# Evaluation of Antidiabetic activity of Costus spicatus flower in wistar albino rat against alloxan induced diabetes

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**Abstract**: *Costus spicatus is a commonly utilized medicinal plant abundant in phytoconstituents such as flavonoid glycosides, terpenoids, tannins, and resins, employed in the management of diabetes. This study sought to assess the hypoglycemic effects of ethanolic extract of Costus spicatus buds (ECSB) given at various dosages over 21 days in alloxan-induced diabetic rats. The administration of the extract at dosages of 100 and 200 mg/kg/bw considerably ($P < 0.001$) enhanced body weight, reduced water and feed consumption, lowered fasting blood glucose levels, and rectified lipid metabolism imbalances in diabetic rats. The ECSB demonstrates antidiabetic properties, with a dosage of 200 mg/kg/bw exhibiting the most effective hypoglycemic action, comparable to glibenclamide, a common hypoglycemic agent. The extract, at all tested dosages, normalized renal function indices and hematological markers in the diabetic rats. This study shown that, along its hypoglycemic effects, ECSB may also safeguard the kidney and blood from damage caused by diabetes. The ECSB's activity is attributed to the abundance of potent antioxidants and anti-inflammatory compounds in the ethanolic extract of Costus spicatus buds.*

***Keywords:*** *Costus spicatus, Antidiabetic, Fasting blood glucose, Hypoglycemia, Alloxan*

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## Introduction

Diabetes significantly contributes to morbidity and mortality in a growing portion of the global population [1]. The World Health Organization predicts a 50% increase in diabetes-related deaths worldwide over the next decade, primarily in developed nations (WHO, 2008) [2]. Diabetes mellitus (DM) is a chronic disorder caused by a genetic and/or acquired deficiency in insulin synthesis by the pancreas, or by the ineffectiveness of the insulin generated [3]. This deficiency causes increased blood glucose levels, which damage various body systems, including the blood vessels and neurons [4].

As the incidence of diabetes rises worldwide, the ailment increasingly accounts for a substantial portion of national and international healthcare costs [5]. It is expected to become a leading cause of disability and mortality worldwide during the next 25 years [6]. Regions with the greatest potential are Asia and Africa, where diabetes mellitus rates could grow two- to thrice compare to current levels [7]. By 2025, India will have the largest population of diabetics worldwide, gaining the designation of the "Diabetic Capital of the World." Alongside current pharmacological therapies, alternative herbal medicines have been proposed for the control of diabetes [8].

Despite considerable progress in understanding and managing diabetes, the incidence of the disease and its related complications continues to increase relentlessly due to numerous shortcomings in its pathogenesis [9]. Simultaneously, the extensive approach of herbal medicine has accelerated global efforts to employ and develop medicinal plants with diverse beneficial effects. Numerous compounds have been evaluated and active substances extracted; yet, the quest for novel antidiabetic therapies continues [9].

*Costus spicatus* Swartz (Costaceae), commonly referred to as “cana-do-brejo,” “canafístula,” or “canarana” in northeastern Brazil, is a medicinal plant indigenous to humid coastal woodlands [10, 11]. The rhizomes of this plant are utilized in traditional medicine as a diuretic [12], a hypoglycemic agent [13], for addressing bladder and urethral ailments [14], and for facilitating the expulsion of kidney stones. An infusion of the aerial components of this plant is administered to alleviate colds, sore throats [15], dysentery, and diarrhea [16]. Flavonol glycosides have been extracted from the leaves and have exhibited inhibitory effects on nitric oxide generation by activated macrophages. Furthermore, the same research demonstrated that polysaccharides extracted from the fresh stems of *C. spicatus* decreased

capillary permeability and exhibited phagocytosis-stimulating effects in rodents [17, 18]. Some flavonol glycosides have been shown to provide an anti-inflammatory effect by inhibiting some macrophage processes associated with the inflammatory process, including nitric oxide generation [10, 19]. Additional results indicate that the plant extract, abundant in flavonoids, exhibits antinociceptive qualities maybe through interaction with the opioid system [20, 10].

Currently, there is no data about the potential antidiabetic effects of *C. spicatus* flower buds in experimental animals. The objective of this study was to assess the antidiabetic effects of the ethanol extract derived from the flower buds of *C. spicatus* in rodents.

## Material and methods

### 2.1. Chemicals and reagents

Alloxan and Glibenclamide were obtained from Sigma, USA. Randox Laboratories Limited of the United Kingdom supplied the assay kits utilized in the biochemical assays. All additional chemicals and reagents utilized were of analytical grade.

### 2.2. Collection of the plant

The flower buds of *Costus spicatus* were collected from the Talakona forest in Chittoor and validated by Dr. Madhav Chetty of Sri Venkateswara University in Tirupati, Andhra Pradesh. After being bench-dried for eleven days, the inflorescence buds were ground into a coarse powder, and then they were stored in polythene bags at room temperature. This was done in order to get them ready for extraction.

### 2.3. Ethanolic extract of *Costus spicatus* buds (ECSB)

The dried inflorescence buds of *Costus spicatus* (300g) were macerated with 1000 ml of ethanol (60-80 °C) for three days at ambient temperature and subsequently filtered [21]. This was carried out twice and subsequently filtered. The filtrate was subjected to distillation via a rotary evaporator and subsequently air-dried to get the ethanol extract. Phytochemical screening was conducted utilizing established protocols, and tests were executed to ascertain the presence of alkaloids, tannins, terpenoids, flavonoids, reducing sugars, anthraquinones, and saponins.

### 2.4. Experimental Animals

Ten-week-old albino Wistar male rats, with a body weight ranging from 180 to 250 grams, were utilized. Animals were maintained under conventional conditions of

temperature (24±2°C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were provided with a regular pellet diet. Animals were managed in accordance with Good Laboratory Practice. Ethical approval was secured by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (Reg no- SECP/2021-22/COL/001).

### 2.5. Groups and experimental protocols

The groups under investigation are Group I received normal saline to healthy albino Wistar rats, Group II was administered alloxan (120 mg/kg, i.p.), Group III and IV were treated with ethanolic extract of *Costus spicatus* flower buds (ECSB - 100 and 200 mg/kg, p.o.), and Group V was given Glibenclamide (10 mg/kg, p.o.). Two doses of ECSB were selected based on the literature, and Glibenclamide is utilized as a standard medication.

### 2.6. Water, Feed, Weight, and Blood Glucose Monitoring

Throughout the 21-day study period, the water consumption, feed consumption, and body weight gain of all the rats were observed. Blood samples were taken every 7 days via retro-orbital plexus puncture, and blood glucose levels were measured using an electronic glucometer (Miles Inc, USA) and glucostix (Bayer Diagnostic India Ltd., Baroda).

### 2.7. Induction of Diabetes and Biochemical analysis

Alloxan monohydrate (120 mg/kg) was administered intraperitoneally to albino wistar rats in a single dose to induce diabetics [22, 23]. Alloxan was initially weighed for each animal based on its weight and subsequently solubilized in 0.2 ml of saline (154 mM NaCl) immediately before injection. Rats exhibiting plasma glucose levels exceeding 200 mg/dl were incorporated into the study two days post-alloxan administration. Treatment with ECSB extracts commenced 48 hours post-alloxan injection. Rats exhibiting glucose levels over 200 mg/dl were chosen for a diabetic investigation and categorized into five groups, each comprising six animals. Group I consisted of normal rats, group II consisted of diabetic rats that had not been treated, group III consisted of diabetic rats that were treated with ECSB 100 mg/kg, orally, group IV consisted of diabetic rats that were treated with ECSB 200 mg/kg, orally, and group V consisted of diabetic rats that were treated with glibenclamide 10 mg/kg, orally. All groups received oral treatment once daily for 21 days. Rats in groups I and II were administered a vehicle. Fasting blood glucose levels were assessed at regular intervals of 1, 7, 14, and 21 days.

Blood samples were collected at weekly intervals until the conclusion of the investigation (i.e., 3 weeks). Fasting blood glucose levels were assessed on days 1, 7, 14, and 21 of the research. On day 21, blood was obtained via heart puncture under moderate ether anesthesia, and serum was isolated for analysis of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. The levels of total protein, creatinine, urea, calcium, and uric acid were assessed.

**2.8. Examination of Total Protein, Lipid Profile, and Kidney Function Tests**

Using an automated analyzer, the serum concentrations of triglycerides, HDL cholesterol, LDL cholesterol, and total cholesterol were measured (Beckman Coulter Inc., Ireland) [3, 5]. Using bovine serum albumin as a reference, the total protein in the serum was calculated. Using Randox Assay kits, the amounts of creatinine, urea, calcium, and uric acid were ascertained in the serum.

**2.9. Estimating the HbA1c level**

After lysis with 5 ml of water, erythrocytes were rinsed with normal saline and then incubated at 37°C for 15 minutes. After centrifuging the contents and discarding the supernatant, 0.5 milliliter of saline was added. After adding 4 milliliters of oxalate hydrochloride solution to 2 milliliters of aliquot, the mixture was heated to 100 degrees Celsius for 4 hours, cooled, and precipitated with 2 milliliters of 40% trichloroacetic acid (TCA) [24]. After centrifuging the mixture, 0.5 ml of the supernatant was added, along with 0.5 ml of 80% phenol and 3 ml of concentrated sulfuric acid. After 30 minutes, the produced color was measured at 480 nm. One percent fructose solution was used to generate working standards (10–50 µg). After 30 minutes, the working standards were refilled with 0.5 ml of 80% phenol and 3 ml of concentrated sulfuric acid, and the reading was taken at 480 nm. The HbA1c concentration was given in milligrams per gram of hemoglobin [25].

**2.10. Histopathology**

For two days, the pancreatic tissue was stored in 10% formalin [26, 27]. Alcohol was used to dehydrate the pancreas for 12 hours at a time, followed by alcohol concentrations of 70, 80, 90%, and absolute. Further, the tissues were cleansed for 15 to 20 minutes with xylene before being infiltrated with paraffin in an automated tissue processing machine. After preparing the tissue blocks, they were sliced into 5 µm-thick pieces using a microtome. The portions were placed on a tiny slide that had been coated with egg albumin, a sticky material, and let to dry. The sections were then stained with hemotoxylin (basic stain) and eosin (acidic stain).

### 2.11. Statistical Analysis

The data were presented as the mean ± standard error of six replicates and underwent one-way analysis of variance (ANOVA) and Duncan's multiple range test to identify significant variations in every parameter. The statistical significance level was set at ***P<0.001, **P<0.01, and *P< 0.05 for the values.

## Results

### 3.1. Phytochemical analysis of ethanolic extract of *Costus spicatus bud*

*Costus spicatus* ethanolic extract contained alkaloids, tannins, flavonoids, saponins, glycosides, and terpenoids, according to a phytochemical examination.

### 3.2. Water/Feed Consumption and Gained Weight

The administration of ECSB dramatically decreased ($P < 0.001$) the amount of water and feed intake in diabetic animals, whereas the untreated diabetic rats (Group II) consumed more water (Table 1). In a similar vein, the untreated diabetic rats displayed polyphagia and ate more feed than the rats in the treatment and control groups. When compared to the control and treatment groups, the weight gain of the untreated diabetic rats was significantly lower ($P < 0.001$). Treatmet with 200 mg/kg body weight of ECSB generally compared favorably to those of glibenclamide, a well-known standard medication for diabetes.

### 3.3. Fasting Blood Glucose Level

Upon completion of the experiment, it was discovered that the continuous administration of ECSB significantly decreased ($p < 0.001$) the fasting blood glucose level in diabetic rats (Table 2). Further, the impact was more noticeable in the rats given 200 mg/kg bodyweight of the ECSB, and it fared better than in the rats given glibenclamide.

### 3.4. Serum Lipid Profile and Total Protein

When comparing the diabetic rats to the control group, there was a substantial increase ($p < 0.001$) in the serum concentrations of LDL, triglycerides, and cholesterol as well as a decrease in HDL and protein (Table 3).After 21 days of treatment, the levels of blood cholesterol, triglycerides, and LDL were dramatically reduced ($P < 0.001$) by the ECSB and glibenclamide, while the concentrations of HDL and protein were elevated to almost normal, as shown in the control group.

### 3.5. Kidney Function Parameters

When comparing the diabetic rats to the control group, there was a substantial increase ($p < 0.001$) in the serum concentrations of LDL, triglycerides, and cholesterol as well as a decrease in HDL and protein (Table 3). After 21 days of treatment, the levels of blood cholesterol, triglycerides, and LDL were dramatically reduced ($P < 0.001$) by the ECSB and glibenclamide, while the concentrations of HDL and protein were elevated to almost normal, as shown in the control group.

### 3.6. Glycated hemoglobin (HbA1c) level assessment

When compared to control animals, diabetic animals' HbA1c levels significantly increased ($p < 0.001$). The HbA1c levels were restored to normal by glibenclamide and ECSB [Table 4].

## Discussion

An indicator of a diabetic state in the animals following alloxan administration is the higher than normal consumption of food and water, together with elevated fasting blood glucose levels and urine output. The results of this investigation showed that the ethanolic extract of *Costus spicatus* buds (ECSB) has antidiabetic activity [18]. Its effectiveness is on par with that of the common hypoglycemia medication glibenclamide.

The plant extract was administered, and the conditions of polydipsia and polyphagia were successfully avoided. Taofik et al. used *Artemisia afra* Aqueous Extract in diabetic rats and obtained similar findings [3]. The untreated diabetic rats consumed a lot of feed and water, but their body weight increased much less than that of the groups who received extracts treatment [28]. The increased metabolic activity of the rats' bodily systems may have contributed to the improvement in body weight in the ECSB-treated group. This demonstrates unequivocally that the extract improved the rats' body weight by increasing glucose metabolism. Once more, Ravi et al. reported same observation. These authors claim that the *Eugenia jambolana* seed kernels caused the diabetic rats' body weight to grow [28]. The fact that the effectiveness of ECSB at a dose of 200 mg/kg body weight contrasted very well with glibenclamide is especially noteworthy.

An essential aspect of the diabetic state is the rise in HbA1c and fasting blood glucose concentrations. On day 21, the glucose and HbA1c levels in the diabetic rats showed a tendency towards normalcy, similar to what was observed in the control

rats, indicating that ECSB had a strong hypoglycemic effect. Flavonal glycosides and terpenoids, which have been shown to have hypoglycemic action in diabetic rats, have been detected by microchemical analysis of ECSB [29]. Therefore, the presence of flavonal glycosides and terpenoids, which may be functioning as a stimulant for the release of insulin following the repair of pancreatic beta cells by the extract, could be responsible for the hypoglycemic action of ECSB shown in this study.

Lipid profile abnormalities are quite prevalent in the diabetes state [30]. While lipoprotein changes are a natural feature of diabetes mellitus, other diabetes-related problems like obesity and kidney illness can also cause these changes [31].

In the current investigation, when compared to the diabetic group that did not get treatment, ECSB was able to lower the levels of LDL, triglycerides, and cholesterol while raising the levels of HDL in diabetic rats to almost normal levels. Diabetes typically results in an increase in serum cholesterol levels, which increases the risk of coronary heart disease [32]. Because insulin inhibits the hormone-sensitive lipase, the abnormally high blood cholesterol concentration associated with diabetes is mostly caused by an increase in the mobilization of free fatty acids from peripheral depots [33]. When ECSB was given to diabetic rats, the plasma cholesterol level dropped dramatically to almost normal, lowering the risk of cardiovascular disease. An elevated risk of myocardial infarction is linked to changes in total cholesterol, LDL cholesterol, and HDL cholesterol concentrations that are seen during diabetes [34]. When diabetic rats were treated with ECSB extract, their levels of HDL and LDL cholesterol increased and decreased, respectively, indicating a lower risk of myocardial infarction. An increasing amount of research from clinical, laboratory, and epidemiological studies suggests that high triglyceride levels are a stand-alone risk factor for cardiovascular disease. Among diabetics, hypertriglyceridemia is a common condition. The fact that the myocardial membrane in this study is intact and not destroyed is indicated by the fact that therapy with ECSB has prevented the increase of triglycerides [35].

Increased protein catabolism and the influx of amino acids into the liver during diabetes stimulate gluconeogenesis [36]. The researchers found that aberrant glucagon-mediated regulation of cyclic AMP production in insulin shortage leads to increased proteolysis of uncontrolled diabetes [37, 38]. This could have explained the observed decline in the overall protein concentration in diabetic rats that were left untreated after being exposed to alloxan [39, 22]. When ECSB was given to

diabetic rats, the proteolysis brought on by insulin shortage was much reduced, and as a result, the plasma protein level rose to almost normal levels. In this investigation, ECSB improved glucose utilization by sharply lowering blood sugar levels in rats given glucose. This could have been accomplished by the extract's potential to increase the amount of insulin secreted by the pancreatic beta cells.

The selection of dosage is a significant issue when using herbs to treat illnesses [40]. The majority of herbal remedies are taken without following a set dosage, which could have harmful effects on the body's essential organs [41]. When given orally, ECSB reduced the increased activity of every enzyme studied in diabetic rats, making them similar to the control group. The increasing amounts of calcium ion, creatinine, urea, and uric acid in the serum are indicative of renal impairment, which most likely caused the decreased functional capacity [35]. It is anticipated that the untreated diabetic rats in this investigation will have higher serum levels of urea, creatinine, and uric acid. Gluconeogenesis is triggered as a substitute glucose supply pathway when there is insufficient insulin, which prevents glucose from entering the extrahepatic tissues. Increased proteolysis, which releases free glucogenic amino acids into the plasma and deamines them in the liver to raise blood urea levels, is what keeps this pathway going [25]. Muscle creatine is converted to creatinine, a metabolite whose quantity in serum is correlated with muscle mass. Since creatinine is easily eliminated by the kidneys, increased levels only signal reduced renal function because the amount is normally stable. The primary metabolic byproduct of purine metabolism is uric acid, whose increased level in the serum is indicative of renal impairment [29].

However, the administration of ECSB resulted in a notable decrease in these three metabolite levels, providing protection against diabetes-related damage. Rats with diabetes that were given extracts from *Terminalia catappa* have shown similar results [4]. The fact that the effects of ECSB at dosages of 100 and 200 mg/kg body weight contrasted very well with the control is especially noteworthy. We studied the effects of glibenclamide on β cell regeneration and pancreatic damage in diabetic control rats treated with alloxan [26]. ECSB also showed a similar regeneration. Flavanol glycosides and terpenoids, which have been discovered to be components *of Costus spicatus* buds, may be the cause of this action. It has previously been documented that flavanol glycosides and terpenoids have a beneficial impact in lowering diabetic sequelae such as glycosylation in rats with diabetes induced by alloxan. Our research's photomicrographic data support the idea that ECSB's antidiabetic action involves pancreatic repair.

## Conclusion

The antidiabetic efficacy of the ECSB at dose levels of 100 and 200 mg/kg is therefore demonstrated by the results of our investigation. As demonstrated by lower levels of fasting blood glucose, HbA1c, total cholesterol, triglyceride, low density lipoprotein (LDL), urea, creatinine, uric acid, and calcium and higher levels of HDL-cholesterol and total protein, the antidiabetic potential of the ECSB is similar to that of glibenclamide. The ECSB, being abundant in flavonoid glycosides, may contribute to the antidiabetic effect in albino Wistar rats.

**Competing interests**
The authors report no conflicts of interest.
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**Contributions**
Radhika Reddy Ganta is responsible for the research study, content, writing the article, conceptualization, design and experimentation, Rakesh K. Jat involved in supervision, editing and revision of the article.

**Data and Code availability**
**No data was used for the research described in the article.**

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**Table legends**

Table 1 Effect of oral administration of ethanolic extract of *Costus spicatus* on water intake, feed intake, and body weight in diabetic rats

Table 2 Effect of oral administration of ethanolic extract of *Costus spicatus* on fasting blood glucose levels in diabetic rats

Table 3 Effect of oral administration of ethanolic extract of *Costus spicatus* on Cholesterol, Triglycerides, HDL, LDL, and Total protein levels in diabetic rats

Table 4 Effect of oral administration of ethanolic extract of *Costus spicatus* on Calcium, Creatinine, Urea, Uric acid, and HbA1c in diabetic rats

**Table 1 Effect of oral administration of ethanolic extract of *Costus spicatus* on water intake, feed intake, and body weight in diabetic rats**

| Groups | Water intake (ml/day) | Feed intake (g/day) | Weight gain (g) |
|---|---|---|---|
| Group I - Control (normal saline) | 20.01 ± 1.92 | 13.89 ± 1.23 | 33.41 ± 2.57 |
| Group II - Alloxan (120 mg/kg, i.p) | 90.32 ± 1.75$^{***}$ | 32.78 ± 0.87$^{***}$ | 4.52 ± 1.28$^{***}$ |
| Group III - *Costus spicatus* buds (ECSB - 100 mg/kg , p.o) | 76.57 ± 2.35$^{\#\#\#}$ | 24.31 ± 1.78$^{\#\#\#}$ | 12.38 ± 0.85$^{\#\#\#}$ |
| Group IV - *Costus spicatus* buds (ECSB - 200 mg/kg , p.o) | 48.65 ± 1.24$^{\#\#\#}$ | 18.54 ± 1.04$^{\#\#\#}$ | 17.42 ± 1.58$^{\#\#\#}$ |
| Group V - Glibenclamide (10 mg/kg, p.o) | 46.32 ± 1.77$^{\#\#\#}$ | 20.78 ± 1.28$^{\#\#\#}$ | 19.15 ± 1.36$^{\#\#\#}$ |

Data are presented as mean ± SE (n=6) and analyzed using one-way ANOVA followed by Duncan's multiple comparison test. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to control. $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, and $^{\#}P < 0.05$ compared to Alloxan (120 mg/kg, intraperitoneally).

**Table 2 Effect of oral administration of ethanolic extract of *Costus spicatus* on fasting blood glucose levels in diabetic rats**

| Groups | Fasting blood glucose level (FBG) | | | |
|---|---|---|---|---|
| | Day 1 (mg/dl) | Day 7 (mg/dl) | Day 14 (mg/dl) | Day 21 (mg/dl) |
| Group I - Control (normal saline) | 82.24 ± 5.32 | 85.21 ± 3.27 | 81.34 ± 1.21 | 78.02 ± 3.24 |
| Group II - Alloxan (120 mg/kg, i.p) | 239.51 ± 10.98$^{***}$ | 275.3 ± 7.97$^{***}$ | 294.0 ± 6.16$^{***}$ | 300.5 ± 5.06$^{***}$ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Group III - *Costus spicatus* buds (ECSB - 100 mg/kg , p.o) | 155.1 ± 7.61$^{###}$ | | 140.3 ± 4.27$^{###}$ | | 129.6 ± 7.78$^{###}$ | | 103.3 ± 2.98$^{###}$ | |
| Group IV - *Costus spicatus* buds (ECSB - 200 mg/kg , p.o), | 130.8 ± 3.50$^{###}$ | | 120.6 ± 4.24$^{###}$ | | 117.8 ± 2.75$^{###}$ | | 91.25 ± 2.87$^{###}$ | |
| Group V - Glibenclamide (10 mg/kg, p.o) | 129.0 ± 2.16$^{###}$ | | 121.3 ± 1.70$^{###}$ | | 9.15 ± 1.26$^{###}$ | | 87.50 ± 1.91$^{###}$ | |

Data are presented as mean ± SE (n=6) and analyzed using one-way ANOVA followed by Duncan's multiple comparison test. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to control. $^{###}P < 0.001$, $^{##}P < 0.01$, and $^{#}P < 0.05$ compared to Alloxan (120 mg/kg, intraperitoneally).

**Table 3 Effect of oral administration of ethanolic extract of *Costus spicatus* on Cholesterol, Triglycerides, HDL, LDL, and Total protein levels in diabetic rats**

| Groups | Cholesterol (mg/dL) | Triglycerides (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | Total protein (g/L) |
|---|---|---|---|---|---|
| Group I - Control (normal saline) | 51.23 ± 2.17 | 94.23 ± 1.98 | 15.92 ± 2.23 | 36.24 ± 1.87 | 79.52 ± 1.58 |
| Group II - Alloxan (120 mg/kg, i.p) | 75.62 ± 1.32$^{***}$ | 152.11 ± 3.29$^{***}$ | 9.17 ± 1.56$^{***}$ | 56.27 ± 1.43$^{***}$ | 65.28 ± 2.54$^{***}$ |
| Group III - *Costus spicatus* buds (ECSB - 100 mg/kg , p.o) | 55.23 ± 2.12$^{###}$ | 102.41 ± 2.65$^{###}$ | 12.27 ± 0.94$^{###}$ | 45.71 ± 3.27$^{###}$ | 72.01 ± 2.29$^{###}$ |
| Group IV - *Costus spicatus* buds (ECSB - | 52.31 ± 1.36$^{###}$ | 96.23 ± 2.91$^{###}$ | 13.21 ± 1.47$^{###}$ | 39.28 ± 2.08$^{###}$ | 75.26 ± 2.84$^{###}$ |

| | | | | | |
|---|---|---|---|---|---|
| 200 mg/kg , p.o), | | | | | |
| Group V - Glibenclamide (10 mg/kg, p.o) | 54.32 ± 1.27### | 95.84 ± 3.01### | 14.04 ± 1.28### | 38.22 ± 2.74### | 76.58 ± 2.88### |

Data are presented as mean ± SE (n=6) and analyzed using one-way ANOVA followed by Duncan's multiple comparison test. ***P < 0.001, **P < 0.01, and *P < 0.05 compared to control. ###P < 0.001, ##P < 0.01, and #P < 0.05 compared to Alloxan (120 mg/kg, intraperitoneally).

**Table 4 Effect of oral administration of ethanolic extract of *Costus spicatus* on Calcium, Creatinine, Urea, Uric acid, and HbA1c in diabetic rats**

| Groups | Calcium (mmol/L) | Creatinine (mg/dL) | Urea (mg/dL) | Uric acid (mg/dL) | HbA1C (%) |
|---|---|---|---|---|---|
| Group I - Control (normal saline) | 1.56 ± 0.02 | 32.31 ± 0.76 | 35.27 ± 3.24 | 6.24 ± 0.87 | 4.86 ± 0.02 |
| Group II - Alloxan (120 mg/kg, i.p) | 3.01 ± 0.07*** | 58.63 ± 0.52*** | 70.28 ± 4.78*** | 9.85 ± 0.25*** | 16.52 ± 0.06*** |
| Group III - *Costus spicatus* buds (ECSB - 100 mg/kg , p.o) | 1.89 ± 0.04### | 45.82 ± 0.94### | 58.24 ± 3.14### | 8.21 ± 0.68### | 8.41 ± 0.08### |
| Group IV - *Costus spicatus* buds (ECSB - 200 mg/kg , p.o), | 1.76 ± 0.03### | 38.41 ± 0.33### | 45.85 ± 2.95### | 7.45 ± 0.95### | 5.02 ± 0.09### |
| Group V - Glibenclamide (10 mg/kg, p.o) | 1.63 ± 0.02### | 34.56 ± 0.68### | 38.29 ± 3.08### | 5.89 ± 0.54### | 5.31 ± 0.07### |

Data are presented as mean ± SE (n=6) and analyzed using one-way ANOVA followed by Duncan's multiple comparison test. $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ compared to control. $^{###}P < 0.001$, $^{##}P < 0.01$, and $^{#}P < 0.05$ compared to Alloxan (120 mg/kg, intraperitoneally).