

Phytochemical profiling and In-vitro antioxidant activity of In-vivo α - amylase and α - glucosidase inhibitory activities of kidney *Costus spicatus* extract in diabetic Albino wistar Rats

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Abstract

The study found that *C. spicatus* extract had a beneficial effect on body weight and blood glucose levels in diabetic rats. Additionally, the extract contained polyphenol compounds with antioxidant, enzyme inhibition, and antiglycation activity. The blood glucose levels of the experimental groups were measured over a 28-day period, and the lipid profile was also assessed. The important result of this study is that the plant extract from *C. spicatus* was able to recover the antioxidant activity parameters, as well as improve the activity of glycolysis enzymes in the kidney of diabetic rats. This suggests that the plant extract has potential as a natural remedy for diabetes and its complications. Diabetic control + *C. spicatus* 500 mg/kg at $0.99 \pm 0.05ab$, Pyruvate kinase as a normal control at $20.01 \pm 0.05a$ and $14.41 \pm 1.2c$, and Glucose-6-Phosphatase Dehydrogenase as a normal control at $0.52 \pm 0.02a$ and Diabetic control + *C. spicatus* 500 mg/kg at $0.66 \pm 0.05c$. The study found that the *C. spicatus* leaf extract was effective in improving the antioxidant activity of important parameters such as SOD, CAT, GPx, and GR, and protected against hyperglycemia and oxidative damage in type 2 diabetes-induced hepatorenal functions both *in vitro* and *in vivo*. The kidney glycolysis enzymes, Hexokinase, Pyruvate kinase, and Glucose-6-Phosphatase Dehydrogenase, also showed improvement in the experimental group. Overall, the *C. spicatus* leaf extract proved to be effective in protecting against hyperglycemia and oxidative damage in type 2 diabetes-induced hepatorenal functions in both *in vitro* and *in vivo* studies.

1. Introduction

Currently, nanotechnology is a highly recognized and dynamic area of research that focuses on creating Diabetes Mellitus (38). This number is expected to increase to 578 million by 2030 and 700 million by 2045. One of the severe complications of diabetes is diabetic nephropathy, which damages the small blood vessels in the kidneys and can lead to kidney failure. It is currently the primary cause of end-stage renal disease (ESRD) (60). The human body activity of α -amylase and α -glucosidase measuring body weight loss of hypoglycaemia half inhibitory (20, 6). "In diabetic rats, the use of ZnO nanoparticles resulted in a significant decrease in blood glucose levels and an increase in glycogen levels (7) [Figure:1].

In diabetic rats, the effects of hydrogen bonding, cyaniding-3-glucoside, and delphinidin-3-glucoside on the activity of α -amylase and α -glucosidase were compared using potential extraction powder (20). To defend the body against the harmful effects of reactive oxygen species (ROS), the antioxidant system functions as a complex network of enzymes and non-enzymatic molecules that work together (47). To evaluate the effects of hypoglycemia, etc., in diabetic rats, measurements of glucose, glutathione, triglycerides, HDL, LDL, and other relevant biomarkers in their serum would be necessary (28, 40). Non-enzymatic glycation, also referred to as the Maillard reaction, is a process where sugar molecules bind to proteins, lipids, and nucleic acids without the involvement of enzymes. (14, 48). AGEs (Advanced Glycation Endproducts) are molecules that result from the binding of glucose to proteins or lipids in the body. It is believed that they contribute to the development of microvascular complications in diabetes, such as neuropathy, nephropathy, and retinopathy (30). Increased administration of IM6E has the potential to reduce the risk of diabetic complications, including nephropathy, retinopathy, neuropathy, and

cardiovascular diseases, by enhancing glucose metabolism and decreasing oxidative stress. Moreover, IM6E could also lower the risk of hypoglycemia by increasing (3). Interventions in diet, such as an increase in the consumption of antioxidants, reduction in saturated fat intake, and an increase in fiber intake, can aid in lowering oxidative stress and enhancing β -cell function (46, 6). This study focused on the antidiabetic properties of the medicinal plant *Costus spicatus*. The α -amylase and α -glucosidase inhibitory activities, as well as hepatorenal alterations, were examined *in vitro* and *in vivo* using albino Wistar rats with induced diabetes.

2. Material and methods

2.1. Chemicals and reagents

The chemical Streptozotocin (STZ) was purchased from Himedia Limited and Sisco Research Limited in India, while Glibenclamide was procured from Sigma-Aldrich. Hematoxylin & Eosin stain, DPX, ethanol, Ethylene Diamine Tetra Acetic Acid (EDTA), and methanol were purchased from local markets in Chennai and Thanjavur in India. Different diagnostic kits were obtained from ERBA Diagnostic Mannheim (TRANSASIA Bio-medicals Ltd. Solan).

2.2. Fresh sample collection and authentication

Costus spicatus, a perennial herb belonging to the Costaceae family, was grown in the Saliyamangalam (Post) area of Thanjavur (Dt), Tamil Nadu. The plant sample was identified and authenticated by Rev Dr S. John Britto SJ, Director of The Rapinat Herbarium and Centre for Molecular Systematics at St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India. A voucher specimen (No. SAM 001) was deposited at the herbarium of St. Joseph's College for future reference [Figure:2].

2.3. Preparation and extraction of phytochemical analysis

The phytochemical analysis of *Costus spicatus* leaf powder was carried out using 500 g of the plant material and the Soxhlet apparatus. Ethanol was used as the solvent for extraction, and each extracted sample was individually filtered using Whatman No. 41 filter paper. The extract was then condensed using a rotary evaporator, and the obtained crude extract was used for further analysis of tannins, flavonoids, alkaloids, steroids, and polyphenols following standard methods (18, 53).

2.4. Ethical statement and animal maintenances

Albino Wistar rats were fed a standard laboratory diet and provided with tap water *ad libitum*. Animal experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC) and were registered under Reg. No: 685/PO/Re/S/2002; dated 21st August 2002 and KMCRET/Ph.D/22/2018-19. Male Albino Wistar rats of different age groups with similar body weight ranges of 180 to 250g were housed under controlled laboratory conditions of light, humidity, and standard temperature in groups of six rats for a total of five groups.

2.5. *In-vivo* studies

2.5.1. Acute oral toxicity studies

The acute toxicity are guidelines studies was Organization for Economic Cooperation and Development [OECD] 420 with a few amendments (37). The animals were fasted overnight and then randomly divided into five groups: a healthy control group, a diabetic control group, a diabetic group treated with glibenclamide (5mg/kg) as the standard, and two diabetic groups treated with different dosages of *C. spicatus* leaf extract (300 mg/kg and 500 mg/kg) via oral gastric gavage. The animals were observed for any physical symptoms of toxicity such as alterations in their beha

avior, respiration, grooming habits, lethality, and diarrhea for up to 14 days, with examinations conducted at one hour and then every 24 hours for the next 5 hours.

2.5.2. Subchronic oral toxicity studies

The subchronic toxicity studies was [OECD] respected Dose for the 28 days, with a few changes (37). The study involved five groups of six animals, including the Healthy control, Diabetic control, diabetic + Glibenclamide (5mg/kg) standard, diabetic + rhizome extract (300 mg/kg), and diabetic + rhizome extract (500 mg/kg). The healthy control group was administered with water every morning for 28 days, while the other groups were given *C. spicatus* plant extract in doses of 300 and 500 mg/kg via oral gavage. The animals' body weight was recorded daily, and their blood glucose levels were measured weekly by obtaining blood from the tail vein using a suitable glucometer.

2.6. Experimental design Induction and confirmation of diabetes

In the study, male Wistar albino rats were used and divided into six groups with six rats in each group. Group 1 received citrate buffer solution (normal), group 2 received diabetic control, group 3 received diabetic + Glibenclamide (5 mg/kg) standard treatment for 4 weeks, group 4 received diabetic + rhizome extract (300 mg/kg), and group 5 received diabetic + rhizome extract (500 mg/kg). All experimental animals were given ketamine chloride (24 mg/kg) and were overnight fasted before blood collection without EDTA. After separating plasma serum, biochemical estimation was performed. At the end of the experimental phase (28 days), the animals were sacrificed and blood glucose levels were assessed from blood collected in test tubes containing EDTA. Insulin levels in plasma were isolated from the collected kidney and washed with iced-cold saline. The chemicals were stored in 10% formalin for further evaluation.

2.7. Collectionn of Serum biochemical kits

The kidney function test including Vizalanine Aminotransferase [ATL], Aspartate Aminotransferase [AST], and Alkaline Phosphatase [ALP], and renal function test of [Creatinine, urea, and uric acid] were commercially procured from Himedia limited and Sisco Research Limited, India. Lipid profiling kits for

Triglyceride (TG), cholesterol (CHOL), and low- and high-density lipoprotein kits (LDL and HDL) were also purchased from Himedia limited and Sisco Research Limited, India. Kits for the estimation of total protein content, albumin, bilirubin, and electrolytes such as sodium (Na⁺) and potassium (K⁺) were also purchased from the same suppliers (33).

2.8. Antidiabetic enzymatic assays

2.8.1. α-amylase inhibitory assay

The potential inhibitory activity of the *C.spicatus* plant extract against α-amylase (Sigma-Aldrich, United States) was assessed using a specific protocol. (23). The *C.spicatus* leaf extract (100 µL prepared in 3% dimethyl sulfoxide) was added to 100 µL of α-amylase solution (0.1 mL prepared in pH 6.9 phosphate buffer solution) and incubated at 37°C for 30 minutes. The reaction was stopped by adding 200 µL of DNS reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. The absorption was measured at 540 nm using a spectrophotometer. Acarbose was used as a positive control, and the α-amylase inhibitory activity was expressed as the percentage of inhibition, which was calculated using the following equation.

$$\text{Inhibition (\%)} = [(AC-ACb)-(AS - ASb) / (AC-ACb)] \times 100 \quad (1)$$

F_s and F_{sb} represent the fluorescence intensity of the mixture and mixture blank (without fructose), respectively. F_c and F_{cb} correspond to the fluorescence intensity of the control mixture and control blank mixture, respectively.

2.8.2. α-glucosidase inhibition *C.spicatus* ethanol extract

The inhibitory effect of *C.spicatus* ethanol extract against α-glucosidase was evaluated by adding 30 µL of each sample prepared in dimethyl sulfoxide [3%] to 20 µL of sodium phosphate buffer solution [pH = 6.7] containing 0.1 mL of α-glucosidase, followed by incubation at 37°C for 10 mins. Then, 40 µL of pNPG solution [1mM] was added to the mixture and incubated at 37°C for 30 mins. Next, 0.1 M NaCO₃ solution was added, and the absorbance was determined using a spectrophotometer at 405 nm. Acarbose was used as a positive control (23).

The inhibitory activity was expressed by % inhibition according to the formula.

2.9. The collection of organs

The albino wistar rats animals organs kidney collected from the biochemical parameters (32).

2.9.1. Histological preparation

The kidney organs were harvested and washed with normal saline solution, and then fixed in Bouin's fluid. After dehydration with graded series of ethanol solutions, the kidney tissues were cleared with xylene and embedded in paraffin wax. Tissue sections of 4–5 µm thickness were cut with a rotary microtome (Leica

RM 2125-RT 5, Biosystems Nussloch GmbH), stained with Hematoxylin and Eosin, mounted with DPX (Dibutyl phthalate Polystyrene Xylene), and examined under a light microscope (purchased from Himedia Limited, Sisco Research Limited, India) for histological analysis.

2.10. Antioxidant enzyme assays

The enzymatic antioxidant activity was determined using spectrophotometry. The activity of antioxidant enzymes, including Superoxide Dismutase (SOD), Glutathione peroxidase (GPx), Glutathione Reductase (GR), and Catalase (CAT), was measured using the methods described (15).

2.11. Oxidative stress markers

2.11.1. Determination of hydrogen peroxide (H₂O₂) level

The determination of H₂O₂ levels, an oxidative stress marker, was performed by crushing cells in a 0.1% trichloroacetic acid solution [TCA]. The homogenate was then centrifuged at 12000g for 15 minutes at 4°C, and the resulting supernatant was mixed with 10 mM phosphate buffer solution [pH 7.0] and 1 M potassium iodide solution. The solution was kept in the dark for 1 hour, and the absorbance was measured at 390 nm. H₂O₂ concentrations were calculated using a standard curve and following established methods (15, 42).

2.11.2. Determination of malondialdehyde [MDA]

The measurement of lipid peroxidation as MDA content was performed by using thiobarbituric acid [TBA] with slight modifications under different conditions. The cell homogenate was mixed with a solution containing 20% trichloroacetic acid and 0.67% TBA, and then heated at 95°C for 1 hour. After cooling, 1 mL of n-butanol solution was added to the mixture, which was then centrifuged at 12,000 rpm for 10 mins. The absorbance of the organic supernatant was measured at 532 nm using standard methods (39).

2.11.3. Carbohydrates metabolites enzymes

The hexokinase assay was performed using pyruvate kinase, and the glucose-6-phosphate dehydrogenase was determined according to standard methods (11).

2.11.4. Determination of protein content

The sample was total protein content for the BSA as a protein following standard methods (20).

Analysis of statistical tools

"The experimental results contain the mean ± S.E. for each group with six animals. The results were analyzed using SPSS [IBM version 20.0] and Graphpad Prism 0.9 software tools. The groups were compared using One-way ANOVA analysis of variance followed by a Student's t-test, with statistical significance set at P < 0.05."

3. Results and Discussion

3.1. Secondary metabolites from ethanol extracts *C.spicatus*

The qualitative analysis of phytochemicals in the ethanolic leaf extract of *C. spicatus* showed the presence of major secondary metabolites, including bioactive compounds such as tannins (anti-inflammatory), alkaloids (anticancer and antidiabetic), flavonoids (anti-microbial and antioxidants), steroids (anti-bacterial), and polyphenols (antimicrobial). These bioactive compounds exhibit various properties, including anti-inflammatory [Table:1].

Table:1.Phytochemical analysis of (Secondary metabolites) ethanol solvent leaf extract

.NO	Secondary metabolites	Ethanol
	Tannin	+
	Alkaloids	+
	Flavonoids	+
	Steroids	+
	Poly-phenol	+

(+) indicates activity and (-) sign indicates low activity

The non-polar nature of hexane makes it unsuitable for extracting polar compounds such as tannins and steroids. However, it is effective in extracting non-polar compounds such as alkaloids, flavonoids, and polyphenols, which are present in *Costus* species (22). The secondary metabolites of *Pergularia tomentosa* and *Silybum marianum* mainly consist of phenolic compounds such as flavonoids, tannins, and phenolic acids. These compounds have been reported to possess antioxidant, anti-inflammatory, antimicrobial, and biochemical properties (52, 4, 9). The secondary phytoconstituents of *C. ingenus* were found to be polyphenols, which showed high concentration and potential antimicrobial properties (51, 34). Secondary metabolites phytochemical analysis using to good activity natural sources of plant *C.spicatus* [Insuin plant] human organic and In-organic elements human bodyes.

3.2. Acute oral toxicity studies

Based on the statement provided, an acute oral toxicity study was conducted on five animals using *C.spicatus* leaf extract. The highest concentration of the extract used was 500 mg/kg, and the animals experienced mild diarrhea during the initial 24-hour period. However, there were no signs of toxicity or lethal reactions observed in animals treated with lower doses of the extract.

3.2.1. Blood glucose levels studies [Sub -chronic oral toxicity]

The study measured the blood glucose levels and body weight changes in experimental animal groups treated with different concentrations of *C. spicatus* leaf extract on days 7, 14, 21, and 28 compared to day 0. The results showed that the extract caused a significant decrease in body weight gain in all treated animals compared to untreated animals. However, there was no significant effect on blood glucose levels observed in the treated animals after 4 weeks of the experiment. These findings suggest that the *C. spicatus* leaf extract may have a potential weight-reducing effect but does not have a significant impact on blood glucose levels [Table:2].

Table:2. Blood glucose level of experimental groups on days 0, 7, 14, 21, and 28 of the treatment in the sub chronic study.

Treatment groups	Change in Blood glucose level (mg/dl)				
	0 day	7th days	14th days	21th days	28th days
1 st	134 ± 08	132 ± 07	130 ± 08	134 ± 3	131 ± 6
II nd	129 ± 10	130 ± 7	131 ± 14	130 ± 9	132 ± 10
III rd	127 ± 8	132 ± 9	131 ± 12	132 ± 10	132 ± 15
IV th	129 ± 12	133 ± 4	131 ± 16	130 ± 8	131 ± 07
V th	121 ± 07	122 ± 8*	119 ± 7*	117 ± 15*	115 ± 9*

3.3. Antidiabetic animals lipid profile experimental *C.spicatus* Leaf extract

"The antidiabetic effect of *C. spicatus* leaf extract was observed on lipid profiling in diabetic albino Wistar animals, showing a decrease in hyperglycemia activity. Total cholesterol (TC) was reduced in diabetic + *C. spicatus* leaf extract animals compared to normal, with values of 131 ± 02 and 120 ± 09, respectively. Low-density lipoprotein (LDL) was also reduced in diabetic + *C. spicatus* leaf extract animals, with values of 65 ± 02 compared to 75 ± 8 in normal animals. Very-low-density lipoprotein (VLDL) was reduced in diabetic + *C. spicatus* leaf extract animals, with values of 131 ± 08 compared to 119 ± 7. Triglycerides (TG) were reduced in animals treated with *C. spicatus* leaf extract, with values of 79 ± 05 compared to 63 ± 15. High-density lipoprotein (HDL) was increased in animals treated with *C. spicatus* leaf extract, with values of 131 ± 06 compared to 131 ± 01 in healthy animals. However, the selected dose of *C. spicatus* leaf extract at 500 mg/kg resulted in a statistically significant decrease in HDL levels in treated animals compared to healthy animals ($P < 0.005$) [Table:3].

Table:3. Lipid profile of experimental groups in the sub chronic toxicity study.

Treatment groups	Lipid profile (mg/dl)				
	Total cholesterol	LDL	VDL	Triglycerides	HDL
1 st	131 ± 02	65 ± 02	131 ± 08	79 ± 05	131 ± 06
II nd	129 ± 10	64 ± 03	130 ± 14	76 ± 12	132 ± 07
III rd	126 ± 02	66 ± 02	130 ± 12	77 ± 07	132 ± 05
IV th	125 ± 17	63 ± 07	129 ± 16	77 ± 04	131 ± 01
V th	120 ± 09	75 ± 8*	119 ± 7*	63 ± 15*	115 ± 07*

Data were expressed as the mean ± standard deviation, n = 6. *p < 0.05

The hypoglycemic effect of *Sambucus wightiana* plant extract was tested on induced diabetic rats at different doses of total alkaloids (50 mg, 100 mg, 200 mg, and 500 mg) for 28 days [Figure:2].

The body weight and food intake of test group animals improved. Administration of TASW reduced the levels of triglycerides, cholesterol, and LDL while increasing HDL and Hb levels (27, 19). The α -glucosidase energy metabolites hydrolyze in the terminal non-reducing 1–4 linked α -glucose to release molecules, which directly reflects the ratio of LDL and VLDL cholesterol to HDL cholesterol level. In the DC, DE, NC, and NE groups, the ratio was 2.8, 0.96, 0.47, and 0.26, respectively. The atherogenic index of the NE group was lower than that of the NC group by 13% and 43%, respectively, in diabetic rats (2). The hypoglycaemia activity of phenolic compounds enzymes inhibitory and antiglycation activity of plant *Oxalis pes-caprae* flower extraction compard to control and diabetic of 500 mg good sources activity. (24). The phtochemical analysis of *S. venulosa* leaf extraction active compunds present in antihyperglycemia activity of diabetic 500 mg normal and diabetic animal rats 28 days (54). Administration of plant rhizome extract at a dose range of 100–300 mg/kg improved the total protein content and body weight in three groups of diabetic rats. Similar results were reported with the administration of 300 mg/kg of the extract in rats (35). In another study, rejuvenation in various parts, such as sinusoidal spaces and nucleus, was observed (16). The present study showed that the activity of flavonoids and polyphenols can be correlated to the improvement in histoarchitecture and recovery in diabetic animals (5). It has been reported that polyphenols have the potential to reduce oxidative stress due to several mechanisms, such as inflammation reduction and fatty acid oxidation (55). Polyphenols also prevent hepatic fibrosis (25), A dose range of 100–300 mg/kg did not show lethal activity in animals (50). Diabetic rats have been shown to have kidney nephropathy (1), and diabetic animals show an increase in the levels of various markers, such as uric acid, urea, and creatinine (59). The dose of *C. spicatus* drug at 500 mg/kg can be compared to that of an antidiabetic drug.

3.4. Antioxidant enzymes of ethanolic extract of *C.spicatus*

The mechanism of antioxidant activity of vitamin C in the treatment of diabetes mellitus (DM) is through its intracellular action (49, 21). This defense mechanism relies on the presence of antioxidant enzymes, such as superoxide dismutase (SOD), catalases (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), which catalyze the decomposition of reactive oxygen species (ROS) and maintain redox homeostasis. SOD is considered the primary barrier against ROS, as it catalyzes the conversion of superoxide anion ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) and oxygen (23, 45). The GR enzyme is a homodimeric protein with a molecular weight of approximately 100 kDa, and each subunit is composed of two domains: a small N-terminal domain and a large C-terminal domain containing the active site of the enzyme(26, 44). DM2 is linked to enhanced pro-inflammatory cytokine production, including IL-1 β , TNF- α , and consequently an increased risk of endothelial dysfunction and development of atherosclerosis (10). The interaction of H_2O_2 with free metals generates hydroxyl radical ($\bullet OH$), the most reactive and hazardous radical (8, 36). PK is the enzyme involved in the last step of glycolysis, as it catalyzes the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP, resulting in the production of pyruvate and ATP (23). In line with these findings, we analyzed the activities of the four antioxidant enzymes in normal mice, untreated diabetic mice, and diabetic mice treated with either 10 mg/kg of glibenclamide, 150 mg/kg of OPC ME, or 250 mg/kg of OPC ME (57). The results obtained in the present study demonstrated that the activities of antioxidant enzymes, including SOD, CAT, GPx, and GR, present in the kidney, decreased significantly in the diabetic mice group compared to the normal mice group. Our results are consistent with several studies that have indicated that the activities of these enzymes are significantly decreased in diabetic patients [Table:4].

Table :4. Antioxidant enzymes in Streptozotocin induced diabetic albino wistar rats of C.spicatus

Antioxidant enzymes Kidney					
Parameters $\mu\text{mol min}^{-1}\text{mg}^{-1}\text{prot}$	Normal control	Diabetic control	Glibenclamide 05 mg/kg	Diabetic control + <i>C.spicatus</i> 300 mg/kg	Diabetic control + <i>C.spicatus</i> 500 mg/kg
SOD	07.89 $\pm$ 0.73a	01.3 $\pm$ 0.14b	06.67 $\pm$ 0.02c	5.75 $\pm$ 0.31c	03.62 $\pm$ 0.01d
CAT	16.37 $\pm$ 0.66a	08.71 $\pm$ 0.16b	10.89 $\pm$ 0.45c	10.22 $\pm$ 0.62c	9.61 $\pm$ 0.31bc
GPx	20.34 $\pm$ 0.12a	18.87 $\pm$ 0.18b	19.81 $\pm$ 0.12b	19.51 $\pm$ 0.09c	19.27 $\pm$ 0.25c
GR	15.69 $\pm$ 0.49a	10.36 $\pm$ 0.79b	14.52 $\pm$ 0.61c	13.68 $\pm$ 0.37c	13.61 $\pm$ 0.16c

All the data have been expressed in mean $\pm$ SEM. SOD: superoxide dismutase. CAT: Catalase. GPx: glutathione peroxidase. GR: glutathione reductase. Different letters in the line indicate significant differences among treatments at $p < 0.05$.

The presence of polyphenols and flavonoids in the *Costus spicatus* extract may be responsible for the reactivation of enzymes involved in glucose metabolism in diabetic rats. However, the extent of restoration varied depending on the concentration of phenolic compounds, the type of enzyme, and the organ studied. Therefore, further research is required to investigate the specific molecular mechanisms underlying the antidiabetic effect of the extract and to isolate its bioactive compounds.

3.5. Carbohydrate metabolic enzymes ethanol extract of *C.spicatus*

We conducted a study to investigate the potential antidiabetic effects of *C. spicatus* leaf extract by examining its impact on the activity of enzymes involved in carbohydrate metabolism, such as hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), and pyruvate kinase (PK). HK is the first enzyme of glycolysis that phosphorylates glucose into glucose-6-P, while G6PD catalyzes the initial step of the pentose phosphate pathway [78 PK, on the other hand, is the enzyme involved in the last step of glycolysis, facilitating the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP, ultimately producing pyruvate and ATP (23, 17). The administration of OPC ME and glibenclamide to diabetic mice led to a significant increase in the activities of HK, PK, and G6PD. The restoration of carbohydrate enzyme activities can be attributed to several factors, such as the reduction of blood sugar levels resulting from the inhibition of α -amylase and α -reductase by OPC extracts, protection against damage to insulin-secreting pancreatic β cells, and modulation of insulin signaling pathways (12). Therefore, the reactivation of the studied enzymes could be linked to the presence of polyphenolic compounds in *C. spicatus* extract, which regulate glucose metabolism in diabetic mice. However, the level of restoration of enzymatic activities varies based on the concentration of phenolic compounds in the extract, the type of enzyme, and the organ studied. Thus, further research is necessary to explore the precise molecular mechanism of *C. spicatus* extract for its antidiabetic effect and to isolate the bioactive compounds responsible for this effect [Table:5].

[Table:5]. Glycolysis enzymes Kidney *C. spicatus* extract

Glycolysis enzymes Kidney					
Parameters $\mu\text{mol min}^{-1}$ $\text{mg}^{-1} \text{ prot}$	Normal control	Diabetic control	Glibenclamide 05 mg/kg	Diabetic control + <i>C.spicatus</i> 300 mg/kg	Diabetic control + <i>C.spicatus</i> 500 mg/kg
Hexokinase	1.10 $\pm$ 0.05ac	0.84 $\pm$ 0.02b	1.21 $\pm$ 0.07c	1.01 $\pm$ 0.06a	0.99 $\pm$ 0.05ab
Pyruvate kinase	20.01 $\pm$ 0.05a	3.23 $\pm$ 0.05b	14.64 $\pm$ 0.54c	19.35 $\pm$ 1.03a	14.41 $\pm$ 1.2c
Glucose-6- Phosphatase Dehydrogenase	0.52 $\pm$ 0.02a	0.22 $\pm$ 0.02b	0.48 $\pm$ 0.03a	0.57 $\pm$ 0.07ac	0.66 $\pm$ 0.05c

All the data have been expressed in mean $\pm$ SEM. Hexokinase, Pyruvate kinase, and Glucose-6-Phosphatase Dehydrogenase. Different letters in the line indicate significant differences among treatments at $p < 0.05$.

The number of hydrogen bonds and hydroxyl groups are important factors in determining the α -amylase inhibitory activity of compounds. When compared to the initial blood glucose levels of the diabetic group, a net reduction in hyperglycemia was found after 21 days of treatment with 100 mg/kg b.w., 200 mg/kg b.w., and 300 mg/kg b.w. IM6E treatments, with reduction percentages of 73.2%, 78.6%, and 86.4%, respectively. The 300 mg/kg b.w. fenugreek seeds caused a 69.4% reduction in overall blood glucose levels of the diabetic rats after 21 days of treatment, which is much lower than the reduced levels (82.3%) caused by the 300 mg/kg b.w. untreated fenugreek sprouts treatment. Interestingly, the combination of 300 mg/kg b.w. of IM6E and 1 mg/kg b.w. voglibose treatment caused a maximum decrease (88.09%) in blood glucose levels of the diabetic group as compared to blood glucose levels observed on day 0. (31). The alpha-amylase inhibition percentage of methanol leaf extracts of the five populations ranged between $21.66 \pm 0.27\%$ and $73.73 \pm 0.70\%$. P1 showed the highest inhibitory activity (73.73%), followed by P5 (71.89%) at a concentration of 6.67 mg mL^{-1} ($p < 0.05$). Hot water infusions of all leaf populations showed a lower ($< 10\%$) alpha-amylase inhibition percentage at a concentration of 3.33 mg mL^{-1} (29). Therefore, this can be a valuable tool for pharmacologic assessment of anti-diabetic foods and drugs because they directly cause irreversible damage to β -cells of pancreatic islets of Langerhans, resulting in degranulation and loss of insulin secretion (58). The IM6E treatments significantly reduced the calculated relative area under the glucose concentration curves (AUCs) of OGTT and OSTT as compared to the untreated glucose-loaded normal control group. The study indicates that IM6E possesses appreciable hypoglycemic effects at 300 mg/kg bw and is almost at par with 1 mg/kg bw of voglibose treatment. Previous studies have shown that fenugreek phytochemicals including quercetin, trigonelline, and diosgenin have glucose-lowering effects by targeting the carbohydrate metabolism pathway (56). The decreased blood glucose levels caused due to plant extract treatment may be attributed to the activation of β -cells and granulation returning to normal and insulinogenic effect (13). The underlying biochemical and molecular mechanisms by which wildy growing *H. perforatum* extract its antihyperglycemic and antidiabetic activities in diabetic rats are that treatment of diabetic rats with HH extract resulted in decreased activity of hepatic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase, increased liver glycogen and glucose-6-phosphate content, resulting in reduced blood glucose concentration up to normoglycemia. Non-significant changes were observed in the activity of hexokinase, glycogen phosphorylase, and glucose-6-phosphate dehydrogenase (43). On the seventh day of treatment, the group of diabetic animals treated with Mm (50 mg/kg bw) showed a 74.7% reduction in glucose levels compared to the beginning of treatment, and this hypoglycemic effect was maintained. The results of biochemical and histopathological parameters, as well as TBARS levels, suggest that the dry extract from *M. multiflora* exerts nephron- and hepatoprotective effects. Additionally, the dry extract obtained from the infusion of *M. multiflora* leaves (25 mg/kg and 50 mg/kg bw) exerted a hypoglycemic effect in streptozotocin-induced diabetic mice compared to the acarbose standard. Histopathological examinations of kidney and liver tissue sections, as well as biochemical parameters of renal and liver

enzymes, revealed a beneficial effect of the antioxidant constituents present in this extract by improving or preventing oxidative stress in the liver (41). *C. spicatus* is very rich in plant extracts and natural polyphenols, which have anti-diabetic, anti-inflammatory, anti-apoptotic, and anticancerous activities, making them valuable for controlling diabetes mellitus. Polyphenols can also decrease other metabolic diseases, such as insulin resistance, hyperglycemia, hyperlipidemia, obesity, and diabetes mellitus and associated complications.

4. Conclusion

The administration of *C. spicatus* leaf extract effectively improved various biochemical parameters, including the antioxidant system, hyperglycemia, hyperlipidemia, and histopathological alterations in the kidneys of diabetic model subjects. Additionally, the secondary metabolites present in the leaf extract may increase the total antioxidant activities, exerting defensive effects against free radical pathogenesis in diabetic kidneys. The ethanolic extract of *C. spicatus* significantly reduced both α -amylase and α -glucosidase activities and had a protective effect against protein glycation. Furthermore, the ethanolic extract, when administered at doses of 500 mg/kg, was able to reduce activity of diabetes in Streptozotocin induced diabetic albino wistar rats after 4 weeks of treatment compared to glibenclamide, indicating that the phenolic compounds present in the methanolic extract have significant antihyperglycemic activity. Therefore, these results validate the potency of *C. spicatus* as an antidiabetic agent and suggest that it could serve as an alternative remedy to ameliorate or protect against hyperglycemia, hyperlipidemia, obesity, and diabetes mellitus and associated complications.

CRedit Authorship Contribution Statement

Azhagu Madhavan Sivalingam:- Work Designed, Writing –Original Draft, Writing-Review & Editing, Grammar Checking, Visualization, Methodology, Formal analysis and Investigation. **Arjun Pandian:-** Editing, Formal analysis, Methodology, Visualization. **Sumathy Rengarajan:-** Formal analysis and methodology. **Raju Ramasubbu:-** Funding sources and formal analysis. All the authors give final approval of the version to be submitted.

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Declarations

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Azhagu Madhavan Sivalingam:- Work Designed, Writing –Original Draft, Writing-Review & Editing, Grammar Checking, Visualization, Methodology, Formal analysis and Investigation. **Arjun Pandian:-** Editing, Formal analysis, Methodology, Visualization. **Sumathy Rengarajan:-** Formal analysis and

methodology.**Raju Ramasubbu:-** Funding sources and formal analysis· All the authors give final approval of the version to be submitted.

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No data was used for the research described in the article.

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Competing Interests

Not applicable.

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Figures

(a) Rhizome part of the *Costus spicatus*, (b) Plant identified and herbarium authentication deposited. (c) Plant collected location.

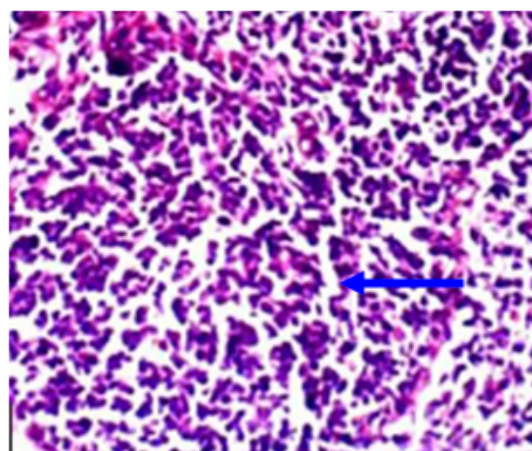
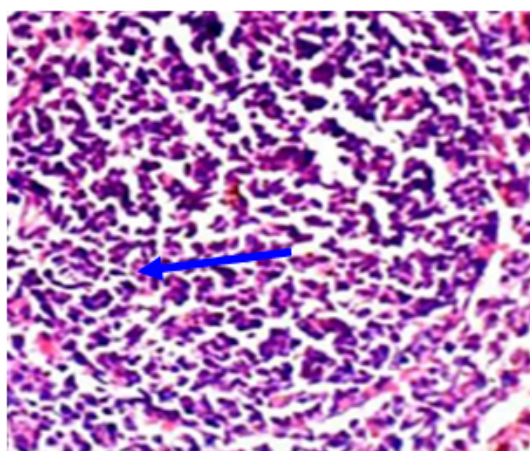
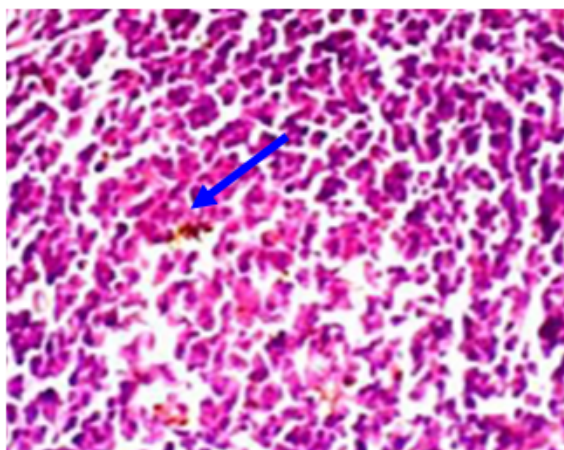
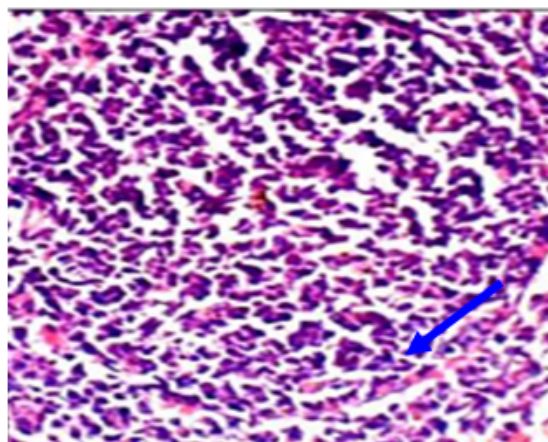
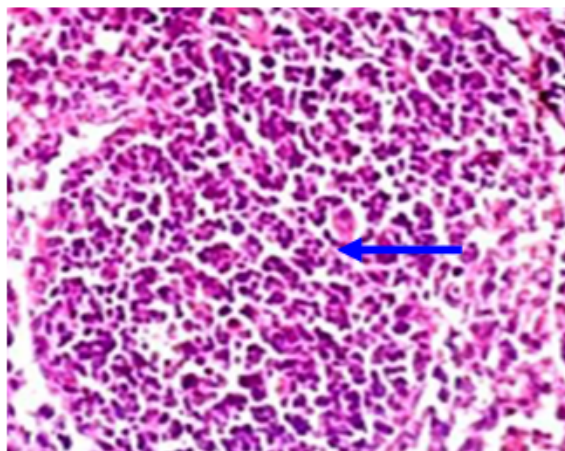


Figure 3

Lipid profile of experimental groups in the sub chronic toxicity study.