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Original Research Article

The Anti-Hyperglycemic and Antioxidative Effects of Aqueous Extracts from *Sphenocentrum jollyanum*: Evidence from *In Vitro* and *In Vivo* Studies

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ABSTRACT

Red medicine (*Sphenocentrum jollyanum*) is a dense forest plant with an ethnomedicinal impact on disease management. The antioxidative and antihyperglycemic effects of aqueous extracts from *Sphenocentrum jollyanum* root and leaf were investigated. The radicals scavenging ability, iron (Fe^{2+}) chelating ability, and the inhibitory effect on carbohydrate metabolizing enzymes (α-amylase and α-glucosidase) were assessed *in vitro*. Thereafter, the effect was evaluated in streptozotocin (35 mg/kg)-induced diabetic rats placed on a high fat diet. The leaf extract was higher in phenol (4.57 mg/g) and flavonoid (2.20 mg/g) which was significantly ($p < 0.05$) different from the root extract with a phenol content of 2.79 mg/g and a flavonoid content of 1.75 mg/g. The radical scavenging and inhibitory properties of aqueous extract from the leaf were greater than the root extract except for α-amylase activity which was inhibited better by the root extract (87.5%) when compared to the leaf (77.2%). The extracts significantly improved the glycemic state, prevented oxidative stress by enhancing the antioxidant status and may be classified as a potential natural anti-hyperglycemic agent.

***Keywords*:** Anti-hyperglycemia, Antioxidants, Aqueous extract, Purinergic enzymes.

Introduction

Metabolic disease interferes with normal metabolism, causing the body to have excess or deficit of molecules necessary to stay healthy. Diabetes mellitus is a prevalent multifactorial disorder globally estimated to affect approximately 537 million in the year 2021, with a future projection of 783 million by the year 2045.[1] Insulin is a regulatory hormone produced by the ß-cells of the pancreas and functions in the transport of glucose from the bloodstream into body cells for conversion into energy. The insufficiency or insensitivity of insulin results in hyperglycemia and uniquely underlines the pathology of diabetes mellitus. These, coupled with uncontrolled production of reactive oxygen species activate signaling mechanisms involved in the pathogenesis of diabetes.[2,3] Consequently, there is a harmful exertion on the vascular system resulting in complications, increased morbidity, and mortality.[4] The burden of diabetes majorly lies with the non-insulin dependent class (type 2; approximately 85%), which the American Diabetes Association (2018)[5] describes as a loss in ß-cell's capability to secrete insulin and or insulin resistance.[6] Understanding diabetes mellitus and its complications has been overviewed in animal models, allowing the combination of genetic and functional characterization of the syndrome.[7] Combination of a diet high in fat and streptozotocin (HFD/STZ) is a modification designed to mimic insulin resistance. High-fat diet induces dyslipidemia with a resulting insulin resistance, and/or glucose intolerance; a low dose of STZ reduces functionality in ß-cell's secretion of insulin.[8]

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Red medicine (*Sphenocentrum jollyanum* Pierre), a perennial plant, is an erect shrub belonging to the Menispermaceae family with an indigenous name "Akerejupon" in the South-Western part of Nigeria and other distinct meanings across different tribes. This shrub (up to 1.5m in height) shares a close habitat with dense forest and some located around deep shades of bamboo trees. Traditional health practitioners in the Western region claim the plant effectively manages diabetes mellitus with exceptional wound healing properties.[9] Similarly, one of the mechanisms through which *Sphenocentrum jollyanum* elicits wound healing potentials in diabetic animal is by managing the glycemic state with evidence of correlation between them.[10,11] It is also generally known from research that natural products with good antioxidant potentials show significant hypoglycemic effect.[12] It becomes imperative to assess the anti-hyperglycemic effect of *Sphenocentrum jollyanum* from different in *vitro* and *in vivo* analysis.

Materials and Methods

Sample collection and identification

Fresh samples of *Sphenocentrum jollyanum* Pierre identified with herbarium number 0263 were sourced around vicinities of Akure community, Nigeria in July, 2022. The identification of the plant was carried out by Mr. Omomoh B.E. at the Centre for Research and Development, Federal University of Technology, Akure, Nigeria. This was deposited at the herbarium unit with voucher number 0263. The roots and leaves of the plant were powdered into fine samples and extracted in distilled water at room temperature. These samples were subsequently freeze-dried and the resulting crude extract was used for analysis after reconstituting in water.

Antioxidant assay: In vitro

The antioxidant properties of the extract were evaluated using standard referenced protocols. The total phenol content, total content of flavonoid, radical scavenging and chelating ability of the extracts were assessed following protocols described and referenced in Oboh et al.[13,14]. The DPPH (1,1-diphenyl2picrylhdrazyl) radical scavenging ability;[15] and the ferric reducing property[16] were also evaluated. The

dinitrosalicylic acid method was used to evaluate inhibition of *α*-amylase;[17] the inhibition of *α*-glucosidase was evaluated following the method of Apostolidis *et al*.[18] and the inhibition of lipid peroxidation was evaluated following the standards of Ohkawa *et al*.[19]

Care and handling of animals

Wistar albino rats (180 - 240 g) acclimatized by standard protocols were used for the *in vivo* analysis of the antioxidant and anti-hyperglycemic effect of *Sphenocentrum jollyanum*. The guide of the National Institute of Health (NIH) was followed to ensure proper handling of the experimental animals. An ethical approval for animal use was given by FUTA-Committee on research (FUTA/ETH/2020/012) before the experiments were carried out.

Induction of diabetes and treatment

The animals were fed a diet high in fat (Table 1) for two weeks and afterward injected with STZ (35 mg/kg b.w; *i.p*).[20] After 72 hours post-administration of streptozotocin, their blood glucose level was checked using a glucometer (Finetest Auto-coding™). Those with glucose levels above 250 mg/dL were considered diabetic.

The doses of extract used for treatment were based on results from previous oral toxicity studies where no toxic effect was observed at these doses.[21] Rats were orally administered the extracts for 14 days as grouped below (n = 6).

Group 1: normal rats administered the vehicle (distilled water); groups II and III are diabetic rats given 100 and 200 mg/kg b.w of the root extract; groups IV and V are diabetic rats given 100 and 200 mg/kg b.w of the leaf extract; group VI are diabetic rats given 25 mg/kg acarbose (standard antihyperglycemic agent); group VII are untreated diabetic rats. They were regularly (3-day intervals) checked for changes in glucose levels and weight during the experimental period.

Biochemical assays

At the end of the 14-day study, blood collected by cardiac puncture was used to evaluate glycated hemoglobin level following step-by-step method of Fortress diagnostic kits (U.K). The pancreas of the rats was excised in 0.9% cold saline; homogenized in phosphate buffer (0.1M, pH 7.4), and afterward centrifuged at 10000 x g for 10 mins. This yielded the supernatant which was analyzed for biochemical markers.

Determination of antioxidants

The activity and level of antioxidants in the pancreas were determined by standard protocols. The activity of glutathione peroxidase (GPx);[22] superoxide dismutase (SOD);[23] catalase activity;[24] reduced glutathione (GSH).[25]

Determination of purinergic enzymes

The activities of purinergic enzymes adenosine deaminase, 5' Nucleotidase and ATPdase were evaluated following standard protocols.[26,27,28]

Statistical analysis

Data sets from the experiment were analyzed and the results were presented as mean ± standard deviation. The significance between each group at $p < 0.05$) was confirmed by one-way ANOVA and Tukey's test for multiple comparisons using graph pad prism 6.0. The IC_{50} values for *in vitro* studies were determined using non-linear regression analysis.

Table 1: Formulation for high fat diet (HFD) and basal (normal) diet

	HFD (g kg^{-1})	Basal (g kg^{-1})
Skimmed milk	500	500
Corn starch	160	360
Lard	300	-
Premix	40	40
Corn oil	-	100

The feed was baked to make the meal appealing to the rats. 20 g baked feed was administered to each rat in their compartmental cage on a daily basis

Result and Discussion

The leaf extract contained a higher content of phenol (4.57 mg/g) and flavonoid (2.20 mg/g) than the root extract (Table 2). Studies on medicinal plants in recent times have showcased their potential in managing and preventing varying degree of ailments. This has confirmed and provided evidence of their use in traditional practices. Most importantly, their pharmacological effect has been well established and correlated with the broad range of phytochemicals present in them. An appreciable total phenol and flavonoid content per gallic acid and quercetin equivalent respectively was found in the extracts from of *S. jollyanum*. They have been linked with protection against free radicals and also in the prevention of oxidative stress related diseases like diabetes.[29] The inhibitory concentrations (IC_{50}) of chelating and scavenging abilities of extracts from *S. jollyanum* is on Table 3. The leaf extract scavenged radicals better than the root extract with a similar result from the Fe^{2+} chelating ability revealed by the IC_{50} values of 1.41 mg/ml and 1.48 mg/ml for the leaf and root extract respectively. The leaf extract scavenged ABTS radical and better reducing power than the root extract (Fig 1). In the prevention of oxidative stress, functional plants employ several distinct mechanisms like metal chelation, radical scavenging, improving antioxidant defense, and breaking chains of lipid peroxide production amongst other well-documented mechanisms.[30] According to Jafri et al.,[31] there is an agreement between antioxidative effect and the phenolic content of a plant. The radical scavenging activity of aqueous extracts from *S. jollyanum* may be because of polyphenols like kaempferol, rutin, quercetin, gallic acid, p-coumaric acid identified in them.[32]

Table 2: Total phenol and total flavonoid content of aqueous extracts from root and leaf of *Sphenocentrum jollyanum*

	Root	Leaf
Total phenol (mg/g GAE)	2.79 ± 0.05^{a}	4.57 ± 0.06^{b}
Total flavonoid (mg/g QE)	1.75 ± 0.01^{a}	2.20 ± 0.02^{a}

Values represent means ± standard deviation of triplicate readings
Values with the same superscript across rows are not significantly different ($p > 0.05$)

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Table 3: IC_{50} values for DPPH, OH radical scavenging abilities and Fe^{2+} chelating ability of aqueous extracts from root and leaf of *Sphenocentrum jollyanum*

	Root	Leaf
DPPH*	3.90 ± 0.01[a]	3.36 ± 0.05[a]
Fe^{2+}	1.48 ± 0.00[a]	1.41 ± 0.00[a]
OH*	1.27 ± 0.01[a]	1.22 ± 0.05[a]

Values represent mean ± standard deviation of triplicate readings (n = 3)
Values with the same superscript across rows are not significantly different (p > 0.05)

Inhibition of enzymes that metabolize carbohydrate and the inhibition of lipid peroxidation is presented in Figure S2. The root and leaf extracts had a significant ($p < 0.05$) effect on enzyme inhibition. According to the inhibitory values presented in Table 4, the root extract (0.50 mg/ml) inhibited α-amylase better than the leaf extract (0.97 mg/ml) while the leaf extract presented a better inhibition of α-glucosidase and malondialdehyde (MDA) than the root extract. The root and leaf extracts reduced the production of MDA from 100 to 38.4% and 40.1 % respectively. Repression of the activities of carbohydrate metabolizing enzymes reveals one of the mechanisms through which medicinal plant elicit its anti-hyperglycemic effect. The inhibition of α-amylase and α-glucosidase slows down/prevents the abnormal degradation of large sugar/starch polymers into its common monomeric unit (glucose). This prevents a surge in blood glucose level of diabetic individuals. *S. jollyanum* prevented the formation of malondialdehyde and repressed α-amylase and α-glucosidase activities *in vitro*. This conforms with studies elucidating enzyme and lipid peroxidation inhibitors of plant origin.[33,34]

Figure 1: ABTS radical scavenging and ferric reducing ability of aqueous extract from root and leaf of *Sphenocentrum jollyanum*
Bars represent mean ± standard deviation of triplicate reading (n = 3)
Bars with different letters are significantly different (p < 0.05)

Normal rats fed a high-fat diet for two weeks significantly ($p < 0.05$) gained weight before streptozotocin (STZ) administration (Table 5). The intraperitoneal administration of STZ caused significant weight loss and increased blood glucose levels above 250mg/dl after 72 hours post-induction (Table 6). The administration of aqueous extract from *Sphenocentrum jollyanum* caused a decline in blood glucose and glycated hemoglobin. The blood glucose ranged below 250 mg/dl implying that they had become normoglycemic or non-diabetic (Figure 2). In line with other studies, glycated hemoglobin is increased in a diabetic state as a consequence of redox imbalance;[35] it was significantly elevated in diabetic rats. In the red cell, glycated hemoglobin spends its life span there and gives trends of glucose in the blood of diabetic patients. The reduction in the percentage of glycated hemoglobin following treatment with *S. jollyanum* root and leaf extract aligns with the control of blood glucose observed in the treated groups. As markers of oxidative stress, there was a compromise in the antioxidant system (reduced activities of SOD, GPx, catalase, and level of glutathione) after administration of STZ. This was significantly ($p < 0.05$) enhanced following administration of aqueous extracts of *S. jollyanum* as revealed by an increase in SOD, GPx, catalase activities, and level of glutathione (Figure 3) in the pancreas. The elevation in the activities of SOD, GPx, catalase, and level of glutathione following the administration of aqueous extracts of *S. jollyanum* have been confirmed[36] stating that potent anti-hyperglycemic plant enhances the antioxidant system to reduce oxidative stress and consequently manage diabetes mellitus. This similar trend was observed for the activities of purinergic enzymes with a reduction in ATPDase, 5' Nucleotidase, and adenosine deaminase after oral administration of aqueous extract from *S. jollyanum* (Figure 4).

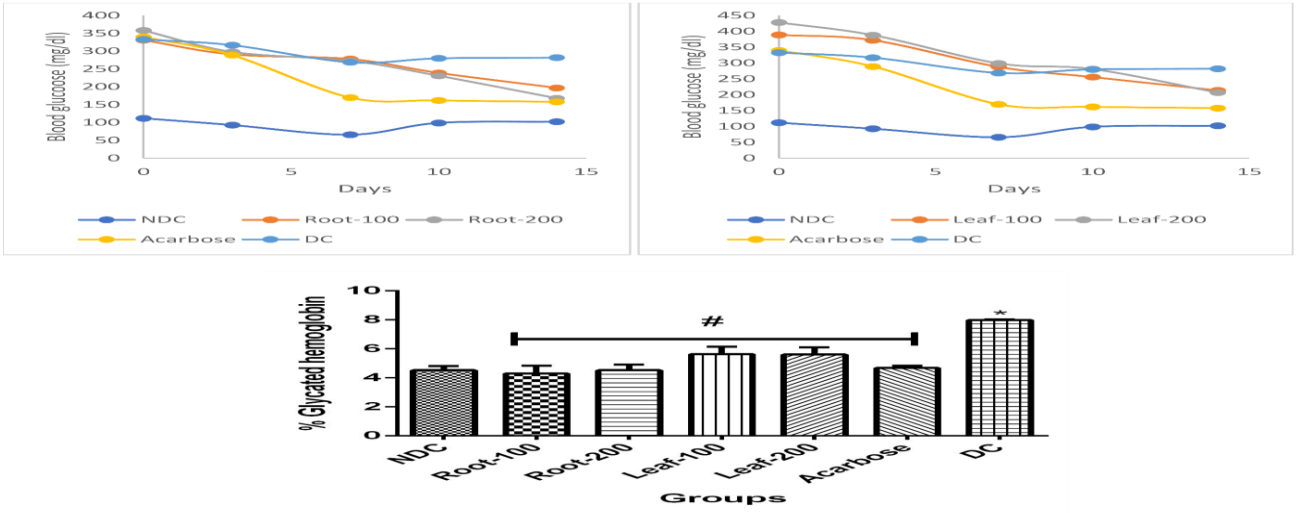

Figure 2: Effect of oral administration of *Sphenocentrum jollyanum* on blood glucose (mg/dl) and glycated hemoglobin (%) level in diabetic rats
NDC: non-diabetic control; **Root-100**: 100 mg/kg root extract; **Root-200**: 200 mg/kg root extract; **Leaf-100**: 100 mg/kg leaf extract; **Leaf-200**: 200 mg/kg leaf extract; **Acarbose:** 25 mg/kg acarbose; **DC**: diabetic control rats untreated
Bars represent mean ± standard deviation (n = 6).
[#]Values significantly different from diabetic control p < 0.05

Purine nucleoside mediate pathological processes in diabetes by promoting insulin resistance responsible for the progression of the disease. Adenosine activates certain receptors and consequently inhibits the secretion of insulin.[37] Adenosine triphosphate (ATP) activates certain receptors on ß-cells to generate a positive signal that results in insulin secretion. In type 2 diabetes, high activity of nucleoside triphosphate diphosphohydrolases (NTPDases) hydrolyzing ATP and ADP have been observed resulting in the formation of AMP acted upon by 5' nucleotidase to give adenosine.[38] The activities of adenosine triphosphate diphosphohydrolases (ATPDase), 5' nucleotidase, and adenosine deaminase which was high in diabetic rats were reduced after administering aqueous extracts of *S. jollyanum*. These reduced activities probably enhanced insulin secretion and regulation of blood glucose levels.

Table 4: IC_{50} values of α-amylase, α-glucosidase and Fe^{2+} induced lipid peroxidation inhibitory effect of aqueous extract of the root and leaf of *Sphenocentrum jollyanum*

	Root	Leaf
LPO (Fe^{2+})	0.76 ± 0.00^{a}	0.74 ± 0.00^{a}
α- amylase	0.50 ± 0.01^{a}	0.97 ± 0.01^{b}
α- glucosidase	1.04 ± 0.04^{b}	0.76 ± 0.02^{a}

Values represent mean ± standard deviation of triplicate readings
Values with the same superscript across rows are not significantly different ($p > 0.05$)

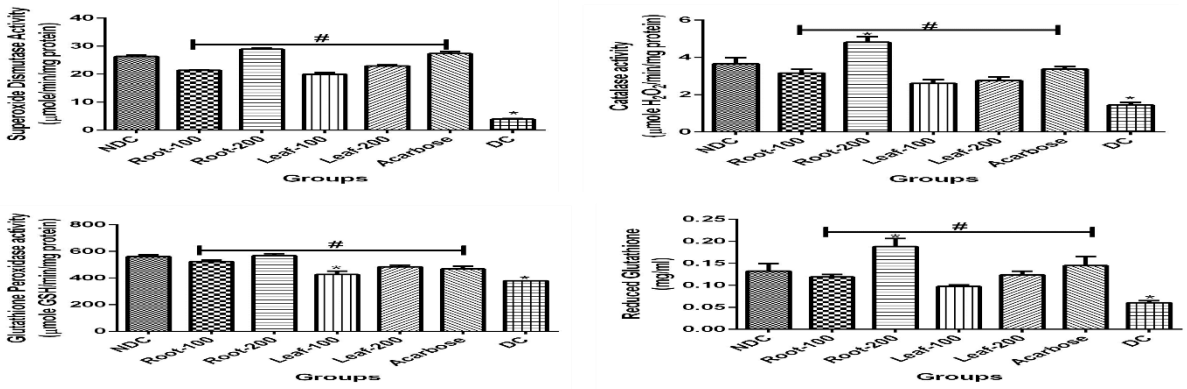

Figure 3: Effect of oral administration of *Sphenocentrum jollyanum* on antioxidant status in the pancreas of diabetic rats
NDC: non-diabetic control; **Root-100**: 100 mg/kg root extract; **Root-200**: 200 mg/kg root extract; **Leaf-100**: 100 mg/kg leaf extract; **Leaf-200**: 200 mg/kg leaf extract; **Acarbose:** 25 mg/kg acarbose; **DC**: diabetic control rats untreated
Bars represent mean ± standard deviation (n = 6).
*Values significantly different from non-diabetic control $p < 0.05$
#Values significantly different from diabetic control $p < 0.05$

Table 5: Average weight gain (%) of normal rats fed High Fat Diet for 14days

Groups	Day 1	Day 14	Weight gain (%)
NDC	168.2 ± 13.1	178.4 ± 10.9	5.73^{d}
Root-100	208.7 ± 9.37	227.5 ± 5.05	8.25^{a}
Root-200	183.7 ± 6.31	203.3 ± 0.82	9.61^{a}
Leaf-100	192.1 ± 10.3	206.7 ± 2.81	7.04^{c}
Leaf-200	184.6 ± 3.97	204.8 ± 8.04	9.86^{a}
Acarbose	181.5 ± 7.72	200.2 ± 3.08	9.33^{a}
DC	210.4 ± 17.7	228.6 ± 16.5	7.93^{c}

Values represent mean ± standard deviation (n = 6)
Values with the same superscript down column are not significantly different ($p > 0.05$)
NDC: non-diabetic control; **Root-100**: 100 mg/kg root extract; **Root-200**: 200 mg/kg root extract; **Leaf-100**: 100 mg/kg leaf extract; **Leaf-200**: 200 mg/kg leaf extract; **Acarbose:** 25 mg/kg acarbose; **DC**: diabetic control rats untreated

Table 6: Effect of aqueous extract from root and leaf of *Sphenocentrum jollyanum* on weight (g) of high fat diet/streptozotocin induced diabetic rats

Groups	Initial weight	Final weight	Weight gain/loss (%)
NDC	178.6 ± 11.0	189.8 ± 15.0	5.90^{a}
Root-100	204.9 ± 11.7	214.4 ± 14.7	4.43^{b}
Root-200	178.9 ± 3.0	188.1 ± 2.0	4.89^{b}
Leaf-100	179.5 ± 5.5	190.1 ± 6.8	5.58^{a}
Leaf-200	175.0 ± 5.9	185.0 ± 8.2	5.41^{a}
Acarbose	184.0 ± 4.1	195.7 ± 1.5	6.12^{a}
DC	208.3 ± 24.4	194.3 ± 38.3	-6.72^{d}

Values represent mean ± standard deviation (n = 6)
Values with the same superscript down column are not significantly different ($p > 0.05$)
NDC: non-diabetic control; **Root-100**: 100 mg/kg root extract; **Root-200**: 200 mg/kg root extract; **Leaf-100**: 100 mg/kg leaf extract; **Leaf-200**: 200 mg/kg leaf extract; **Acarbose:** 25 mg/kg acarbose; **DC**: diabetic control rats untreated

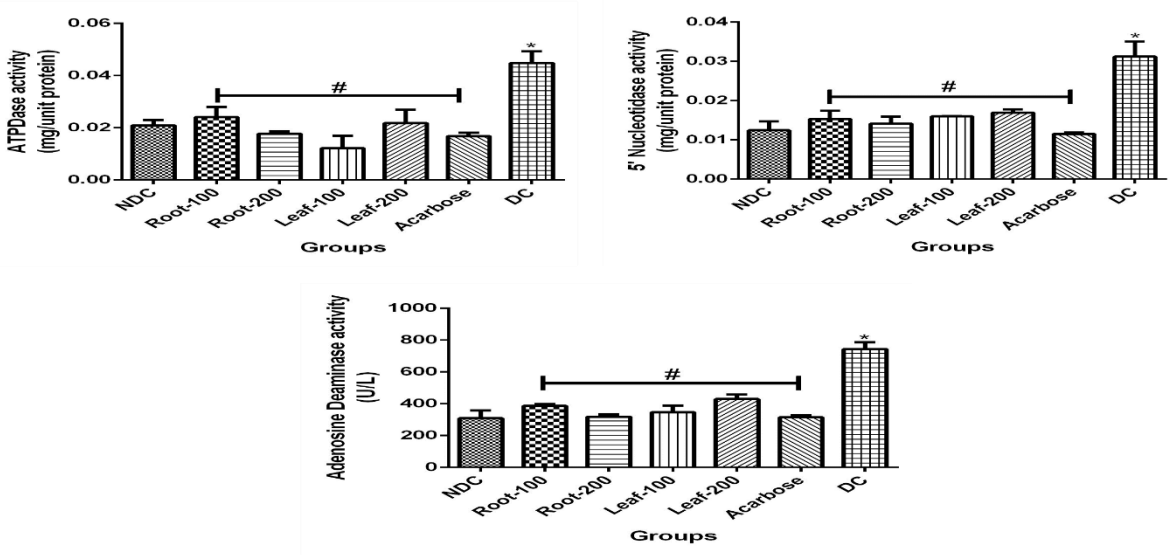

Figure 4: Effect of oral administration of *Sphenocentrum jollyanum* on activities of purinergic enzymes in the pancreas of diabetic rats
NDC: non-diabetic control; **Root-100**: 100 mg/kg root extract; **Root-200**: 200 mg/kg root extract; **Leaf-100**: 100 mg/kg leaf extract; **Leaf-200**: 200 mg/kg leaf extract; **Acarbose:** 25 mg/kg acarbose; **DC**: diabetic control rats untreated
Bars represent mean ± standard deviation (n = 6).
*Values significantly different from non-diabetic control $p < 0.05$
#Values significantly different from diabetic control $p < 0.05$

Conclusion

The antihyperglycemic effect of *Sphenocentrum jollyanum* has been determined and can be correlated largely to the phenolic compound present in them. This research showed that *S. jollyanum* greatly improved the glycemic state of diabetic rats by increasing the activities of antioxidant enzyme against oxidative stress and regulating the actions of α-amylase, α-glucosidase that are key in the management of diabetes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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