaves were rinsed in clean water, dried at room temperature, ground into powder and then stored in an airtight container for further analysis.

Proximate analysis: Proximate analysis was carried out to determine moisture, crude fibre, crude lipid, crude protein and total ash by the AOAC methods[18].

Determination of total phenol: The test tubes were filled with 1 mL of aliquots of the sample and gallic acid as a standard (10, 20, 40, 60, 80 and 100 g mL^{-1}) and then 5 mL of distilled water and 0.5 mL of Folin Ciocalteu's reagent were added and agitated. After 5 min of incubation, 10 mL of distilled water and 1.5 mL of 20% sodium carbonate were added, followed by re-incubation for 2 hrs at room temperature.

After incubation, the absorbance of the sample was assessed using UV-visible Jasco V-630 Spectrophotometer, JASCO International CO. Ltd., 4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan equipment at 750 nm. The analysis was performed in triplicates. A reagent blank with solvent was used as a blank sample. Gallic acid was used to plot the calibration curve. The total phenolic content of *Cucurbita maxima* leaves was estimated as mg of gallic acid equivalent (GAE)/100 g of dry mass[19].

Determination of tannins: Mujeeb *et al.*[20] modified version approach was used to estimate tannins quantitatively. *Cucurbita maxima* leaves that had been finely pulverized were placed in a beaker with 20 mL of 50% methanol, covered with parafilm and heated for one hour in a water bath at 80°C with constant stirring. The extract was filtered using double-layered Whatman No. 1 filter paper with 50% methanol. One milliliter of extract was mixed with 20 mL of distilled water, 2.5 mL of Folin-Denis reagent and 10 mL of 17% Na_2CO_3 and left to stand for 20 min to produce a bluish-green color. The tannin content was determined by comparing the measured absorbance by UV-visible Jasco V-630 Spectrophotometer (JASCO International CO. Ltd., 4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan) at 760 nm to a reference curve established for a range of 0-10 ppm.

Determination of saponins: The Mujeeb *et al.*[20] method determined the saponin content. One gram of finely powdered leaves was added with 100 mL of isobutyl alcohol and agitated for 5 hrs. The mixture was mixed with 20 mL of a 40% saturated magnesium carbonate solution and then filtered. One milliliter of solution was mixed with 2 mL of a 5% $FeCl_3$ solution and 50 mL of distilled water and the mixture was left to stand for 30 min to develop the blood-red colour. The absorbance was measured at 380 nm using a UV-visible Jasco V-630 Spectrophotometer. A standard saponin curve was established for a 0-10 ppm range.

Determination of total flavonoid: Test tubes were filled with 1 mL of aliquots of the sample, 1 mL of standard quercetin solution (100, 200, 400, 600, 800 and 1000 g mL^{-1}), 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. After 5 min of incubation, 0.3 mL of 10% aluminum chloride was added and then 2 mL of 1 M sodium hydroxide was added at the 6th min. The volume was finally made up to 10 mL with distilled water and thoroughly mixed to produce an orange-yellow tint. The absorbance was measured using a UV-visible Jasco V-630 Spectrophotometer. Distilled water was used as a blank. Analysis was performed in duplicate. Standard quercetin was used to establish the calibration curve. The total flavonoid in the leaf powder was expressed as quercetin equivalent/g dry mass[21].

Determination of antioxidant activity by DPPH: Two milliliters of a 1.0 mM DPPH radical solution in methanol were combined with one millilitre of a standard or extract solution ranging in concentration from 10 to 500 μg mL^{-1}. After a quick stir, the solution was left to incubate in the dark at 37°C for 20 min. The UV-visible light was used to monitor the decline in absorbance of each solution. The spectrophotometer reading was taken at 517 nm on a Jasco V-630 from JASCO International Co. Ltd., at 4-21 Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan. The positive control was ascorbic acid, while the blank was a mixture of 1 millilitre of ethanol and 2 mL of a 1.0 mM DPPH radical solution[22].

The degree of radical scavenging was measured by:

$$\text{Antioxidant capacity (\%)} = \frac{AC - AS}{AC} \times 100$$

Where:

Ac = Control absorbance

As = Sample absorption

By plotting the percentage of inhibition against the various concentrations, the concentration of sample needed to scavenge 50% of the free radical DPPH (IC_{50}) was estimated.

Experimental diet: The experimental diet used in this study was grower mash feed produced by UAC Company from Jos, Plateau State. The feed served as the standard diet and was also used to mix with the powdered leaves of *Cucurbita maxima* to formulate dietary inclusion of 5, 10, 15 and 20% w/w) for the experimental group[15].

Grouping of experimental animals: Sixty healthy male albino rats of the Wistar strain weighing 80-100 g were obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The experimental animals were kept in conventional laboratory settings (24 2°C, 12/12 hrs light-dark cycle), fed a standard grower mash pellet and given unlimited access to water for 28 days. The acclimatization period was carried out for two weeks before the experiment. All animals were weighed after the acclimatization period and assigned into 6 groups of 5 animals each for prevention and ameliorative treatments.

Induction of diabetes and treatments: The procedure of diabetic induction follows the method of Gurumallu *et al.*[23]. There are six treatment groups, each consisting of five animals. A single intraperitoneal injection of freshly prepared streptozotocin (STZ, 45 mg kg^{-1} b.wt.) in 0.1 M citrate buffer of pH 4.5 induced diabetes after an overnight fast.

Streptozotocin is a glucosamine derivative of nitrosourea that selectively destroys pancreatic islets of β-cells resulting in hyperglycemia and glycosuria. The STZ-induced rats with early-stage diabetes were treated with a 5% (w/v) glucose solution overnight to prevent death from hypoglycaemia. Rats' blood sugar levels were tested 48 hrs later by pricking their tails and using a glucometer (Glucocard-01 Mini, Bengaluru).

Diabetic rats were those with fasting blood glucose levels >250 mg dL^{-1} that were included in the study. At the time of induction, the control group of rats received a single injection of 0.2 mL of vehicle (0.1 M citrate buffer at pH 4.5). Animals treated with STZ were given unlimited access to food and drink.

Group 1: Normal control group received an equal volume of vehicle orally (P.O.)+normal (unformulated) feed
Group 2: Induced diabetic group received normal feed (unformulated feed without supplementation of *Cucurbita maxima* leaf
Group 3: Induced 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*

During the experimental period, the rats' blood glucose levels and body weights were measured weekly, i.e., on days 0, 7, 14, 21 and 28. Biochemical parameters were analyzed on the 28th day. The rats were anesthetized with ether and the blood samples were collected and preserved for further analysis.

In the preventive study, STZ-induction was carried out after the experimental animals received a *Cucurbita maxima* leaf-formulated diet for four weeks. Consequently, the rats were sacrificed and the blood samples were collected and preserved for further analyses.

Determination of liver function: Liver function was assessed by analyzing serum concentrations of Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), total protein and albumin and direct and indirect bilirubin using an auto-chemistry analyzer (Cobas C111, Germany, Land wind LW E60B, China).

Ethical approval, statement of human and animal rights and statement of informed consent: All ethical protocols concerning the use of animals in scientific investigations of University of Agriculture Makurdi, Benue State, Nigeria were duly followed when conducting this experiment. All necessary permissions were taken through formal request and were duly granted before the conduct of the study.

Statistical analysis: The ANOVA in SPSS version 20 was used to examine the data (version 20 SPSS Inc., Chicago, Illinois, USA). The Bonferroni multiple comparison (*post hoc*) Test was used to compare differences among the various animal groups. Data was represented as Standard Deviation±Mean. The data with a p-<0.05 was significantly different.

RESULTS

Proximate composition of *Cucurbita maxima* leaves: The proximate composition of *Cucurbita maxima* leaves, including moisture, ash, moisture, ash, lipid, fibre, protein and carbohydrate, were 9.85±0.12, 5.43±0.05, 2.54±0.01, 8.56±0.03, 3.165±0.04 and 70.455±1.78 g/100 g dry matter, respectively as presented in Table 1. The result showed that the highest content of *C. maxima* leaf was carbohydrate.

Quantitative analysis of phytochemical content in *Cucurbita maxima* leaves: The quantitative analysis was carried out on total phenol, flavonoids, tannins and saponins. Total phenol was evaluated using the Folinciocalteu reagent method against a gallic acid standard curve using the linear regression equation

$$y = 0.0745x+18.348$$
$$R^2 = 0.8976$$

Total flavonoid was calculated using the aluminum chloride technique and the quercetin standard curve:

$$y = 0.0499x+6.7813$$
$$R^2 = 0.864$$

The total phenol, flavonoids, tannins and saponins contents are presented in Table 2. *Curcuma maxima* leaves higher phenol concentration (6.32±0.41 mg GAE g^{-1} of extract), followed by flavonoid content with 3.21±0.09 mg QE g^{-1} extract.

Table 1: Proximate compositions of *Cucurbita maxima* Leaves

Parameter	Composition (dry matter (%))
Moisture	9.85±0.12
Ash	5.43±0.05
Lipid	2.54±0.01
Fibre	8.56±0.03
Protein	3.165±0.04
Carbohydrate	70.455±1.78

n = 3 and Results are in Mean±Standard Deviation

Table 2: Quantitative phytochemical constituents of *Cucurbita maxima* leaves

Parameter	Amount (mg g^{-1})
Phenol	6.32±0.41
Tannins	0.91±0.01
Saponins	1.26±0.01
Flavonoids	3.21±0.09

n = 3 and Results are in Mean±Standard Deviation

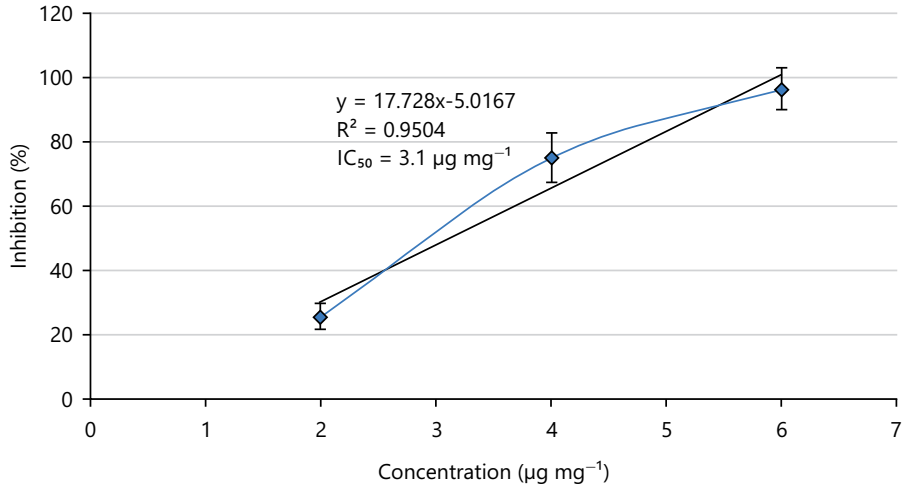

Fig. 1: DPPH free radical scavenging and IC_{50} of *Cucurbita maxima* Leaves

Values plotted are the mean of triplicate observations

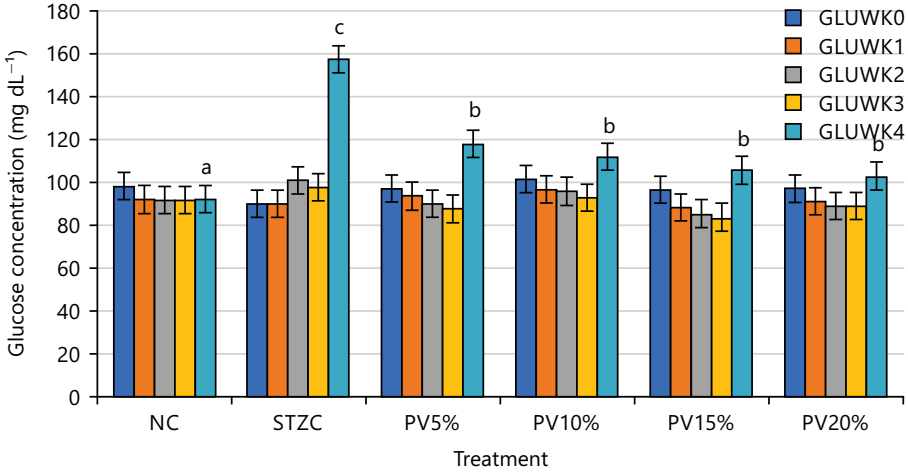

Fig. 2: Preventive Effects of *Cucurbita maxima* Leaves fortified diet on Blood Glucose of STZ-induced Diabetic Rats

n = 5, Results are Mean±Standard Deviation, different alphabet indicates significant difference at $p<0.05$, NC: Normal control, STZC: Streptozotocin control and PV: Preventive treatment

Antioxidant activity of *Cucurbita maxima* leaves: The ability of *Cucurbita maxima* leaves to scavenge DPPH free radicals was estimated by determining its IC_{50} using the linear regression equation in Fig. 1:

$$y = 17.728x-5.0167$$

The free radical scavenging activity of *Cucurbita maxima* leaves increased as the extract doses increased. The IC_{50} of *C. maxima* was 3.1 µg mg^{-1}.

Preventive and ameliorative effects of feed supplemented with *Cucurbita maxima* leaves on blood glucose level in STZ-induced diabetic rats: Figure 2 depicts the weekly mean blood glucose of all rat groups in the preventative therapies from week 0 to week 4. All groups' weekly glucose levels in weeks 0, 1, 2 and 3 were not significantly different ($p>0.05$). However, the STZ control group had a significantly ($p<0.05$) higher mean weekly blood glucose value in week 4 than the groups that received a *C. maxima* leaves-supplemented diet.

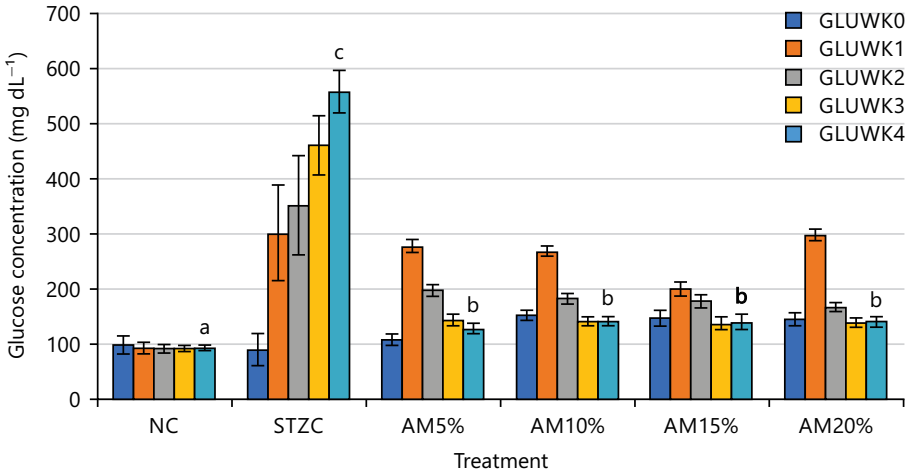

Fig. 3: Ameliorative effects of *Cucurbita maxima* leaves fortified diet on blood glucose of STZ-induced diabetic rats

n = 5, results are Mean±Standard Deviation, different alphabet indicates significant difference at p<0.05, NC: Normal control, STZC: Streptozotocin control, AM: Ameliorative treatment and GLU: Glucose

Table 3: Preventive effects of *Cucurbita maxima* leaves supplemented feed on liver function of STZ-induced diabetic rats

Treatment	ALT (IU L^{-1})	AST (IU L^{-1})	ALP (IU L^{-1})	TB (mg dL^{-1})	DB (mg dL^{-1})	TP (mg dL^{-1})
NC	43.00±8.27^{a}	58.47±7.95^{a}	110.45±3.43^{a}	1.50±0.54^{a}	0.08±0.00^{a}	59.83±5.43^{a}
STZC	109.14±5.91^{c}	114.85±2.25^{c}	226.17±5.14^{c}	5.88±0.06^{c}	4.18±0.01^{c}	33.50±1.63^{c}
PV5%	81.47±10.71^{b}	74.56±3.02^{b}	160.42±27.27^{b}	3.81±0.46^{b}	2.36±0.68^{b}	43.50±0.01^{b}
PV10%	67.54±9.06^{b}	60.32±1.53^{a}	175.36±1.03^{b}	2.45±0.56^{a}	0.50±0.01^{a}	40.75±0.54^{b}
PV15%	62.24±6.74^{a}	69.79±0.60^{a}	155.12±5.11^{b}	2.17±0.03^{a}	0.14±0.02^{a}	43.25±0.06^{b}
PV20%	59.41±7.86^{a}	48.69±5.52^{a}	142.77±9.92^{b}	2.49±0.11^{b}	0.07±0.00^{a}	42.76±0.45^{b}

n = 5, Results are in Mean±Standard Deviation, values with different superscripts down the column are significantly different at (p<0.05), NC: Normal control, STZC: Streptozotocin, PV: Preventive treatment, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, DB: Direct bilirubin and TP: Total protein

Table 4: Ameliorative effects of *Cucurbita maxima* leaves supplemented feed on liver function of STZ-induced diabetic rats

Treatment	ALT (IU L^{-1})	AST (IU L^{-1})	ALP (IU L^{-1})	TB (mg dL^{-1})	DB (mg dL^{-1})	TP (mg dL^{-1})
NC	43.00±8.27^{a}	58.47±7.95^{a}	110.45±3.43^{a}	1.50±0.54^{a}	0.08±0.00^{a}	59.83±5.43^{b}
STZC	109.14±5.91^{b}	114.85±2.25^{c}	226.17±5.14^{b}	5.88±0.06^{c}	4.18±0.01^{b}	33.50±1.63^{c}
AM5%	47.08±5.49^{a}	81.39±10.75^{b}	115.06±7.53^{a}	2.94±0.55^{b}	0.04±0.01^{a}	40.15±0.05^{a}
AM10%	41.39±17.69^{a}	68.47±7.14^{a}	108.61±8.48^{a}	2.53±0.50^{b}	0.15±0.00^{a}	43.53±0.14^{a}
AM15%	53.20±5.80^{a}	55.83±11.05^{a}	102.86±3.11^{a}	2.44±0.52^{a}	0.05±0.00^{a}	45.62±0.54^{a}
AM20%	58.00±12.57^{a}	57.16±10.59^{a}	109.11±10.57^{a}	2.12±0.44^{a}	0.17±0.00^{a}	43.01±0.57^{a}

n = 5, Results are in Mean±Standard Deviation, values with different superscripts down the column are significantly different at (p<0.05), NC: Normal control, STZC: Streptozotocincontrol, AM: Ameliorative treatment, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP = Alkaline phosphatase, TB: Total bilirubin, DB: Direct bilirubin and TP: Total protein

Figure 3 represents the mean blood glucose in the ameliorative treatments from week 0 to 4. The weekly mean glucose levels of the groups did not differ significantly (p>0.05) in week 0. However, the glucose level in the STZ control group increased significantly (p<0.05) from week 2 to 4, whereas the groups that received a *C. maxima* leaves-supplemented diet reduced significantly (p<0.05) from week 2 to 4.

Preventive and ameliorative effects of *Cucurbita maxima* leaves supplemented diet on liver function parameters of STZ-induced diabetic rats: Table 3 reveals that STZ induction did not cause significant (p>0.05) changes in the liver function parameters in the supplemented groups of preventive and ameliorative treatments except for the STZ control group when compared to the normal control group after 28 days of *C. maxima* leaves supplementation.

Table 4 shows a substantial (p<0.05) reduction in ALT, AST, ALP, TB and DB levels in the rats that received a *C. maxima* leaves-supplemented diet compared to the diabetic non-supplemented control group (STZ control). Total protein, on the other hand, increased significantly (p<0.05) in the supplemented groups compared to the diabetic non-supplemented control group.

DISCUSSION

Supplemented diets of *Cucurbita maxima* provided efficient protective and ameliorative properties against streptozotocin-induced diabetes in rat experimental models by restoring glucose metabolism anomalies and normalizing liver function parameters. All the experimental rats fed with diets supplemented with *C. maxima* demonstrated significant reversal of the biochemical parameters evaluated which was comparable with normal control. On the other hand, the negative control showed significant variations in all the biochemical indices measured when compared with the normal control. The study therefore provided possible agents for the management of diabetes and its associated risks such as distortion of the liver function.

The adverse effects of synthetic medications encourage the search for new medicinal sources for metabolic diseases. According to Okur *et al.*[24], herbal medicine has emerged as a promising treatment for effectively managing diabetes. The present research revealed that the *C. maxima* leaf had high amounts of carbohydrates, moisture and fibre. It was consistent with the previous study by Jahan *et al.*[25]. *Cucurbita maxima's* edible sections may aid a person in consuming the appropriate amount of micronutrients daily[26].

Bioactive components of plants could be used to treat oxidative stress, chronic illnesses and aging. Nutraceuticals of plant origin, often known as phytochemical compounds, have been suggested by Cicero and Colletti[27] as a potential treatment for metabolic syndrome. The phytochemicals and their effects on diabetes and associated consequences were also listed in the report. A preliminary phytochemical study was conducted to determine the presence of flavonoids, phenols, tannins and saponins in the powder of *C. maxima* leaves. Phenols and flavonoids exhibit antioxidant, antibacterial, anti-inflammatory, anti-diabetic and anti-cancer properties[28].

The risk of metabolic syndrome and its associated complications, such as type 2 diabetes, may be reduced by consuming phenolic substances such phenolic acids and flavonoids. However, little is known about the processes through which the various classes of phenolic chemicals protect illness[29].

By using the radical DPPH (2,2-diphenyl-1-picrylhydrazyl), which transforms into a stable diamagnetic molecule[30], it is possible to measure the strength of free radicals that are created by DPPH[31]. Results on IC_{50} showed that, *C. maxima* leaf supplemented diet demonstrated antiradical activity. Increasing antiradical activity is indicated by the smaller IC_{50} value[32].

According to Nasr *et al.*[33], oxidative stress is caused by oxidant formation and relates to diabetic problems. In the current investigation, albino rats were given an intraperitoneal injection of STZ at a dosage of 45 mg kg^{-1} body weight. According to Sunil *et al.*[23], streptozotocin (STZ) is frequently used to induce diabetes in animal models and a single STZ injection can cause diabetes by damaging Langerhans Islets in the pancreas. Streptozotocin specifically recognizes the abundantly present glucose transporter two receptors on plasma membranes of cells[23].

The blood glucose levels of all diabetic rat groups receiving preventative and ameliorative therapies were considerably ($p<0.05$) higher than those of the negative control rat group. Streptozotocin administration results in insulin suppression, followed by increasing blood glucose excessively which agreed with the observation of Damasceno *et al.*[34].

Results in this study showed that in the preventative and ameliorative treatments, the *C. maxima* leaf supplementation diet in diabetic rats lowered the blood glucose levels significantly ($p<0.05$) when compared to the diabetic control rat group that had not received the *C. maxima* leaf supplemented diet. The drop in blood glucose levels in STZ-induced diabetic rats provided with a supplemental diet containing *C. maxima* leaf shows the hypoglycemic effect of the feed additions. The *C. maxima* leaf

supplementation may have an anti-diabetic impact because the phytochemical compounds might have an insulin-like effect or they cause the pancreatic islet β-cells to secrete more insulin after consuming the diet-supplemented *C. maxima* leaf[35].

Persistent hyperglycemia and insulin insufficiency or resistance usually damage liver and renal tissue[36]. The standard liver function parameters for identifying liver disease in clinical practice are bilirubin, ALT, AST and ALP. Bilirubin is usually processed in the liver as a by-product of heme catabolism and expelled from the body. The liver's reduced function causes an increase in bilirubin in the blood. Albumin gene transcription is decreased by insulin insufficiency, which lowers serum albumin levels[37]. According to Saleh *et al.*[38], liver injury is indicated by high levels of bilirubin, ALT, AST and ALP and low levels of albumin and protein in the blood.

In the current investigation, hepatocyte injury was indicated by a substantial rise in liver function parameters ($p<0.05$) in the serum of the STZ-induced diabetic control rat group which collaborated with the report of Pulivarthi *et al.*[39]. According to Al-Bahrani[40] and Saleh *et al.*[38], high levels of these enzymes in the serum of STZ-induced diabetic rats represent hepatocyte damage. Streptozotocin specifically recognizes glucose transporter 2 receptors on the cell plasma membrane[23] and these receptors are also present on the liver and kidney cell membranes[41]. Therefore, STZ induction in animal models may also interfere with hepato-renal function[41].

When compared to the diabetic control rat group, the levels of bilirubin, ALT, AST and ALP in the serum of STZ-induced diabetic rats who received a diet supplemented with *C. maxima* leaves were reduced significantly ($p<0.05$). Compared to the diabetic control rat group following treatment with a supplemented diet, the serum levels of the liver enzymes trended downward to normal levels, which may be attributed to cell membrane stability and cellular regeneration brought about by the interaction of bioactive components in the plant material with the cellular receptors[42]. These findings show that plant-based supplements have hepatoprotective properties.

The results suggest that *C. maxima* inhibits STZ-induced hyperglycemia and hepatotoxicity in diabetic rats and that these effects may be mediated by interacting with multiple receptors to raise the levels of antioxidant enzymes in the system. However, the actual mechanism of biological activities of the plant's active components was not elucidated in the present study and could be a limiting factor in harnessing the full potential of the plant.

CONCLUSION

The *Cucurbita maxima* leaf is abundant in phytochemicals. It has potential antihyperglycemic activity and can protect against and treat biochemical changes related to diabetes. The current study provided scientific support for the traditional use of *C. maxima* leaf as a blood glucose-lowering plant. Further study to evaluate the precise mechanism of the active constituents of *C. maxima* leaf, determine various bioactive compounds in the *C. maxima* leaf and isolate active compounds responsible for lowering blood glucose should be considered.

SIGNIFICANCE STATEMENT

Diabetes is yet to have a permanent cure and therefore often poses a severe burden to the patient and society in general. On this premise, *Cucurbita maxima* were investigated for antidiabetic properties to increase the horizon of the available treatment options for diabetes. Supplemented diets of *Cucurbita maxima* provided efficient protective and ameliorative properties against streptozotocin-induced diabetes in rat experimental models by restoring glucose metabolism anomalies and normalizing liver function parameters. Hence, the study has added possible agents in the management of diabetes and its associated risks such as distortion of the liver function.

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