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Research Article

Peptide Hydrolysate of *Telfairia occidentalis* Hook f. Seed Protein Promotes Effective Glucose Homeostasis by Improving β-cell Dysfunction and Abating Carbohydrate Metabolic Disturbance in Diabetic Rats

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Rising concerns with the use of synthetic antidiabetic drugs have promoted a shift towards the use of natural products. This study therefore investigated the antidiabetic activity of peptide hydrolysate of *Telfairia occidentalis* (*T. occidentalis*) seed protein (PHTOSP) in streptozotocin-induced diabetic rats. Thirty-six (36) experimental animals were randomly distributed into six groups (A–F) of six rats each ($n = 6$). Group A served as normal control while groups B, C, D, E, and F were treated with streptozotocin (STZ, 50 mg/kg body weight, b.w, i.p) dissolved in cold citrate buffer (0.1 M, pH 4.5) to induce type 2 diabetes. Groups C, D, and E were administered 50, 100, and 150 mg/kg b.w PHTOSP, respectively, while groups B and F were diabetic untreated and 5 mg/kg b.w glibenclamide-treated controls, respectively, in the experiment that lasted for 21 days. Subsequently, the analysis of biochemical parameters demonstrated a significant ($p < 0.05$) increase in serum insulin, hepatic glycogen, hexokinase, and glucose-6-phosphate dehydrogenase activities, accompanied by a significant ($p < 0.05$) reduction in fasting serum glucose, glucose-6-phosphatase, and fructose-1,6-bisphosphatase, along with an enhancement in relative body weight. Similarly, PHTOSP demonstrated a significant ($p < 0.05$) improvement on high-density (HDL) and low-density (LDL) lipoproteins, triglyceride (TG), total cholesterol (TC), and atherogenic index (AI) significantly ($p < 0.05$). In addition, histoarchitectural analysis revealed a reversal of congestion and proliferation of inflammatory cells in the pancreatic tissue following treatment with PHTOSP. Therefore, PHTOSP might possess potential antidiabetic properties such that it improves glycolytic pathway and promotes cell survival that are helpful in the management of diabetes mellitus (DM).

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent high blood sugar levels and disruptions in the metabolism of carbohydrates, lipids, and proteins due to abnormalities in insulin production, insulin action, or both [1, 2]. It occurs when the pancreas does not produce enough insulin or when the body is unable to effectively use the insulin it produces [3]. In the twenty-first century, diabetes has emerged as one of the most challenging health conditions [4]. It affects approximately 4% of the global population and is projected to increase by 27% by 2025 [5]. In

many high-income nations, it ranks as the fourth leading cause of mortality, while in economically developing and industrialized countries, it poses a significant burden [6]. It is estimated that by 2030, the number of people with diabetes will exceed 552 million, with a 176% increase in developed nations [7, 8]. This rise can be attributed to factors such as rapid urbanization, westernization, and associated lifestyle changes [9].

DM is classified into two types: insulin-dependent diabetes mellitus (type 1) and non-insulin-dependent diabetes mellitus (type 2) [2]. Type 1 diabetes is an autoimmune disease characterized by inflammation and selective destruction of insulin-secreting cells in and around the islets of Langerhans [10]. Type 2 diabetes is characterized by peripheral insulin resistance and dysfunction. Both types of diabetes pose significant risks to affected individuals and society due to their potential life-threatening complications. Complications associated with DM, including disturbances in body protein balance, can lead to conditions such as retinopathy, nephropathy, neuropathy, and atherosclerotic vascular disease [11–13]. Possible risks associated with diabetes include heart attack, stroke, kidney failure, lower limb amputation, vision loss, and nerve damage [11].

Cardiovascular diseases are a prevalent complication that often arises in association with DM [14]. These diseases are responsible for approximately 65% of diabetes-related deaths attributed to heart disease and stroke [15]. Managing DM involves various approaches, including dietary modifications, medication, physical activity, regular screening, and care for complications that may arise [16]. While several oral hypoglycemic drugs and insulin are available to control diabetes, there is currently no definitive treatment to cure the condition [17]. Furthermore, many of these drugs are costly and can lead to significant side effects such as hypoglycemia, weight gain, headaches, and gastrointestinal discomfort. Although medicinal plants have been traditionally used to manage DM, only a limited number of them have undergone scientific and medical evaluation to assess their effectiveness [18–20].

T. occidentalis is a tropical vine cultivated for its edible seeds and leafy vegetable in West Africa [21]. Common names include *Ugu*, fluted gourd, and fluted pumpkin. It is a creeping vegetative shrub, with big lobed leaves and long twisting tendrils spreading low across the ground [22]. The plant belongs to the Cucurbitaceae family and has a simple dark green veined leaf that is up to 35 cm long and as wide as 18 cm. Nutrients such as vitamins, proteins, carbohydrates, fiber, and minerals are reportedly found in *T. occidentalis* [23]. More so, studies have highlighted antidiabetic properties of the plant [24–26], as well as hepatoprotective effect against oxidative stress caused by garlic (*Allium sativum*) in rats [25]. More so, with an increase in the use of this plant as one of the herbal remedies in the folkloric management of DM, this study therefore aimed at investigating the antidiabetic potential of peptide hydrolysate of *T. occidentalis* seed protein in STZ-induced diabetic rats.

2. Materials and Methods

2.1. Chemicals Used. Pepsin, trichloroacetic acid (TCA), NaOH, bovine serum albumin (BSA) (standard protein), streptozotocin, and glibenclamide were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. All other chemicals were of analytical grade and prepared in distilled water using all-glass apparatus.

2.2. Collection and Authentication of Plant Sample. Fresh *T. occidentalis* seeds were purchased from a merchant at Oja Oba market in Otun Ekiti, Ekiti State, Nigeria. They were authenticated at the herbarium unit, Department of Plant Biology, University of Ilorin, Kwara State, Nigeria, where a voucher specimen was deposited with a given number UILH/001/959.

2.3. Sample Preparation. The fat-free flour sample of *T. occidentalis* was prepared according to the method of Siddeeg et al. [27]. The seeds obtained from *T. occidentalis* were decorticated and air-dried until a consistent weight was obtained. The seeds were blended to a powdery form and sieved to obtain fine flour. The resulting *T. occidentalis* flour was subsequently defatted with n-hexane at room temperature (25°C). Seed flour was dispersed in n-hexane (1 : 10 w/v) and continuously agitated for 7 h (this was repeated twice). The residue of n-hexane was removed by placing the defatted *T. occidentalis* flour in a fume hood overnight. The fat-free flour was then dried and stored at −20°C for further treatments.

2.4. Preparation of T. occidentalis Seed Protein Isolate. Protein was extracted from the defatted *T. occidentalis* seed flour using acid precipitation method described by Adebowale et al. [28] with some modifications by Arise et al. [29] and a modified isoelectric precipitation procedure [30]. Defatted flour was dispersed in ultrapure water, Milli-Q water (MQW) (1 : 10 w/v). The pH was adjusted to 9.0 using 1.0 M NaOH in order to solubilize the protein. The mixture was then stirred for 1 h at 1,000 rpm and centrifuged (5000 × g, 4°C) for 20 min. The supernatant was stored at −4°C, and the procedure was repeated with a pellet to MQW (1 : 5 w/v). Supernatants from both extractions were combined and pH was adjusted to 4.6 with 1.0 M HCl to achieve protein precipitation. The precipitate was collected after centrifugation (5000 × g, 4°C) for 20 min, washed with 25 mL MQW, frozen at −80°C, and freeze-dried to obtain a free-flowing powder.

2.5. Preparation of Peptide Hydrolysate of T. occidentalis Seed Protein (PHTOSP). The preparation of peptide hydrolysate of *T. occidentalis* seed protein was carried out using the modified method of Radha et al. [31]. *T. occidentalis* seed protein isolate was enzymatically hydrolyzed using pepsin

under optimal conditions at an enzyme/substrate ratio of 1 : 100 (w/w). The resulting mixture was stirred and incubated for 8 h at 25°C. The enzyme was deactivated after hydrolysis by heating the mixture at 100°C for 10 min in a water bath. This was centrifuged for 10 min at 7000 × g, and the supernatant was collected. The hydrolysate was collected, lyophilized, and stored at −20°C for further bioassays.

2.6. Ethics Approval.

The use of experimental animals involved in this study was carried out with a strict compliance to the ethical guidelines for the best practice issued by Ethical Review Committee (UERC) of the University of Ilorin, Ilorin, Kwara State, Nigeria, on the use of laboratory animals with protocol approval number of UERC/ASN/2017/903.

2.7. Animal Treatment Protocol.

Thirty-six (36) healthy male Wistar albino rats weighing between 150 and 160 g used for this study were obtained from the Animal Breeding Unit at the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were acclimatized for 2 weeks before the experiment and housed in clean wooden cages with a standard diet and distilled water *ad libitum*.

2.8. Induction of Diabetes.

Diabetes induction (type 2 DM) was carried out through single intraperitoneal injection of freshly prepared STZ (50 mg/kg b.w) dissolved in cold citrate buffer (0.1 M, pH 4.5) [32]. The rats were fasted overnight prior the induction of diabetes. After 72 h of STZ injection, diabetic status was established following an overnight fasting using an Accu-Chek glucometer to measure blood glucose concentration (mg/dl). Rat tail was punctured to acquire blood sample for fasting serum glucose (FSG). Animals with blood glucose levels ≥ 200 mg/dl were classified as diabetic. The 21-day treatment commenced after diabetes has been established.

2.9. Animal Grouping.

Thirty-six (36) experimental animals were randomly distributed into six groups (A–F) of six rats each ($n = 6$) as follows. Treatment with PHTOSP was administered orally. The choice of different doses (moderately lower doses of 50–150 mg/kg b.w) used was based on the pilot studies (*in vitro* and *in vivo*) conducted prior to the commencement of the experiment.

 (i) Group A: normal control animals without treatment.

 (ii) Group B: diabetic control animals without treatment.

 (iii) Group C: diabetic animals treated with 50 mg/kg PHTOSP.

 (iv) Group D: diabetic animals treated with 100 mg/kg PHTOSP.

 (v) Group E: diabetic animals treated with 150 mg/kg PHTOSP.

 (vi) Group F: diabetic animals treated with 5 mg/kg glibenclamide.

2.10. Collection and Preparation of Tissue and Homogenate.

Tissue and homogenate samples were obtained according to the method of Akanji et al. [33]. The experimental animals were euthanized by mild exposure to chloroform in an airtight chamber, after which they were dissected. Blood samples were immediately collected through a jugular vein cut. Blood samples were then allowed to stand and clot for 30 min to obtain sera. Liver and pancreatic tissues were immediately isolated, washed (in normal saline), weighed, and placed on ice. Subsequently, the tissues were homogenized in 0.25 M sucrose solution (1 : 5 w/v) at 3000 × g for 10 min. The supernatant was aspirated into sample bottles and appropriately diluted and later kept frozen at 4°C before being used for various biochemical parameters analyzed. Pancreatic tissue was also cut out and preserved (in 10% neutral buffered formalin solution) at 25°C for histopathological examination.

2.11. Biochemical Assays.

Biochemical parameters such as serum insulin concentration were determined using the method described in Dallak et al. [34], liver glycogen content was determined by the method of Murat and Serfaty [35], hexokinase activity was determined as described by Zhang et al. [36], glucose-6-phosphate dehydrogenase activity was determined by the modified method of Maurya et al. [37], fructose-1,6-bisphosphatase activity was determined by the method of Riou et al. [38], and glucose-6-phosphatase activity was determined by the method of Maurya et al. [37]. Also, serum lipid profile such as TC was determined by the method of Siedel et al. [39], HDL was determined by the precipitation method of Hirano et al. [40], and TG level was determined by the method described by Fossati et al. [41], while LDL and atherogenic index (AI) were calculated as follows [42, 43]:

$$\text{LDL} - \text{cholesterol} = (\text{total cholesterol}) - (\text{HDL} - \text{cholesterol}) - \left(\frac{\text{triglyceride}}{5}\right),$$

$$\text{AI} = \left(\frac{\text{TC} - \text{HDL} - c}{\text{HDL} - c}\right). \tag{1}$$

2.12. Histopathological Examination. The histological status of the pancreatic tissues of the rats was assessed using the method described by Oloyede et al. [44].

2.13. Statistical Analyses. Data were analyzed using one-way ANOVA, followed by Tukey's test for post hoc analysis, and graphical representation of results was performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). All values were expressed as mean ± SEM ($n = 6$). Statistical differences were considered at $p < 0.05$.

3. Results

3.1. Effect of PHTOSP on Fasting Serum Glucose (FSG) Levels of STZ-Induced Diabetic Rats. The results in Table 1 illustrate the effect of PHTOSP on fasting serum glucose (FSG) levels in STZ-induced diabetic rats. The results revealed that the FSG levels of untreated STZ-induced diabetic rats were significantly ($p < 0.05$) higher than those of the normal control. However, rats treated with PHTOSP at doses of 50, 100, and 150 mg/kg for 7, 14, and 21 days exhibited a significant ($p < 0.05$) decrease in FSG levels compared to both the untreated STZ-induced diabetic control and the normal control. Additionally, the effect of PHTOSP treatment was found to be comparable to that of the 5 mg/kg glibenclamide-treated control group.

3.2. Effect of PHTOSP on Body Weight of STZ-Induced Diabetic Rats. The results in Table 2 demonstrate the effect of PHTOSP on the body weight of in STZ-induced diabetic rats. The data show a significant ($p < 0.05$) decrease in body weight (% change in body weight) in the untreated STZ-induced diabetic control group compared to the normal control group. However, treatment with PHTOSP at doses of 50, 100, and 150 mg/kg for 21 days resulted in a significant ($p < 0.05$) increase in body weight in the treated groups compared to both the untreated STZ-induced diabetic control and the normal control. After 21-day treatment, it was discovered that this effect was comparable to that of the control group receiving 5 mg/kg glibenclamide.

3.3. Effect of PHTOSP on Lipid Profile and Atherogenic Index (AI) of STZ-Induced Diabetic Rats. Table 3 illustrates the effect of PHTOSP on the lipid profile and atherogenic index (AI) of STZ-induced diabetic rats. The results revealed a significant ($p < 0.05$) rise in the levels of serum TC, LDL, TG, and AI, as well as a significant ($p < 0.05$) reduction in HDL in the untreated STZ-induced diabetic control in contrast to the normal control. However, treatment with 50, 100, and 150 mg/kg PHTOSP for 21 days resulted in a significant ($p < 0.05$) decrease in the levels of serum TC, LDL, TG, as well as a significant ($p < 0.05$) reduction in AI compared to the untreated STZ-induced diabetic control, normal control with a beneficial comparison to the group treated with 5 mg/kg of glibenclamide.

3.4. Effect of PHTOSP on the Serum Insulin Levels of STZ-Induced Diabetic Rats. Figure 1 shows the effect of PHTOSP on serum insulin levels in STZ-induced diabetic rats. The results demonstrate a significant ($p < 0.05$) reduction in serum insulin levels of the untreated STZ-induced diabetic rats when compared to the normal control. Conversely, groups that received treatment with 50, 100, and 150 mg/kg PHTOSP for 21 days showed a significant ($p < 0.05$) dose-dependent increase in serum insulin levels when compared to the untreated STZ-induced diabetic control and normal control. This effect was also found comparably beneficial to diabetic groups treated with 5 mg/kg glibenclamide for 21 days.

3.5. Effect of PHTOSP on the Hepatic Glycogen Levels of STZ-Induced Diabetic Rats. Figure 2 presents the effect of PHTOSP on hepatic glycogen levels of STZ-induced diabetic rats. As indicated in the result, there was an observed reduction significantly ($p < 0.05$) in the hepatic glycogen level of untreated STZ-induced diabetic control compared to the normal control. However, following the 21-day treatment with 50, 100, and 150 mg/kg PHTOSP, there was a dose-dependent non-related significant ($p < 0.05$) increase in the hepatic glycogen level in the treated groups compared to untreated STZ-induced diabetic control and normal control. This observation demonstrated a favorable comparison with the effect seen in the diabetic group treated with 5 mg/kg glibenclamide after 21-day treatment.

3.6. Effect of PHTOSP on the Hepatic Hexokinase Activity of STZ-Induced Diabetic Rats. Figure 3 illustrates the effect of PHTOSP on the activity of hepatic hexokinase in STZ-induced diabetic rats. According to the results, there was a noteworthy ($p < 0.05$) reduction in hepatic hexokinase activity in the untreated STZ-induced diabetic control compared to the normal control. However, groups treated with 50, 100, and 150 mg/kg PHTOSP for 21 days exhibited a significant ($p < 0.05$) reduction in hepatic hexokinase activity, compared to both the untreated STZ-induced diabetic control and normal control. Additionally, this effect contrasted favorably with the findings in the group that received 5 mg/kg glibenclamide over the period of 21 days.

3.7. Effect of PHTOSP on the Hepatic Glucose-6-Phosphate Dehydrogenase Activity of STZ-Induced Diabetic Rats. Figure 4 depicts the effect of PHTOSP on the hepatic glucose-6-phosphate dehydrogenase activity of STZ-induced diabetic rats. In the results, a significant ($p < 0.05$) decrease was evident in the activity of hepatic glucose-6-phosphate dehydrogenase of untreated STZ-induced diabetic control compared to the normal control. Conversely, groups treated with different doses of 50, 100, and 150 mg/kg PHTOSP showed an increase significantly ($p < 0.05$) in the activity of glucose-6-phosphate dehydrogenase compared to that of untreated STZ-induced diabetic control as well as

TABLE 1: Effect of PHTOSP on fasting serum glucose levels of STZ-induced diabetic rats.

Groups	Initial day	After 72 h	Day 7 mg/dL	Day 14	Day 21
A	76.63 ± 1.01^a	74.33 ± 0.06^a	82.67 ± 0.86^a	89.00 ± 1.23^a	86.21 ± 0.33^a
B	83.22 ± 1.44^a	390.00 ± 0.09^b	393.20 ± 0.45^d	401.67 ± 1.02^b	415.40 ± 1.08^b
C	78.96 ± 0.91^a	417.24 ± 0.08^c	202.00 ± 0.85^c	94.33 ± 1.03^a	89.00 ± 1.03^a
D	85.55 ± 1.22^a	384.20 ± 0.9^b	189.67 ± 0.53^b	88.00 ± 0.09^a	83.00 ± 0.26^a
E	86.01 ± 1.06^a	421.40 ± 0.07^c	181.00 ± 0.86^b	84.33 ± 1.63^a	88.80 ± 0.18^a
F	80.03 ± 1.11^a	393.20 ± 0.07^b	173.00 ± 0.51^b	86.33 ± 1.14^a	84.60 ± 0.67^a

Values are expressed as mean $\pm$ SD ($n = 6$). Alphabets (a–c) indicate statistical differences. Values in the same column with different letters are significantly ($p < 0.05$) different. A: normal control; B: diabetic control; C: diabetic + 50 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; D: diabetic + 100 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; E: diabetic + 150 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; F: diabetic + 5 mg/kg glibenclamide.

TABLE 2: Effect of PHTOSP on body weight of STZ-induced diabetic rats.

Groups	Initial weight (g)	Final weight	% change in body weight
A	153.73^a	162.21^b	5.32^b
B	157.71^a	121.41^a	-29.90^a
C	155.61^a	163.62^b	4.90^b
D	155.36^a	165.31^b	6.02^b
E	155.43^a	164.41^b	5.46^b
F	156.02^a	166.12^b	6.08^b

Values are expressed as mean $\pm$ SD ($n = 6$). a and b indicate statistical differences between the groups. Values in the same column with different letters are significantly ($p < 0.05$) different. A: normal control; B: diabetic control; C: diabetic + 50 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; D: diabetic + 100 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; E: diabetic + 150 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; F: diabetic + 5 mg/kg glibenclamide.

TABLE 3: Effect of PHTOSP on serum lipid profile of STZ-induced diabetic rats.

Group	TC	HDL	LDL (mg/dL)	TG	AI
A	132.53 ± 1.40^a	41.38 ± 1.81^b	62.90 ± 2.49^a	129.42 ± 1.49^a	1.52 ± 0.05^a
B	263.71 ± 1.61^b	26.92 ± 1.50^a	131.23 ± 2.40^b	259.34 ± 2.11^b	4.88 ± 0.07^b
C	154.31 ± 1.33^a	38.69 ± 1.28^b	76.62 ± 1.54^a	151.91 ± 1.60^a	1.97 ± 0.04^a
D	147.28 ± 1.50^a	40.77 ± 1.05^b	66.44 ± 1.33^a	142.41 ± 1.31^a	1.62 ± 0.05^a
E	150.12 ± 1.77^a	40.07 ± 2.90^b	70.74 ± 1.60^a	146.63 ± 2.01^a	1.76 ± 0.04^a
F	143.09 ± 1.20^a	43.38 ± 0.57^b	65.23 ± 1.16^a	132.19 ± 2.18^a	1.50 ± 0.05^a

Values are expressed as mean $\pm$ SD ($n = 6$). Alphabets (a and b) indicate statistical differences. Values in the same column with different letters are significantly ($p < 0.05$) different. A: normal control; B: diabetic control; C: diabetic + 50 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; D: diabetic + 100 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; E: diabetic + 150 mg/kg peptide hydrolysate of *T. occidentalis* seed protein; F: diabetic + 5 mg/kg glibenclamide.

a favorable comparison with the normal control and 5 mg/kg glibenclamide-treated group after 21 days.

3.8. Effect of PHTOSP on Hepatic Fructose-1,6-Biphosphatase Activity of STZ-Induced Diabetic Rats.

Figure 5 represents the effect of PHTOSP on hepatic fructose-1,6-biphosphatase activity of STZ-induced diabetic rats. As indicated in the results, there was a significant ($p < 0.05$) increase in the hepatic activity of fructose-1,6-biphosphatase of the untreated STZ-induced diabetic control compared to that of normal control. However, treatment with 50, 100, and 150 mg/kg PHTOSP resulted in a significant ($p < 0.05$) dose-dependent reversal compared to the untreated STZ-induced diabetic control group after 21 days, as well as a favorable comparison with the normal control group. Similarly, this

effect was favorably compared to the observation in the group treated with 5 mg/kg glibenclamide for 21 days.

3.9. Effect of PHTOSP on the Glucose-6-Phosphatase Activity of STZ-Induced Diabetic Rats.

Figure 6 reveals the effect of PHTOSP on the glucose-6-phosphatase activity of STZ-induced diabetic rats. The results demonstrate that the untreated STZ-induced diabetic control group displayed a significant ($p < 0.05$) increase in hepatic glucose-6-phosphatase activity compared to the normal control. However, treatment with PHTOSP at doses of 50, 100, and 150 mg/kg for 21 days caused a significant ($p < 0.05$) dose-dependent reduction in hepatic glucose-6-phosphatase activity compared to the untreated STZ-induced diabetic control and normal control groups. Furthermore, this observation
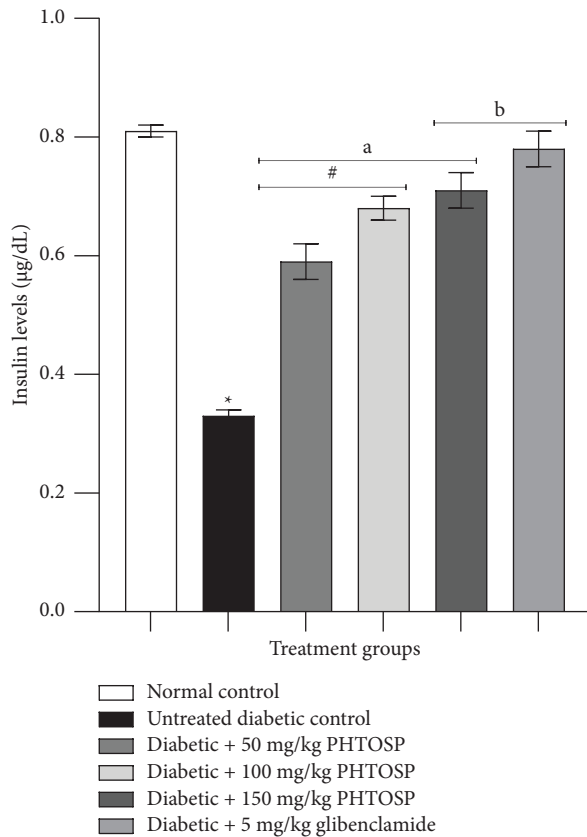
Figure 1: Effect of PHTOSP on the insulin levels of STZ-induced diabetic rats. Results were expressed as mean ± SD of six trials ($n = 6$). *A significant difference at $p < 0.05$ vs. normal control; #, a$p < 0.05$ vs. untreated diabetic control and vs. within treatment groups; b$p < 0.05$ vs. 150 mg/kg PHTOSP treated group.

showed a good comparison with the group treated with 5 mg/kg glibenclamide.

3.10. Effect of PHTOSP on Histopathological Status of the Pancreatic Tissue of STZ-Induced Diabetic Rats. In Figures 7(a)–7(f), the effect of PHTOSP on the histopathological condition of the pancreatic tissue of rats with STZ-induced diabetes is presented. The results indicate that the untreated STZ-induced diabetic control group (Figure 7(b)) exhibited pancreatic histology with pockets of congestion within the islet cells, which differs from the normal control (Figure 7(a)) that showed normal pancreatic histology characterized by normal islet cells, acinar cells (AC), and interstitium free from inflammatory cells. However, treatment with PHTOSP at doses of 50, 100, and 150 mg/kg for 21 days (Figures 7(c)–7(e)) revealed a pancreatic histology free from congestions and inflammatory cells with a favorable comparison with the normal and 5 mg/kg glibenclamide-treated groups.

4. Discussion

Several methods have been used to screen for antidiabetic activity of natural products and synthetic drugs in
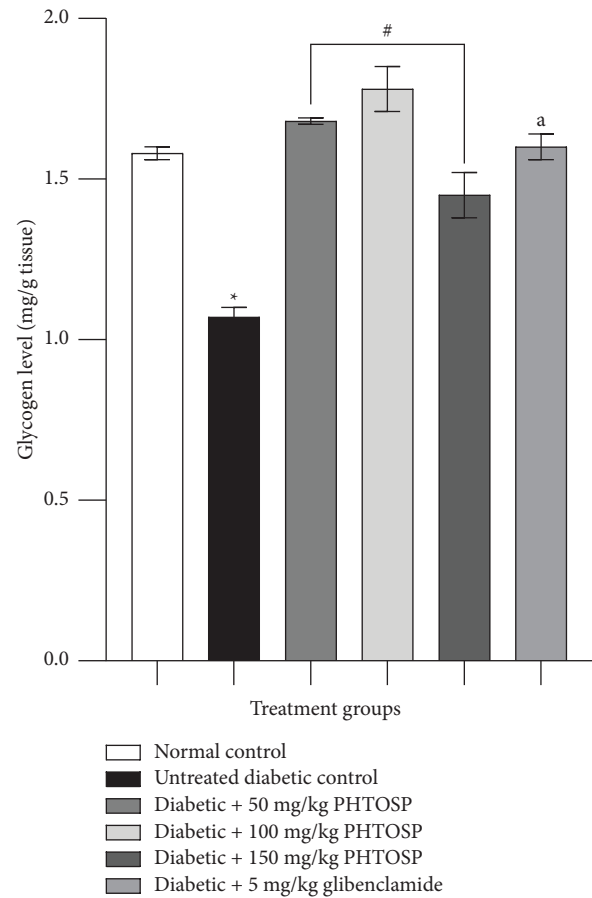

Figure 2: Effect of PHTOSP on the hepatic glycogen levels of STZ-induced diabetic rats. Results were expressed as mean ± SD of six trials ($n = 6$). *A significant difference at $p < 0.05$ vs. normal control; #$p < 0.05$ vs. untreated diabetic control; a$p < 0.05$ vs. untreated diabetic control and $p < 0.05$ vs. 150 mg/kg PHTOSP treated group.

experimental animal models following diabetes induction [45, 46]. STZ, a diabetogenic agent, well known for its selective pancreatic β-cell cytotoxicity is commonly used for the induction of diabetes [47]. It causes alkylation in the β-cell via glucose transporter 2 (GLUT 2) and thereby induces poly ADP-ribosylation, which causes cellular NAD$^+$ and ATP depletion [48]. As a result, pancreatic β-cell necrosis is induced by the generation of free radicals [49]. The increased glucose levels observed in the STZ-induced diabetic rats in this study (Table 1) may be due to the detrimental effect of STZ on pancreatic β-cells, resulting in a significant reduction in insulin secretion [50, 51]. However, administration of PHTOSP revealed a significant glucose-lowering effect, which could be credited to its ability to stimulate insulin secretion [52].

Uncontrolled diabetes has been linked to severe muscle breakdown and wasting, which can result in weight loss [53, 54]. The results in Table 2 indicate that untreated STZ-induced diabetic animals experienced significant weight loss, possibly due to STZ-induced muscle wasting as reported in previous studies [55, 56]. This weight loss could be attributed to insulin resistance or deficiency, which is known to
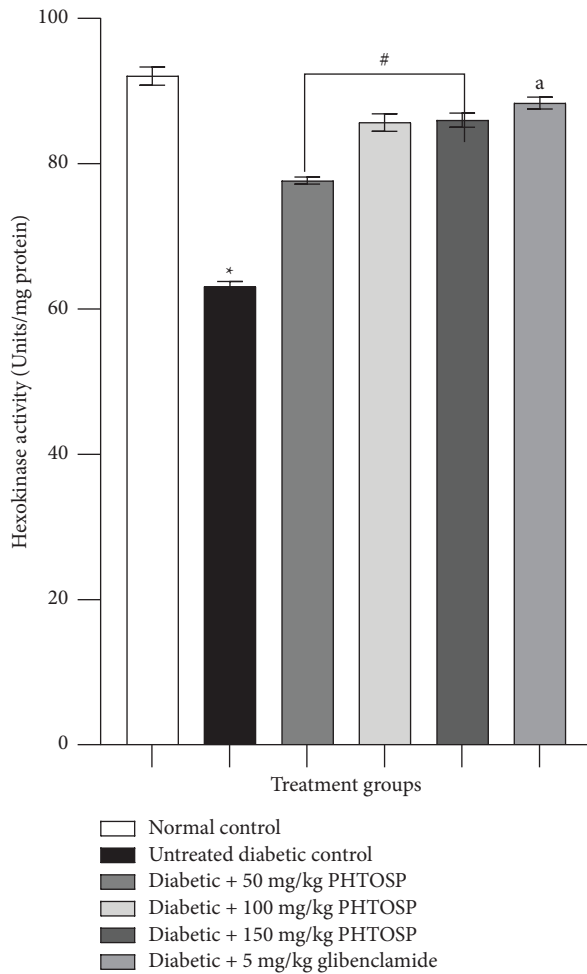
FIGURE 3: Effect of PHTOSP on the hepatic hexokinase activity of STZ-induced diabetic rats. Results were expressed as mean ± SD of six trials ($n = 6$). *A significant difference at $p < 0.05$ vs. normal control; #$p < 0.05$ vs. untreated diabetic control; a$p < 0.05$ vs. untreated diabetic control and $p < 0.05$ vs. PHTOSP treated groups.

FIGURE 4: Effect of PHTOSP on the hepatic glucose-6-phosphate dehydrogenase activity of STZ-induced diabetic rats. Results were expressed as mean ± SD of six trials ($n = 6$). *A significant difference at $p < 0.05$ vs. normal control; #$p < 0.05$ vs. untreated diabetic control; a$p < 0.05$ vs. untreated diabetic control and $p < 0.05$ vs. PHTOSP treated groups.

decrease muscle protein turnover and contribute to muscle wasting [57]. However, the observed increase in body weight after treatment with PHTOSP may be due to its ability to reduce muscle wasting, which may be exacerbated by STZ-induced destruction of pancreatic β-cells.

Lipids play a crucial role in the development of cardiovascular diseases (CVDs) that are associated with DM [58]. Disorder in lipid metabolism, often described as dyslipidemia, has been reported to contribute majorly to CVDs [59]. Also, according to recent studies, insulin resistance and islet dysfunction are significantly worsened by variations in the plasma and islet lipoprotein levels [60, 61]. According to the result in Table 3, there was an alteration in the levels of serum lipoproteins in the untreated diabetic control, which perhaps indicates STZ-induced diabetic dyslipidemia according to the report of Afolabi et al. [58]. More so, it has been reported in a previous study that changes in total cholesterol (TC), total triglycerides (TG), HDL/LDL ratio, and atherogenic index (AI) are indicators of susceptibility to CVDs in DM [62]. These changes have
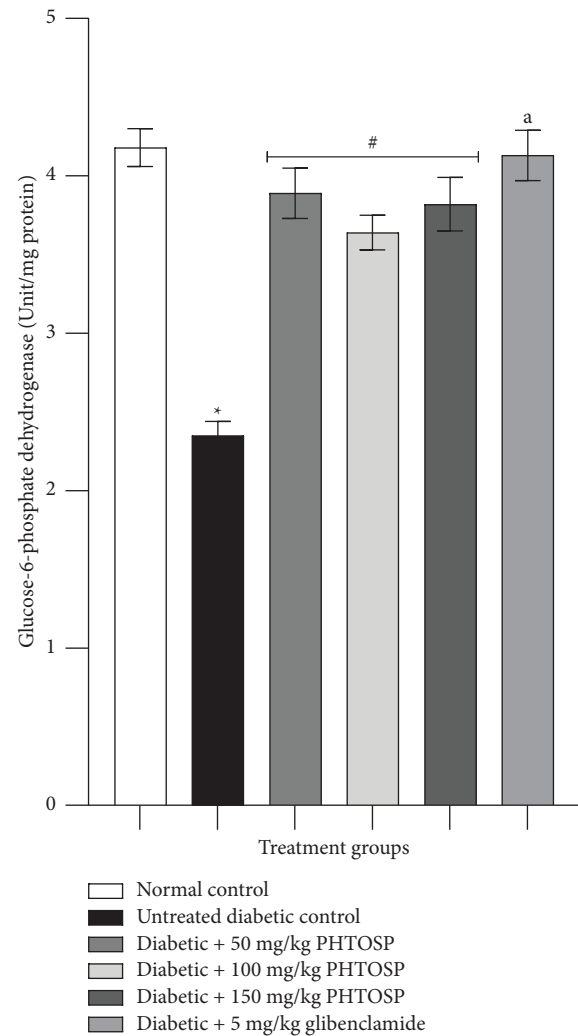
been linked to the activation of hepatic lipase, which leads to lipolysis. However, the administration of PHTOSP significantly mitigated the STZ-induced variations in lipoproteins in treated diabetic rats. This suggests that the hydrolysate may have the ability to enhance pancreatic release of insulin and reduce lipolysis-induced insulin resistance by inhibiting hepatic lipase [63]. Likewise, this study found that the untreated STZ-induced diabetic control group exhibited an increase in the AI, a lipid-related indicator of the potential for developing cardiovascular diseases such as arteriosclerosis [64]. However, the reduction in AI levels observed after PHTOSP administration may suggest the hydrolysate's effectiveness in preventing the risk of CVDs and related complications in STZ-induced diabetes, possibly by restoring normal lipid parameters. This effect aligns with the findings of Nasri et al. [65].
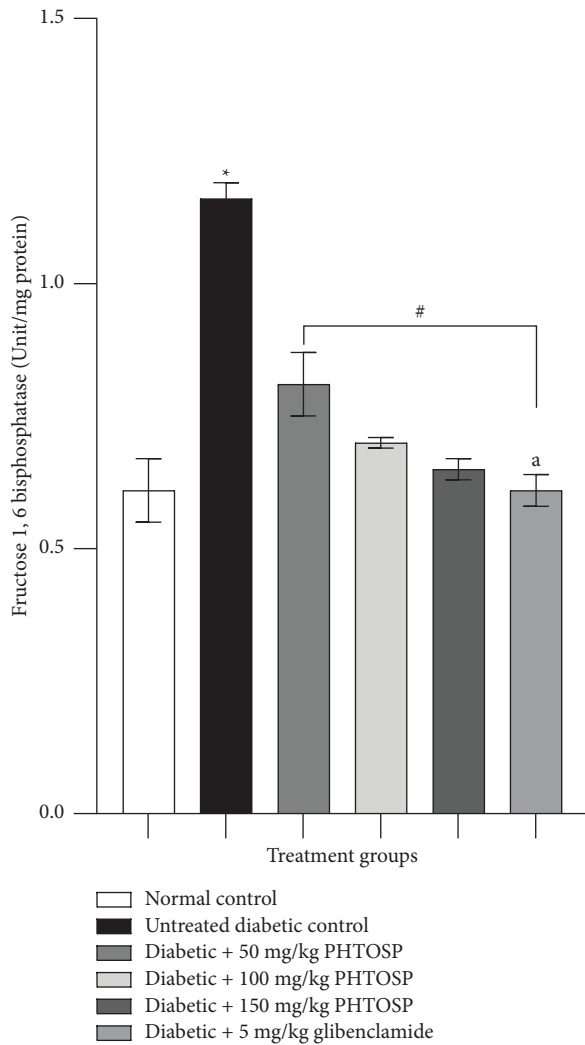
FIGURE 5: Effect of PHTOSP on hepatic fructose-1,6-biphosphatase activity of STZ-induced diabetic rats. Results were expressed as mean ± SD of six trials ($n = 6$). *A significant difference at $p < 0.05$ vs. normal control; #$p < 0.05$ vs. untreated diabetic control; a$p < 0.05$ vs. untreated diabetic control and $p < 0.05$ vs. PHTOSP treated groups.

FIGURE 6: Effect of PHTOSP on hepatic hepatic glucose-6-phosphatase activity of STZ-induced diabetic rats. Results were expressed as mean ± SD of six trials ($n = 6$). *A significant difference at $p < 0.05$ vs. normal control; #$p < 0.05$ vs. untreated diabetic control; a$p < 0.05$ vs. PHTOSP treated group.

It is also worthy of note that insulin regulates proper glucose homeostasis by enhancing its transport to peripheral tissues and muscles [66]. Chronic exposure of β-cells to high level of reactive oxygen species (ROS) has been shown to cause a progressive loss of β-cells and deterioration of its activities and failure [67]. In this condition, the capacity of the β-cell to produce sufficient insulin needed for proper glucose uptake in the physiological milieu is hampered [68]. As indicated in the result (Figure 1), there was a reduction significantly in insulin levels of the untreated STZ-induced diabetic control, an effect that could possibly reflect STZ-induced pancreatic β-cell dysfunction [69]. However, oral administration of PHTOSP evidently reversed this condition either by the ability of the hydrolysate to stimulate proper secretion of insulin or mitigate STZ-occasioned ROS implicated in the deleterious activities of STZ, thereby protecting the β-cell from STZ-induced oxidative damage [70].
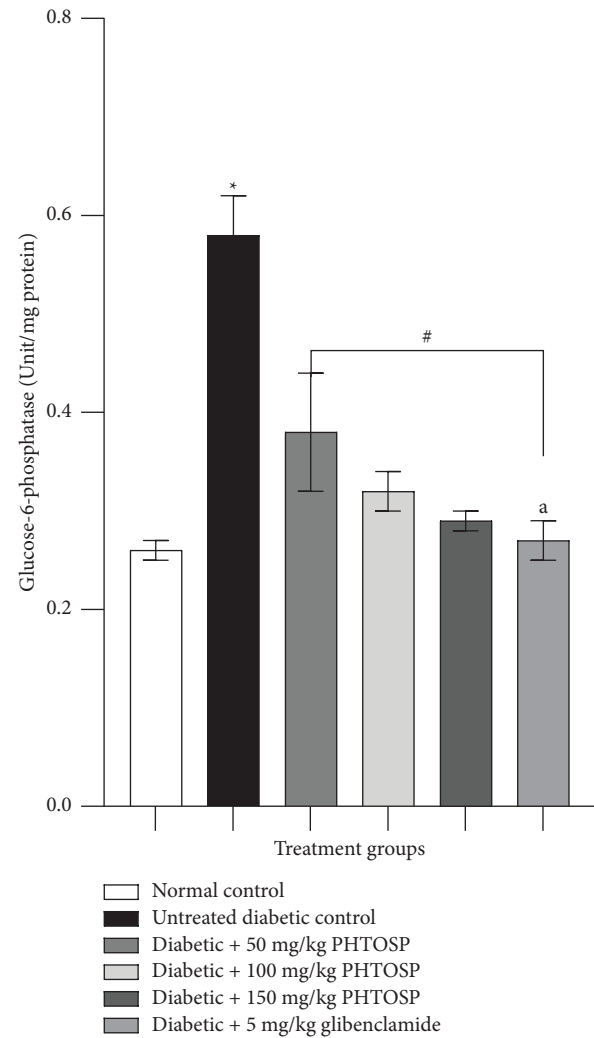
Hepatocytes are the major site for the storage and metabolism of glycogen [71]. Glycogen is a branched polymer of glucose, which acts as a store for glucose units [72]. Imbalances in the hepatic glycogen accumulation and production of glucose through glycogenolysis have been reported in DM [73, 74]. The decrease observed in the hepatic glycogen levels among the STZ-induced diabetic control could possibly be a reflection of STZ-stimulated insulin reduction in insulin, resulting in uncontrollable glycogenolysis probably by activating hepatic glycogen phosphorylase activity [75]. More so, an increase in glycogen phosphorylase and failure of glycogen synthase activating mechanism has been documented in DM [76]. However, treatment with PHTOSP significantly improved hepatic glycogen possibly by its ability to potentiate glycolysis and thereby enhancing secretion of insulin as well as inhibiting the first rate-limiting enzyme of glycogenolysis. The finding of this report is consistent with the findings of Chatterjee et al. [77].
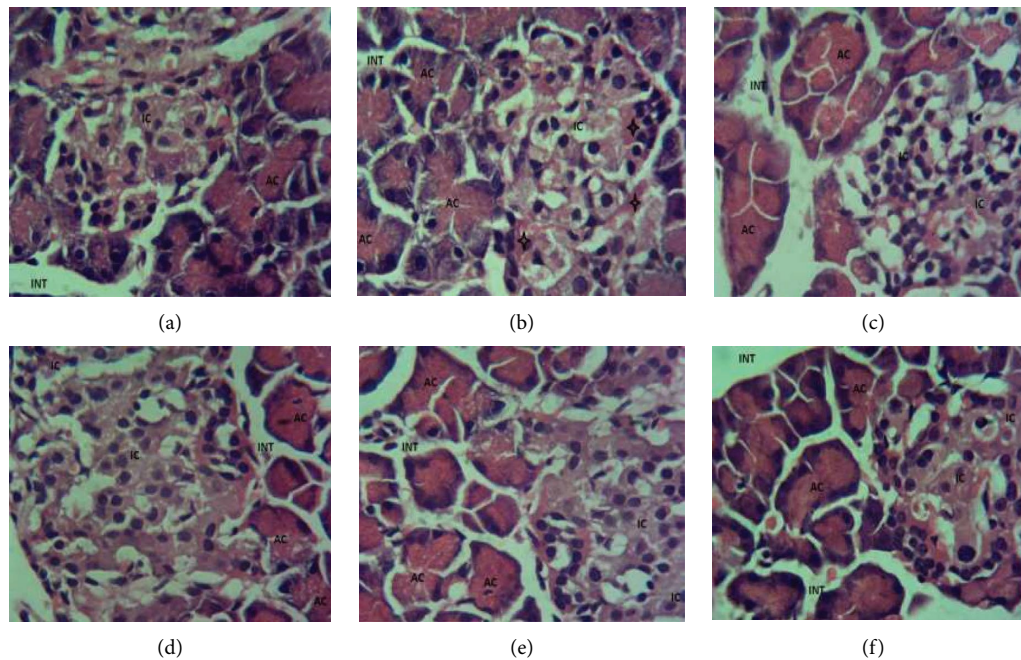
Figure 7: (a–f) Effect of PHTOSP on pancreas histoarchitectural status of STZ-induced diabetic rats (stained with H&E, Mag. ×400). INT, interstitium; AC, acinar cells; IC, islet cells. (a) normal control; (b) untreated diabetic control; (c) diabetic + 50 mg/kg PHTOSP; (d) diabetic + 100 mg/kg PHTOSP; (e) diabetic + 150 mg/kg PHTOSP; (f) diabetic + 5 mg/kg b.w glibenclamide.

A defect in carbohydrate-metabolizing enzymes as a result of deteriorated endocrinal control has been implicated in DM [78]. Hexokinase is a rate-limiting enzyme of glycolysis and plays a central role in the maintenance of glucose homeostasis by catalyzing the phosphorylation of glucose to glucose-6-phosphate [79]. As observed in our study, a reduction was noticed in the activity of this protein among the untreated STZ-induced diabetic control, an effect that could be linked to the STZ-induced glucose metabolic derangement [2]. Besides, a decrease in the activity of hexokinase being an insulin-dependent enzyme has been reported in DM [80]. In the result, the administration of PHTOSP however significantly increased hexokinase activity. This observation might possibly suggest the ability of the extract to restore proper glucose utilization via glycolysis resulting in the reduction of blood glucose [81].

Glucose-6-phosphate dehydrogenase (G6PDH) is the rate-limiting enzyme in the pentose phosphate pathway (PPP), which leads to the production of ribose-5-phosphate and NADPH equivalents [82]. The PPP is an alternate pathway for glucose-6-phosphate from glycolysis, and NADPH is the only source of antioxidant defense mechanisms, heavily relying on G6PDH activity [83]. Our findings showed a reduction in hepatic G6PDH activity in untreated STZ-induced diabetic rats, which is consistent with the report of Karuna et al. [84]. Additionally, previous studies have reported a reduction in hepatic G6PDH activity in DM [85], leading to a decrease in the production of NADPH equivalents necessary for maintaining cellular reduced glutathione [86].

The decrease in hepatic G6PDH activity in untreated STZ-induced diabetic rats leads to an accumulation of glucose-6-phosphate, which acts as a potential glycosylating agent that exacerbates GSH depletion and promotes the final step of gluconeogenesis. This ultimately results in oxidative auto-glycation [87, 88]. However, administration of PHTOSP in different doses to diabetic groups led to a significant increase in hepatic G6PDH activity (Figure 4). This suggests that the hydrolysate has the ability to enhance the activation of hepatic G6PDH, possibly through a mechanism that promotes cell proliferation and survival and restores proper insulin-mediated hepatic glucose oxidation and PPP. This pathway may play a role in regulating glucose levels in DM [89].

Gluconeogenesis is the primary cause of an increased hepatic glucose release in diabetic condition [90]. As seen in this report, fructose-1,6-biphosphatase, a rate-controlling enzyme of gluconeogenesis [90], and glucose-6-phosphatase that catalyzes the final stage of hepatic glucose production were significantly increased among the untreated STZ-induced diabetic rats. Previous documented reports have shown that the absence of insulin in DM results in the activation of these gluconeogenic enzymes [91, 92]. In a normal physiological condition, insulin acts as a suppressor of hepatic gluconeogenic enzymes [93]. However, following the administration of PHTOSP, there was a substantial reduction in the activities of these enzymes, an effect that perhaps signals the ability of the hydrolysate to control hepatic glucose production, thus suppressing gluconeogenesis possibly via a mechanism that stimulates insulin secretion and facilitates proper glucose utilization in the peripheral tissues [94].

Histological changes in pancreatic islet have previously been noted in STZ-treated experimental animals [95]. These

alterations are reportedly mediated by the reduction in nicotinamide adenine dinucleotide (NAD^+) of the β-cells [96]. As shown in this study, a marked vacuolation of Langerhans islets with β-cells revealing an unstained vacuolated cytoplasm and dark-stained degenerated nuclei was noticeable in the pancreatic tissues of the untreated STZ-induced diabetic rats, an effect that could possibly indicate STZ-occasioned inflammation and cytotoxicity to the β-cells according to a previous report of Sangi et al. [97] and Gundala et al. [98]. However, STZ-induced diabetic groups treated with PHTOSP showed a reversal of the STZ-induced cytotoxic effect with a normal population of β-cells in the islets of Langerhans. This observation could also suggest cytoprotective ability of the hydrolysate against STZ-induced pancreatic oxidative damage.

5. Conclusion

This study evaluated the possible antidiabetic potentials of peptide hydrolysate of *Telfairia occidentalis* seed protein (PHTOSP) in streptozotocin-induced diabetic rats through the assessment of some diabetes-related biochemical parameters and pancreatic histological examination. According to our findings, PHTOSP lowered fasting serum glucose and improved insulin levels, lipid profile, and liver parameters such as glycogen contents and glycolytic enzymes as well as suppressed gluconeogenetic enzymes. Similarly, PHTOSP modulated body weight and pancreatic histo-architecture. Therefore, PHTOSP might possess drug-able antidiabetic properties such that it restores glycolytic pathway and promotes cell survival that are helpful in the management of diabetes mellitus (DM).

Abbreviations

PHTOSP:	Peptide hydrolysate of *Telfairia occidentalis* seed protein
DM:	Diabetes mellitus
STZ:	Streptozotocin
G6PDH:	Glucose-6-phosphate dehydrogenase
PPP:	Pentose phosphate pathway
GSH:	Reduced glutathione
NADPH:	Nicotinamide adenine dinucleotide phosphate (reduced)
ROS:	Reactive oxygen specie
M:	Molar
AI:	Atherogenic index
CVDs:	Cardiovascular diseases
w/v:	Weight per volume
ATP:	Adenosine triphosphate
SEM:	Standard error of mean
FSG:	Fasting serum sugar
mg/kg:	Milligram per kilogram
min:	Minute
GLUT 2:	Glucose transporter 2.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Olasehinde Oluwaseun Ruth and Arise Rotimi Olusanya conceptualized the research. Olasehinde Oluwaseun Ruth and Afolabi Olakunle Bamikole were responsible for data curation. Olasehinde Oluwaseun Ruth, Arise Rotimi Olusaanya, and Afolabi Olakunle Bamikole were responsible for reagents and analytical tools. Arise Rotimi Olusanya supervised the study. Olasehinde Oluwaseun Ruth and Afolabi Olakunle Bamikole drafted the original manuscript. All authors were responsible for formal analysis and data validation and reviewed, edited, and confirmed the authorship of the final manuscript.

Supplementary Materials

This illustrates STZ-induced diabetic rats administered with peptide hydrolysate of *Telfairia occidentalis* (*T. occidentalis*) seed protein (PHTOSP). Evaluated biochemical parameter indicated an increase in serum insulin, hepatic glycogen, hexokinase, and glucose-6-phosphate dehydrogenase activities, accompanied by a reduction in fasting serum glucose, glucose-6-phosphatase, and fructose-1,6-bisphosphatase. Also, rats treated with PHTOSP demonstrated an improved lipid profile and relative body weight. (*Supplementary Materials*)

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