

Research Article

Antihyperglycemic, Antidiabetic, and Antioxidant Effects of *Garcinia pedunculata* in Rats

Md. Yousuf Ali,¹ Sudip Paul,^{1,2} E. M. Tanvir,^{1,3} Md. Sakib Hossen,^{1,4}
Nur-E Noushin Rumpa,¹ Moumoni Saha,¹ Nikhil Chandra Bhoumik,⁵ Md. Aminul Islam,¹
Md. Sabir Hossain,¹ Nadia Alam,⁶ Siew Hua Gan,⁶ and Md. Ibrahim Khalil^{1,6}

¹Laboratory of Preventive and Integrative Biomedicine, Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh

²Metabolomics Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC 3004, Australia

³Veterinary Drug Residue Analysis Division, Institute of Food & Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka 1349, Bangladesh

⁴Department of Biochemistry, Primeasia University, Banani, Dhaka 1213, Bangladesh

⁵Wazed Miah Science Research Center, Jahangirnagar University, Dhaka 1342, Bangladesh

⁶Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Correspondence should be addressed to Md. Ibrahim Khalil; drmikhalil@gmail.com

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The antihyperglycemic, antidiabetic, and antioxidant potentials of the methanolic extract of *Garcinia pedunculata* (GP) fruit in rats were investigated. The acute antihyperglycemic effect of different doses of GP was studied in normal male Wistar rats. Diabetes was induced by streptozotocin (STZ) injection in another cohort of male Wistar rats and they showed significantly higher blood glucose and glycated hemoglobin (HbA1c) levels, altered lipid profiles, and lower insulin levels compared to nondiabetic control animals. There were increased lipid peroxidation and reduced levels of cellular antioxidant enzymes in different tissues of diabetic rats. However, oral administration of GP extracts, especially the highest dose (1000 mg/kg), significantly ameliorated hyperglycemia (42%); elevated insulin levels (165%); decreased HbA1c (29.4%); restored lipid levels (reduction in TG by 25%, TC by 15%, and LDL-C by 75% and increase in HDL-C by 4%), liver and renal function markers, and lipid peroxidation (reduction by 52% in the liver, 39% in the kidney, 44% in the heart, and 46% in the pancreas); and stimulated tissue antioxidant enzymes to near normalcy. Overall, the findings suggest that GP fruit is effective against hyperglycemia and could be used in the treatment of diabetes and its complications and other oxidative stress-mediated pathological conditions.

1. Introduction

Diabetes mellitus (DM) is a multifarious, degenerative endocrine disease associated with reduced insulin secretion and activity due to damage to pancreatic β -cells and/or reduced insulin sensitivity, ultimately affecting carbohydrate, fat, and protein metabolism [1]. DM is a growing public health concern all over the world. The WHO has declared that approximately 220 million people will be affected in the year 2020 [2, 3]. Hence, the issue of how to control this complex disorder warrants much concern.

Several antidiabetic drugs are available, but all of them have many adverse side effects such as lactic acidosis, hyperglycemia, diarrhea, or flatulence, which impose an economic burden [4–6]. Therefore, researchers around the world are doing extensive research to find alternative therapies for DM with low side effects and low cost. Medicinal plants and their products have been considered an excellent source for alternative medicine to treat DM by virtue of their active phytochemical constituents. The benefits of a natural medicinal product may be due to a single phytochemical or, more preferably, a synergistic effect of multiple phytochemicals.

To date, more than 800 medicinal plants have been reported to show antihyperglycemic activity [7]. The most common benefits of medicinal plants are better safety profiles, good effectiveness, wide availability, acceptability, and affordability [8].

Garcinia pedunculata (GP) is an indigenous medicinal plant that belongs to the family Clusiaceae and is commonly known as “Taikor” in Bangladesh and “Amlavetasa” in India. Availability is especially common in Sylhet district in Bangladesh and in Assam, Arunachal Pradesh, India. The mature GP fruit is greenish yellow and is consumed as a cooked vegetable, a raw fruit, or an ingredient for pickle preparation [9]. It has been used locally for the ailments of chronic catarrh, asthma, cough, bronchitis, dysentery, maldigestion, and fever [10]. The fruit extract has also been reported to possess a variety of beneficial effects such as antioxidant [9, 11], antimicrobial [12], anti-inflammatory [13], hypolipidemic [14], hepatoprotective [15], nephroprotective, and cardioprotective activity [16]. GP fruit has been reported to contain moisture (88.20%), protein (0.50%) β -carotene (45.00 mg/100 g), thiamine (0.03 mg/100 g), and riboflavin (0.02 mg/100 g) as well as minerals (sodium 1.80, potassium 106.00, calcium 18.00, magnesium 23.00, iron 0.08, zinc 0.15, manganese 0.23, copper 0.12, and phosphorus 17.00 mg/100 g, resp.) [9]. Recently, the antioxidant constituent of the fruit was analyzed and was reported to contain phenolics (5.86 mg/g of catechin), flavonoids (5.60 mg/g of quercetin), and total antioxidant activity (504 nmol/g of ascorbate) as well as 6.67 mg of anthocyanin and 142.83 mg of ascorbic acid per 100 g, respectively [9, 14]. In addition, the presence of a number of benzophenones including pedunculol, garcinol, and cambogin and other organic acids, such as citric acid, hydroxycitric acid, hydroxycitric acid lactone, and oxalic acid has also been identified [17, 18]. Further insights into GP's efficacy as free radical scavengers and capacity to protect cells from lipid peroxidation have been confirmed in several studies, thus uncovering its medicinal importance related to chronic or degenerative diseases including diabetes [9, 19].

In our previous study, we reported the potentials of GP fruit extracts in the regulation of blood glucose level and positive effects on pancreatic and liver functions as well as lipid profile in rats [20].

Therefore, considering the traditional use of the fruit as an alternative medicine and its inherent phytoconstituents, we aim to evaluate the antihyperglycemic, antidiabetic, and antioxidant effects of a GP fruit extract in rats and also to identify its potential compounds using high-performance liquid chromatography (HPLC) analysis.

2. Materials and Methods

2.1. Chemicals. Streptozotocin (STZ) and methanol were purchased from Sigma (Sigma-Aldrich, St. Louis, USA). Glibenclamide was purchased from Square Pharmaceuticals Ltd., Bangladesh. Ketamine was acquired from Popular Pharmaceuticals Ltd., Dhaka, Bangladesh. Glucose oxidase/peroxidase reactive strips were purchased from Abbott Diabetes Care, Inc., USA. All chemicals and reagents used in this experiment were of analytical grade.

2.2. GP Fruit Sample Preparation. Fresh, mature GP fruits were collected from Sylhet district of Bangladesh in March 2016. A methanolic fruit extract (25%) was prepared according to a method described by Lanjhiyana et al. [21] with slight modifications. Briefly, the fresh, cleaned mature fruits were thoroughly rinsed with cold sterile water. The edible parts were isolated carefully, cut into small pieces using a sharp stainless steel knife, and dried in the sunlight. When the samples were free from moisture and appeared crunchy, they were ground into a fine powder using a blender (Jaipan Commando, Mumbai, India).

The fine powder (200 g) was mixed with sufficient absolute methanol to dissolve it (800 mL) and was kept in a shaker (IKA400i, Germany) at 150 rpm and 30°C for 72 hours. The mixture was filtered with Whatman No. 1 filter paper, and the methanol solvent was completely evaporated using a rotary evaporator (R-215, BUCHI, Switzerland) under reduced pressure (100 psi) at a controlled temperature (40°C). The concentrated extract was preserved at –20°C for subsequent use.

2.3. HPLC Analysis of Phenolic Compounds. The phenolic compounds in GP extract were detected following previous methods [22, 23] with slight modifications. Briefly, 5% GP extract solution was prepared in methanol, followed by filtration through a 0.45 μ m syringe filter (Sartorius AG, Germany). 20 μ L of the filtrate was loaded onto the HPLC system (SPD-20AV, serial number: L20144701414AE, Shimadzu Corporation, Kyoto, Japan) equipped with a UV detector (SPD-20AV, serial number: L20144701414AE, Shimadzu Corporation, Kyoto, Japan). A Luna Phenomenex C18 100A (150 \times 4.60 mm, 5 μ m) HPLC column was used. A linear gradient at a flow rate of 0.5 mL/min was employed throughout with a total analytical time of approximately 35 min.

The binary mobile phase consisted of solvent A (ultrapure water with 0.1% phosphoric acid) and solvent B (pure methanol with 0.1% phosphoric acid). Elution from the column was achieved with the following gradient: 0 to 10 min of solvent B, increased from 35% to 55%; 10 to 25 min of solvent B, increased to 62%; 25 to 30 min of solvent B, increased to 85%; and the final composition was kept constant up to 35 min. All solvents were of HPLC grade. The detection wavelength was fixed between 200 and 450 nm, with specific monitoring conducted at 265 nm. The identification of phenolic and flavonoid compounds was performed by comparing the retention times of the analytes with the reference standards. Phenolics which included tannic, gallic, pyrogallol, vanillic, benzoic, and *trans*-cinnamic acids as well as flavonoids (including catechin, naringin, rutin, and quercetin) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and were used as reference standards.

2.4. Experimental Animals. Male Wistar rats ($n = 48$) weighing between 175 and 250 g (aged 16–18 weeks) were used in this study. The animals were maintained under controlled environmental conditions in an animal housing facility at the Department of Biochemistry and Molecular Biology, Jahangirnagar University, at an ambient temperature of 22 \pm 3°C and relative humidity of 44% to 56% under a 12 h/12 h light/dark cycle. They were fed with a standard pellet rat diet

along with water *ad libitum*. The study was conducted in accordance with the internationally established principles of the US guidelines (NIH publication #85–23, revised in 1985). The experimental protocol was approved by the Biosafety, Biosecurity & Ethical Committee of Jahangirnagar University.

2.5. Induction of Diabetes in Experimental Animals. Induction of diabetes in experimental animals was done after 8–10 hours of fasting by intraperitoneal injection of STZ dissolved in 0.1 M cold citrate buffer, pH 4.5, at a single dose of 60 mg/kg [24]. After STZ injection, the animals were allowed to drink a 10.0% glucose solution overnight to overcome the initial drug-induced hypoglycemia. After seven days, rats with glycemia ≥ 250 mg/dL were selected for further experimentation.

2.6. Experimental Design

2.6.1. Evaluation of the Antihyperglycemic Effect of GP Extracts in Normal Rats. An oral glucose tolerance test (OGTT) was performed to observe the acute antihyperglycemic effects of the GP fruit extracts using different sets of normal rats. Five groups of six rats each were used in this study. Briefly, group 1 served as a normal control; group 2 served as a glibenclamide control, orally treated once with glibenclamide at 10 mg/kg; and groups 3, 4, and 5 were orally treated once with fruit extracts (250, 500, and 1000 mg/kg, resp.).

In overnight-fasted rats, fasting blood glucose level was measured; this time point was defined as 0 min. Then, different doses of the fruit extract (250, 500, and 1000 mg/kg) were administered orally to the appropriate groups of rats. Blood glucose levels were measured 30 min later. The animals were then given glucose solution (2 g/kg) orally, and their glucose tolerance was measured hourly. Blood glucose levels were estimated using glucose oxidase/peroxidase reactive strips.

2.6.2. Experimental Groups for Antidiabetic Study. A total of 48 male Wistar rats were acclimatized one week prior to the experiment and randomly divided into six groups (each group containing 8 rats) as follows:

Group 1 (Normal Control). Animals receiving only a normal diet for 28 days.

Group 2 (Diabetic Control). Diabetic animals receiving a normal diet for 28 days.

Group 3 (Diabetic + Glibenclamide). Diabetic animals treated with glibenclamide at a dose of 10.0 mg/kg body weight for 28 days.

Group 4 (Diabetic + GP 250). Diabetic animals treated with methanolic extract of GP at a dose of 250 mg/kg body weight for 28 days.

Group 5 (Diabetic + GP 500). Diabetic animals treated with methanolic extract of GP at a dose of 500 mg/kg body weight for 28 days.

Group 6 (Diabetic + GP 1000). Diabetic animals treated with methanolic extract of GP at a dose of 1000 mg/kg body weight for 28 days.

All animals received a standard laboratory diet and drinking water *ad libitum* during the experimental period. In addition, the rats' body weights and fasting blood glucose levels were measured on a weekly basis. The animals were also monitored for any behavioral changes during the study period.

2.6.3. Evaluation of the Antihyperglycemic Effect of GP Extracts in Rats with STZ-Induced Diabetes. An OGTT was performed to investigate the effects of the GP fruit extracts on glucose-induced hyperglycemia in rats with STZ-induced diabetes. The blood glucose levels were measured in overnight-fasted rats at a time point designated as 0 min. The animals were then orally dosed with glucose solution (2 g/kg) using a gavage needle, and their glucose tolerance was measured hourly for 4 hours. Blood glucose levels were estimated by glucose oxidase/peroxidase reactive strips.

2.7. Sacrifice of Animals. At the end of the experimental period, all rats were fasted for 15 hours before being sacrificed by intraperitoneal injection of ketamine hydrochloride (500 mg/kg) [25]. Blood samples (5 mL) were collected from the inferior vena cava. In addition, selected tissue samples including the liver, kidneys, heart, and pancreas were immediately excised from the surrounding tissues and were subsequently weighed.

The relative organ weight was calculated by dividing the individual weight of each organ by the final body weight of each rat according to the following formula [26]: relative organ weight (%) = wet organ weight/body weight $\times 100$.

2.8. Serum Preparation. Blood samples (4 mL) were placed in dry test tubes and were allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min.

2.9. Tissue Homogenate Preparation. Immediately following collection, the tissue samples were washed with ice-cold phosphate-buffered saline (PBS). The samples were homogenized in phosphate buffer (25 mM, pH 7.4) to make approximately 10% w/v homogenates. The homogenates were centrifuged at 1700 rpm for 10 min, and the supernatant was collected and stored at -20°C for further biochemical analysis. Part of each tissue sample was preserved in formalin solution (10%) for histopathological examination.

2.10. Biochemical Analysis of Serum. Biochemical parameters for liver function such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ -glutamyltransferase (GGT), total bilirubin (TB), total protein (TP), albumin (ALB), globulin (GLB), and albumin/globulin (A/G) ratio; parameters for kidney function such as creatinine, urea, and uric acid; and parameters for lipid profile including total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were determined following standard procedures using an automated chemistry analyzer (Dimension EXL with LM Integrated Chemistry System, Siemens Medical Solutions Inc., USA). Another lipid profile parameter, serum

low-density lipoprotein cholesterol (LDL-C) level, was estimated based on the following Friedewald formula [27]:

$$\text{LDL-C} = \frac{\text{TC} - \text{HDL-C} - \text{TG}}{5}. \quad (1)$$

Serum insulin level was measured using a rat-specific insulin ELISA assay kit, while hemoglobin (Hb) level was analyzed using an automated hematology analyzer (8000i, Sysmex, Japan). Glycosylated hemoglobin (HbA1c) levels were measured in whole blood using an automated chemistry analyzer (Dimension EXL with LM Integrated Chemistry System, Siemens Medical Solutions Inc., USA).

2.11. Biochemical Analysis of Tissue. Malondialdehyde (MDA) levels were analyzed to detect lipid peroxidation (LPO) products in the liver, kidney, heart, and pancreas tissues of normal and treated rats. MDA is referred to as a thiobarbituric acid reactive substance (TBARS) and was measured with thiobarbituric acid (TBA) at 532 nm according to the method described by Ohkawa et al. [28]. The levels of TBARS were expressed as mmol of MDA per mg of protein.

The levels of endogenous antioxidant or antiperoxidative enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), were determined in the rats' liver, kidney, heart, and pancreas tissues using rat-specific SOD and GPx ELISA assay kits (CUSABIO, USA). To this end, the tissue homogenates were recentrifuged at 12,000 rpm for 10 min at 4°C using an Eppendorf 5415D centrifuge (Hamburg, Germany). The clean supernatants were used for analysis. The levels of SOD and GPx were expressed as pg/mL and mIU/mL, respectively.

2.12. Histopathological Analysis. The tissue samples were fixed in 10% neutral formalin and processed by the paraffin embedding technique. The histopathological specimens were cut into 5 µm thick sections using a rotary microtome and subsequently stained with hematoxylin and eosin (H&E) dye [29]. Photomicrographs at 6100x magnification were captured by a normal-spectrum fluorescence microscope (Olympus DP 72) with an attached digital camera (Olympus, Tokyo, Japan). The pathologist performing the histopathological evaluation was blinded to the treatment assignments of the different study groups.

2.13. Statistical Analysis. All results were represented as the mean ± standard deviation (SD). Data were analyzed using Microsoft Excel 2007 (Redmond, Washington, USA), GraphPad Prism version 6 (GraphPad Software, Inc., USA), and SPSS (Statistical Package for the Social Sciences, version 16.0, IBM Corporation, New York, USA). All the data from the treatment groups (including Diabetic + Glibenclamide, Diabetic + GP 250, Diabetic + GP 500, and Diabetic + GP 1000) were compared with the results from the diabetic control group using a one-way ANOVA followed by Dunnett's multiple comparison test. A *p* value of less than 0.05 was considered statistically significant.

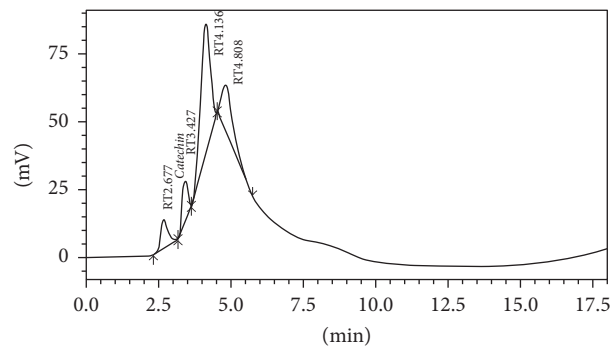


FIGURE 1: Chromatogram of GP extract showing the presence of catechin (RT: 3.427). RT: retention time.

3. Results

3.1. HPLC Analysis. A total of eleven phenolic standards were used in this study in which seven were phenolic acids and four were flavonoid compounds. Only a flavonoid and catechin (13.59 mg/g) were detected in GP extract while some other peaks remained unidentified since there were no proper standards available (Figure 1).

3.2. Acute Effects of GP Extract on Glucose-Induced Hyperglycemia in Normal Rats. Blood glucose levels returned to baseline or even lower 1 hour after glucose administration in all animals. Animals treated with glibenclamide and fruit extracts showed marked decreases in blood glucose level compared with normal control animals, beginning only 1 hour after glucose administration. In particular, the rats given glibenclamide and GP at 500 and 1000 mg/kg doses showed significant reductions in blood glucose level compared with the normal control rats at each time point (Table 1).

3.3. The Effects of GP Extract on Glucose-Induced Hyperglycemia in Rats with STZ-Induced Diabetes. Blood glucose levels were significantly higher in rats with STZ-induced diabetes than in normal control rats one hour after oral administration of glucose solution. However, the administration of glibenclamide and GP at three doses caused gradual decreases in the blood glucose levels compared with those of diabetic control rats. The effects were significant in rats treated with glibenclamide and GP extract at 1000 mg/kg as observed up to 4 hours after treatment (Table 2).

3.4. The Effects of GP Extract on Body Weight and Relative Organ Weight Parameters. The effects of GP fruit extract on body weight and relative organ weights are summarized in Figure 2 and Table 3, respectively. During the experimental period, the body weight of diabetic control rats gradually decreased; however, treatment with GP extract and glibenclamide improved the body weight of the animals. Rats treated with GP at 1000 mg/kg showed a significant increase in the body weight on the last week of the experiment. On the other hand, relative organ weights were increased in diabetic control rats but decreased in GP-treated rats. Administration of GP extract at a dose of 1000 mg/kg significantly reduced

TABLE 1: The effects of GP fruit extract and glibenclamide on glucose-induced hyperglycemia in normal rats.

| Groups | Blood glucose level (mg/dL) | | | | | |
|-----------------|-----------------------------|---------------|---------------|-----------------|---------------|---------------|
| | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h |
| Control | 108.0 ± 17.10 | 106.2 ± 14.58 | 109.8 ± 13.50 | 124.20 ± 9.90 | 115 ± 10.44 | 112.6 ± 8.82 |
| Glibenclamide | 104.40 ± 4.86 | 51.48 ± 4.50* | 71.28 ± 2.70* | 76.14 ± 4.50* | 67.68 ± 4.50* | 54.54 ± 2.07* |
| GP (250 mg/kg) | 102.60 ± 1.98 | 90.00 ± 7.74 | 104.40 ± 2.07 | 117.00 ± 4.50 | 113.4 ± 4.51 | 104.4 ± 1.03 |
| GP (500 mg/kg) | 99.00 ± 14.04 | 87.48 ± 10.44 | 94.14 ± 7.27 | 101.88 ± 10.80* | 99 ± 6.48* | 95.40 ± 6.48* |
| GP (1000 mg/kg) | 104.40 ± 1.98 | 80.64 ± 4.50* | 101.34 ± 9.0 | 108.59 ± 4.50* | 101.98 ± 3.6* | 98.28 ± 5.49* |

Data are represented as the mean ± SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

TABLE 2: The effects of GP fruit extract and glibenclamide on glucose-induced hyperglycemia in rats with STZ-induced diabetes.

| Groups | Blood glucose level (mg/dL) | | | | |
|----------------------------|-----------------------------|----------------------------|-----------------|----------------------------|----------------------------|
| | 0 h | 1 h | 2 h | 3 h | 4 h |
| Normal Control | 110.31 ± 4.05 [#] | 115.12 ± 4.17 [#] | 113.32 ± 3.21 | 111.00 ± 6.41 [#] | 110.72 ± 5.46 [#] |
| Diabetic Control | 335.43 ± 19.05 | 353.46 ± 21.11 | 359.00 ± 16.30 | 351.4 ± 19.44 | 347.80 ± 27.81 |
| Diabetic + Glibenclamide | 421.90 ± 36.08 | 456.40 ± 45.18* | 385.40 ± 45.18* | 356.00 ± 16.48* | 334.68 ± 20.45* |
| Diabetic + GP (250 mg/kg) | 435.20 ± 38.07 | 467.40 ± 31.31* | 435.40 ± 39.52 | 415.20 ± 26.20 | 407.84 ± 32.38 |
| Diabetic + GP (500 mg/kg) | 343.80 ± 18.50 | 374.60 ± 29.56* | 359.50 ± 21.48 | 335.20 ± 9.45 | 325.30 ± 8.30 |
| Diabetic + GP (1000 mg/kg) | 320.80 ± 15.23 | 351.60 ± 12.09* | 324.40 ± 18.72* | 304.40 ± 35.43* | 276.48 ± 24.82* |

Data are represented as the mean ± SD ($n = 6$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group; # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

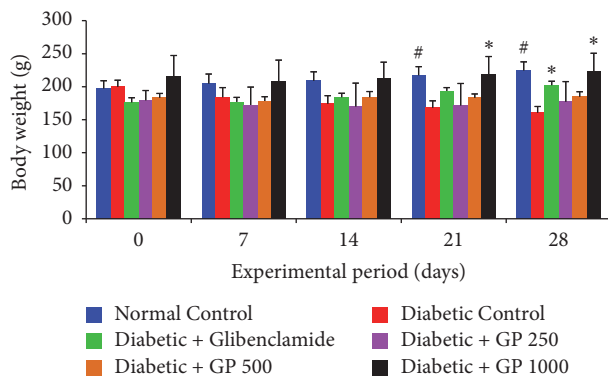


FIGURE 2: The effects of GP fruit extract and glibenclamide on body weight in normal and diabetic rats. Data are represented as the mean ± SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

the relative weights of the liver, kidney, heart, and pancreas compared with the diabetic control group.

3.5. The Effects of GP Extract on the Weekly Measured Blood Glucose Levels of Normal and STZ-Induced Diabetic Rats. Figure 3 represents the effects of GP extract on the weekly measured blood glucose levels of normal and STZ-induced diabetic rats during the experimental period of 28 days. The administration of STZ injections caused a sharp increase in

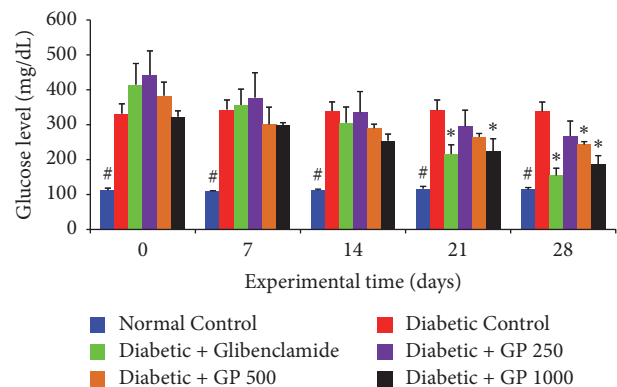


FIGURE 3: The effects of GP fruit extract and glibenclamide on weekly measured blood glucose levels in normal and STZ-induced diabetic rats. Data are represented as the mean ± SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

the blood glucose levels of diabetic control and treated rats compared with those of normal controls. However, treatment with GP extract at the three selected dose levels resulted in a marked decrease in blood glucose following the first week and later in the experimental period. In addition, rats treated with GP extract at 1000 mg/kg and glibenclamide exhibited a significant reduction in blood glucose levels in the third and fourth weeks compared with diabetic control rats ($p < 0.05$).

TABLE 3: The effects of GP fruit extract and glibenclamide on relative organ weight profile.

| Groups | Relative organ weight (%) | | | |
|----------------------------|---------------------------|--------------|--------------------------|--------------------------|
| | Liver | Kidney | Heart | Pancreas |
| Normal Control | 3.41 ± 0.29 | 0.61 ± 0.06 | 0.39 ± 0.02 [#] | 0.24 ± 0.04 [#] |
| Diabetic Control | 4.27 ± 0.03 | 0.85 ± 0.09 | 0.45 ± 0.04 | 0.42 ± 0.06 |
| Diabetic + Glibenclamide | 3.30 ± 0.32 | 0.77 ± 0.06 | 0.31 ± 0.02* | 0.29 ± 0.07 |
| Diabetic + GP (250 mg/kg) | 3.58 ± 0.45 | 0.84 ± 0.13 | 0.31 ± 0.05* | 0.20 ± 0.07* |
| Diabetic + GP (500 mg/kg) | 3.99 ± 0.61 | 0.85 ± 0.05 | 0.32 ± 0.02* | 0.27 ± 0.07 |
| Diabetic + GP (1000 mg/kg) | 2.80 ± 0.46* | 0.55 ± 0.09* | 0.32 ± 0.04* | 0.23 ± 0.06* |

Data are represented as the mean ± SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group; # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

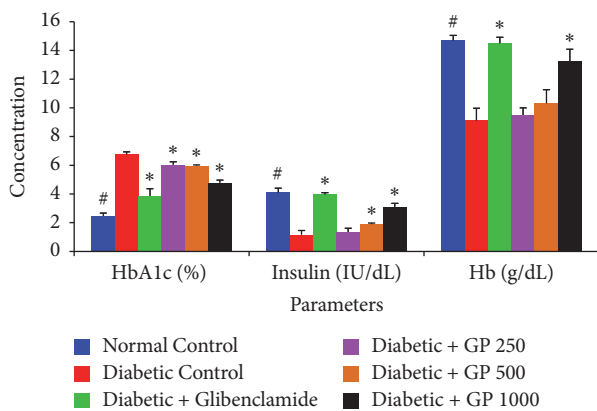


FIGURE 4: The effects of GP fruit extract and glibenclamide on insulin, HbA1c, and Hb levels in normal and STZ-induced diabetic rats. Data are represented as the mean ± SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

3.6. Effect of GP on Serum Insulin, Glycosylated Hemoglobin (HbA1c), and Hemoglobin Levels in Normal and STZ-Induced Diabetic Rats. The effects of GP on serum insulin, HbA1c, and Hb levels are presented in Figure 4. A significant decrease in serum insulin and Hb and an increase in HbA1c levels were observed in diabetic control rats compared with the normal control group. However, treatment with GP extract ameliorated these parameters in a dose-dependent manner. Moreover, GP extract administered at the highest dose (1000 mg/kg) showed the highest potential in restoring insulin levels and decreasing HbA1c levels compared with those of the diabetic control group.

3.7. The Effect of GP Fruit Extract on the Lipid Profiles of Normal and STZ-Induced Diabetic Rats. A marked increase in circulating levels of TG, TC, and LDL-C and a decrease in HDL-C level were observed in diabetic control rats compared with normal control animals (Figure 5). Treatment with GP extract was observed to significantly reduce the levels of TG, TC, and LDL-C while increasing the HDL-C level compared

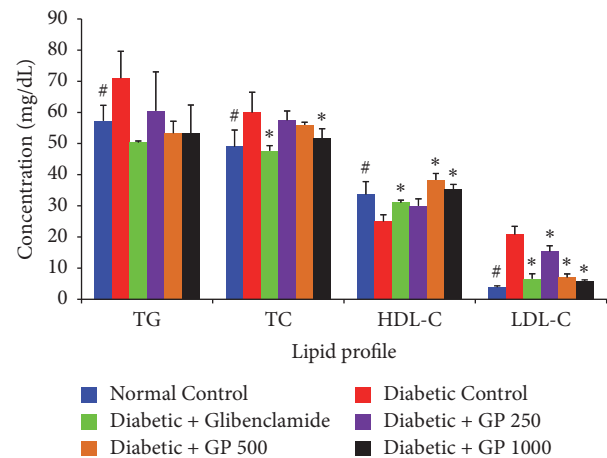


FIGURE 5: The effects of GP fruit extract and glibenclamide on the lipid profiles of normal and STZ-induced diabetic rats. Data are represented as the mean ± SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

with the levels measured in the STZ-challenged diabetic control groups.

3.8. The Effects of GP on Renal Function in Normal and STZ-Induced Diabetic Rats. The effects of GP extract and glibenclamide on the renal function were investigated by measuring serum creatinine, urea, and uric acid levels (Figure 6). There were significant increases in serum urea and uric acid and a nonsignificant increase in creatinine levels in the diabetic rats compared with normal controls. However, the serum levels of these kidney function markers were improved in animals that received GP extracts. Moreover, the animals treated with GP extract (500 and 1000 mg/kg) and glibenclamide had significantly reduced serum urea and uric acid levels compared with the diabetic controls.

3.9. The Effects of GP on Liver Function in Normal and STZ-Induced Diabetic Rats. The effects of oral GP administration

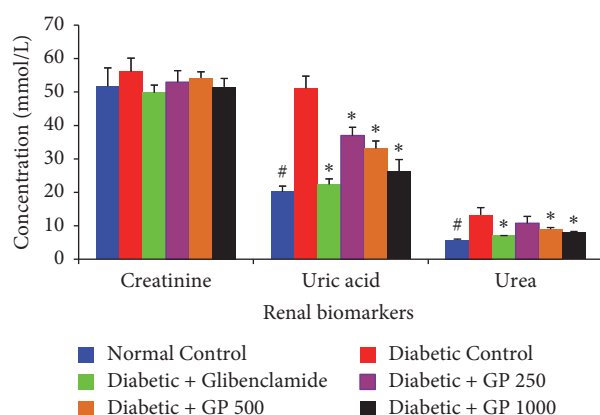


FIGURE 6: The effects of GP fruit extract and glibenclamide on renal function in normal and STZ-induced diabetic rats. Data are represented as the mean \pm SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

on liver function in diabetic rats were investigated by measuring serum ALT, AST, ALP, GGT, and LDH activity as well as TB, TP, ALB, and GLB levels and A/G ratio (Table 4). Serum ALT, AST, ALP, and GGT activity and LDH and TB levels were significantly higher in STZ-challenged diabetic rats than in normal control rats. The rats receiving GP treatment at 500 or 1000 mg/kg or glibenclamide had significantly lowered ALT, AST, ALP, GGT, LDH, and TB levels compared with the STZ-induced diabetic control rats. Significant reductions in serum ALT, ALP, and LDH levels were also observed in the 250 mg/kg GP-treated group compared with the diabetic control group.

3.10. The Effects of GP on Lipid Peroxidation (LPO) in Liver, Kidney, Heart, and Pancreas of Normal and STZ-Induced Diabetic Rats. Based on the investigation of oxidative stress biomarkers, there was a significant increase in LPO levels in STZ-induced diabetic control rats as evidenced by increases in liver, kidney, heart, and pancreas MDA levels compared with those of the normal control group. However, GP tended to confer a protective effect: the animals that were treated with fruit extracts showed a dose-dependent reduction in MDA levels compared with the diabetic controls. Significantly reduced LPO levels were observed in liver, kidney, heart, and pancreas tissues from the rats treated with 1000 mg/kg GP and with glibenclamide, and the rats treated with 500 mg/kg GP had significantly reduced LPO levels in both liver and heart tissues compared with diabetic control rats (Figure 7).

3.11. The Effects of GP on Tissue Antioxidant Enzymes (SOD and GPx) in the Liver, Kidney, Heart, and Pancreas of Normal and STZ-Induced Diabetic Rats. The effects of GP supplementation on the activity of cellular antioxidant enzymes including SOD and GPx in the liver, kidney, heart, and pancreas of rats are shown in Figure 8. Diabetic rats treated with STZ alone exhibited a significant decrease in SOD

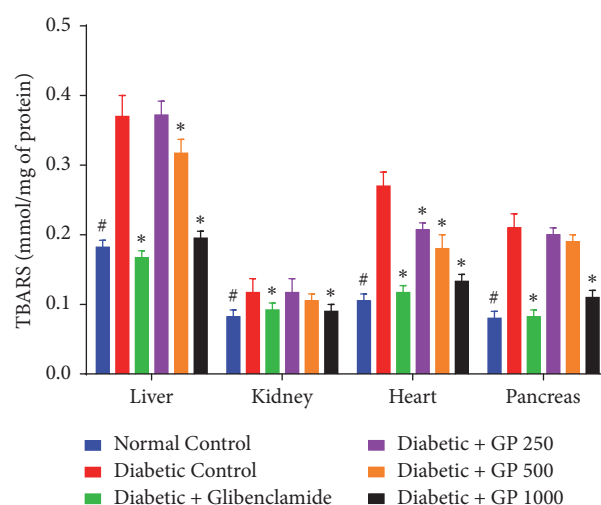


FIGURE 7: The effects of GP fruit extract and glibenclamide on lipid peroxidation in liver, kidney, heart, and pancreas tissues. Data are represented as the mean \pm SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

and GPx activity compared with the levels measured in the control group. However, treatment with GP extract significantly restored the levels of SOD activity compared with the levels measured for the diabetic control rats. On the other hand, diabetic rats treated with 500 mg/kg or 1000 mg/kg GP showed significantly increased GPx activity in the kidney and heart, while rats treated with 1000 mg/kg GP exhibited significantly increased liver GPx activity, as did glibenclamide-treated rats, compared with the diabetic control group.

3.12. Histopathological Examination of the Liver. Normal control rats had normal livers whose hepatic lobules had a uniform pattern of polyhedral hepatocytes radiating from the central vein (CV) towards the periphery (Figure 9(a)). In contrast, the livers of the GP-treated animals exhibited histopathological changes. The rats treated with STZ alone showed a disrupted arrangement of hepatocytes around the CV, congestion in the central vein (CV), degeneration of hepatocytes at the peripheral area of the central vein, and massive vacuolization in the lobules (Figure 9(b)). The livers of the animals in groups 3 to 6 showed improvements in all of the histopathological features (Figures 9(c)–9(f)), with a mild to moderate degree of hepatocyte degeneration, vascular congestion, and edema at all investigated doses.

3.13. Histopathological Examination of the Kidney. Normal control rats showed normal renal parenchymal morphology with well-defined glomeruli and tubules (Figure 10(a)). In contrast, the rats treated with STZ alone (Figure 10(b)) showed some pathological changes including degenerated tubular epithelium, vacuolization in some spaces, and abnormally structured glomeruli (GMs) with infiltration of inflammatory cells. However, diabetic rats treated with GP extract at

TABLE 4: The effects of GP fruit extract and glibenclamide on liver function in normal and STZ-induced diabetic rats.

| Groups | Liver function markers | | | | | | | | | |
|----------------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|--------------------------|--------------------------|---------------------|---------------|----------------|--------------------------|
| | ALT (U/L) | AST (U/L) | ALP (U/L) | LDH (U/L) | GGT (U/L) | Total bilirubin (mg/dL) | Total protein (g/L) | Albumin (g/L) | Globulin (g/L) | A/G ratio |
| Normal Control | 44.67 ± 2.87 [#] | 81.67 ± 2.87 | 231.67 ± 9.53 [#] | 104.33 ± 18.37 [#] | 1.33 ± 0.47 [#] | 0.10 ± 0.01 [#] | 59.73 ± 1.52 | 11.53 ± 0.59 | 48.2 ± 1.60 | 0.23 ± 0.01 |
| Diabetic Control | 82.67 ± 16.21 | 86.00 ± 8.64 | 973.67 ± 31.71 | 290.00 ± 28.85 | 6.33 ± 0.47 | 0.23 ± 0.04 | 65.50 ± 4.54 | 10.46 ± 1.65 | 55.03 ± 5.10 | 0.19 ± 0.04 |
| Diabetic + Glibenclamide | 42.67 ± 3.40 [*] | 68.00 ± 5.34 [*] | 608.67 ± 7.59 [*] | 163.67 ± 3.68 [*] | 3.67 ± 0.47 [*] | 0.10 ± 0.04 [*] | 62.60 ± 1.31 | 11.53 ± 0.60 | 51.06 ± 0.88 | 0.22 ± 0.01 |
| Diabetic + GP (250 mg/kg) | 55.25 ± 5.53 [*] | 77.25 ± 4.81 | 625.00 ± 46.18 [*] | 135.50 ± 32.53 [*] | 5.00 ± 1.22 | 0.18 ± 0.04 | 62.63 ± 4.69 | 10.55 ± 0.45 | 52.07 ± 4.40 | 0.20 ± 0.01 |
| Diabetic + GP (500 mg/kg) | 47.33 ± 3.29 [*] | 64.33 ± 1.69 [*] | 572.00 ± 35.44 [*] | 142.00 ± 23.37 [*] | 4.67 ± 1.24 | 0.10 ± 0.01 [*] | 60.67 ± 2.11 | 9.33 ± 0.74 | 51.33 ± 2.05 | 0.18 ± 0.01 |
| Diabetic + GP (1000 mg/kg) | 44.50 ± 3.35 [*] | 72.50 ± 2.50 [*] | 397.75 ± 42.41 [*] | 110.75 ± 15.36 [*] | 4.00 ± 0.71 [*] | 0.08 ± 0.04 [*] | 63.00 ± 2.14 | 12.57 ± 0.54 | 50.42 ± 2.51 | 0.25 ± 0.02 [*] |

Data are represented as the mean ± SD (*n* = 8). * denotes a significant difference (*p* < 0.05) between the treatment groups and the diabetic control group; # denotes a significant difference (*p* < 0.05) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

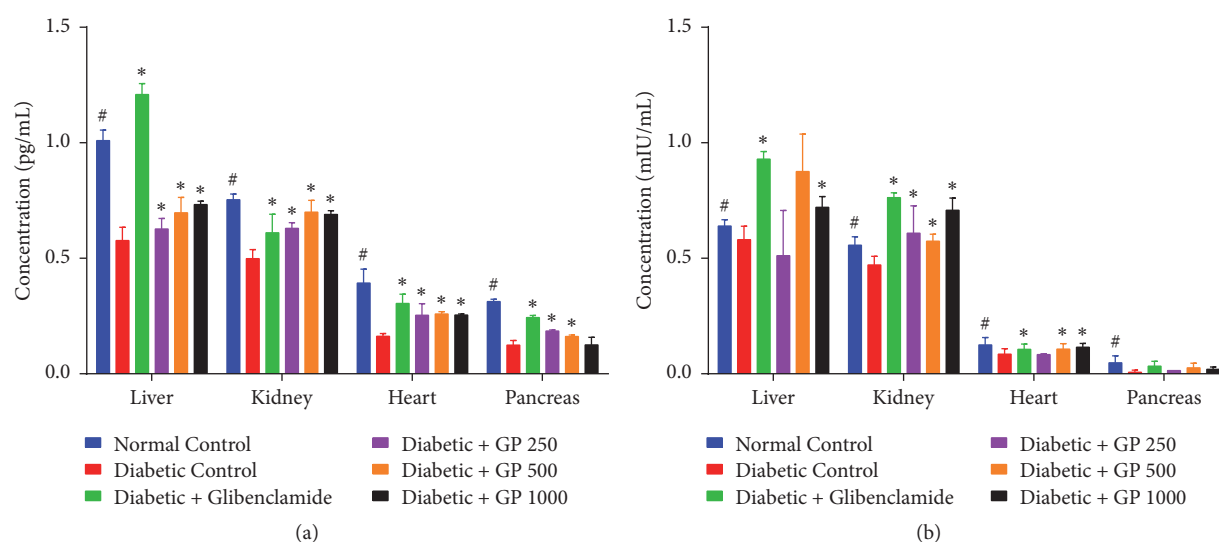


FIGURE 8: Effect of GP on the tissue antioxidant enzymes (a) SOD and (b) GPx in the liver, kidney, heart, and pancreas of rats in different experimental groups. Data are represented as the mean \pm SD ($n = 8$). * denotes a significant difference ($p < 0.05$) between the treatment groups and the diabetic control group. # denotes a significant difference ($p < 0.05$) when the normal control group was compared to the diabetic control group. The comparisons were made by one-way ANOVA followed by Dunnett's multiple comparison test.

each of the three doses and glibenclamide-treated rats showed significant preservation of glomeruli architecture compared with rats treated with STZ alone, although mild tubular degeneration, epithelial vacuolization, and inflammatory cell infiltration were still present (Figures 10(c)–10(f)).

3.14. Histopathological Examination of the Heart. Normal control rats showed normal cardiac muscle fibers (Figure 11(a)). However, myocardial structural changes including separation of cardiac muscle fibers, coagulative necrosis, and edematous intramuscular spaces were found in the diabetic control rats (Figure 11(b)). GP-treated rats showed marked protection of the cardiac muscles, with increased areas of normal muscle fibers (Figures 11(c)–11(f)).

3.15. Histopathological Examination of the Pancreas. Normal control rats showed normal islets of Langerhans (IL) (Figure 12(a)). The rats treated with STZ alone had some pathological changes such as IL disruption, degenerative and necrotic changes, and vacuolization. Treatment with 250 or 500 mg/kg GP caused the IL to shrink and conferred only mild or moderate protection, respectively. Rats treated with 1000 mg/kg GP exhibited significant protection, with markedly decreased necrosis of IL cells.

4. Discussion

To our knowledge, this is the first study to report on the antihyperglycemic, antidiabetic, and antioxidant potentials of GP *in vivo*. Weekly measurement of body weight of the animals gives us an overview of the changes in their body weight. Gradual weight loss is a common symptom in DM due to degradation of structural protein and abnormal carbohydrate metabolism [30, 31]. In this study, significant weight loss was observed in the diabetic control group. In

contrast, significantly improved body weights compared with the diabetic control group were found in the glibenclamide and 1000 mg/kg GP groups. This restoration of body weight might be due to reversal of gluconeogenesis, glycogenolysis, and proteolysis [32]. The relative organ weights (liver, kidney, heart, and pancreas) of the diabetic control rats and the diabetic rats treated with glibenclamide and GP extracts were higher than those of the normal control group. Daily administration of GP extract (1000 mg/kg) significantly decreased the relative organ (liver, kidney, heart, and pancreas) weights compared with those of the diabetic control group, indicating that GP has the potential to ameliorate diabetes by maintaining or regenerating hepatocytes, renal tissue, cardiac muscle, and islet of Langerhans histoarchitecture.

The OGTT measures the body's ability to utilize blood glucose. The GP fruit extract exhibited a dose-dependent effect on the glycemic status of rats. An OGTT study in normal rats and diabetic rats showed that administration of GP extract reduced blood glucose levels significantly, as we also observed in rats treated with a standard drug, glibenclamide. In the normal rats, administration of GP at 500 mg/kg and 1000 mg/kg doses significantly reduced blood glucose levels within 3 to 5 hours. Diabetic rats treated with 1000 mg/kg GP showed significantly reduced blood glucose levels within 2 to 4 hours of treatment. The hypoglycemic activity of GP might be the result of an improved insulin level, which is observed in the GP-treated rats. This suppression of hyperglycemia may result from the inhibition of α -glucosidase and α -amylase enzymes by GP as reported by Sarma et al. [14].

In the present study, we found a continuous increase in blood glucose in the diabetic control group. However, oral administration of GP fruit extract showed significant improvement in blood glucose levels. Our findings indicate that the 1000 mg/kg GP dose significantly reduced blood glucose within the third week of the experiment. This finding strongly

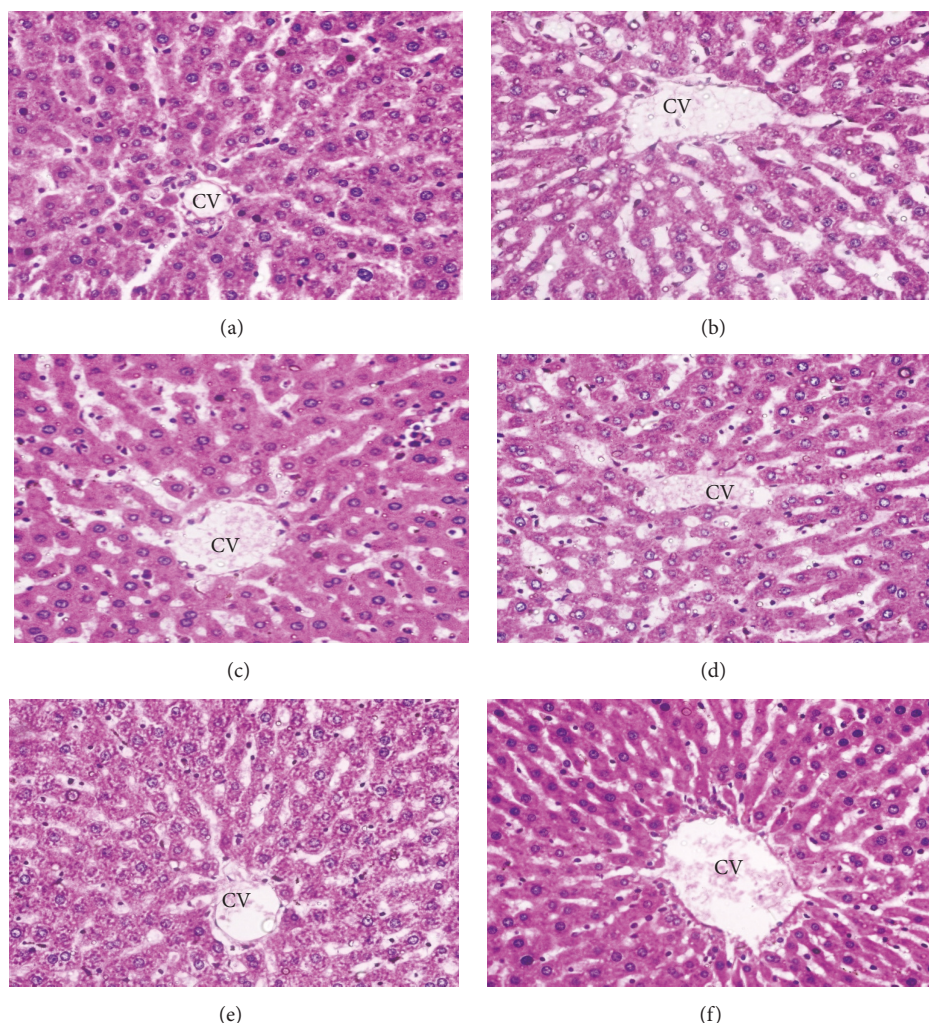


FIGURE 9: Histopathological photomicrographs of liver tissue (6100x magnification; scale bar: 20 μm). (a) Normal control rats had normal livers whose hepatic lobules had a uniform pattern of polyhedral hepatocytes radiating from the central vein (CV) towards the periphery. (b) Diabetic control rats showed disrupted arrangement of hepatocytes around the CV, congestion in the CV, degeneration of hepatocytes at the peripheral area of the central vein, and massive vacuolization in the lobules. (c–f) Glibenclamide- and GP-treated rats showed a remarkable degree of preservation of the cellular arrangement with only mild vacuolization in the lobules.

supports the antidiabetic effects of GP. The antidiabetic role of GP may result from insulin-like action such as improving the uptake of cellular glucose or enhancing glycogenolysis.

Both insulin deficiency and resistance are responsible for the pathogenesis of DM. Hence, increasing insulin secretion and maintain its level within the normal physiological range are very important for antidiabetic therapy. In this study, administration of STZ caused damage to pancreatic β -cells and consequently decreased the serum fasting insulin level in rats. However, oral administration of GP extract significantly increased serum insulin levels compared with those of the diabetic control group in a dose-dependent manner. The insulin levels in GP-treated diabetic rats were found to be in the normal physiological range for rats. This indicates that GP extract might have effects on both insulin secretion and insulin action. In this case, the phytochemicals of GP extract might act as stimulating agents for both insulin secretion and

insulin action. Among the compounds present in GP, garcinol has been reported to enhance insulin action by increasing the expression of GLUT2 and GLUT4.

Hb and HbA1c are closely associated with DM, and HbA1c is a diagnostic marker used to predict DM progression in individuals [33]. HbA1c is also widely used as a reliable marker for estimating the degree of protein glycation during DM [34] to indicate poorer long-term control of blood glucose levels, leading to chronic hyperglycemic status, which is correlated with various complications associated with DM. We observed an elevated level of HbA1c in STZ diabetic control rats, which was also directly proportional to the decreased levels of total Hb. This is similar to some reports in diabetic control experimental animals [35, 36]. However, the oral administration of GP extract significantly improved HbA1c and Hb levels in the rats treated with 1000 mg/kg. This effect might be due to the effective control of hyperglycemia

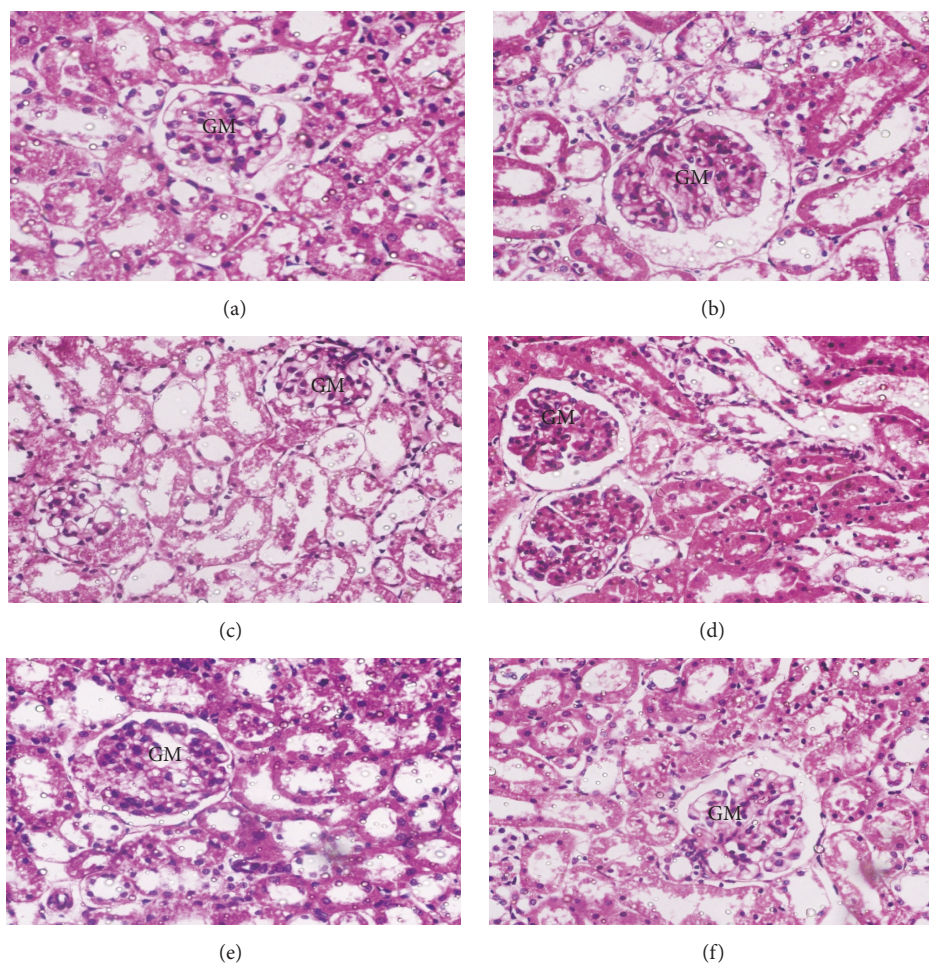


FIGURE 10: Histopathological photomicrographs of kidney tissue (6100x magnification; scale bar: 20 μ m). (a) Normal control rats showed normal renal parenchymal morphology with well-defined glomeruli and tubules. (b) Diabetic control rats showed abnormally structured glomeruli (GMs) with marked degeneration of the tubular epithelium, vacuolization, and inflammatory cell infiltration. (c–f) Glibenclamide- and GP-treated rats showed a remarkable degree of morphological preservation with only moderate to mild degenerative changes.

by GP. This also indicates that GP might be very effective for long-term control of DM and preventing further complications in diabetic individuals.

Catechin was detected in this study by HPLC analysis while significant amounts of some other polyphenols were also identified in some other studies [17, 18]. It is plausible that the polyphenols found in GP such as catechin and garcinol may ameliorate diabetes and its complications by reduction in oxidative stress due to their strong antioxidant properties [37, 38]. Different studies have reported that dietary polyphenols including flavonoids and phenolic acids may inhibit α -amylase and α -glucosidase, inhibit glucose absorption in the intestine by sodium-dependent glucose transporter 1 (SGLT1), stimulate insulin secretion, and reduce hepatic glucose output. Polyphenols may also increase insulin-dependent glucose uptake, activate 5' adenosine monophosphate-activated protein kinase (AMPK), modify the microbiome, and have anti-inflammatory effects. Detailed experimentation should be conducted to identify the exact mechanism.

The most common lipid abnormalities in DM are hypertriglyceridemia and hypercholesterolemia [39]. Elevated levels of certain lipids are major risk factors for cardiovascular diseases [40]. In DM, the increased level of serum lipids is due to the uninhibited activity of lipolytic hormone on the fat stores, primarily because of the low activity of insulin. In the normal state, insulin activates the lipolytic actions of the hormones on the peripheral fat depots, hydrolyzing triglycerides and preventing mobilization of free fatty acids [41, 42]. In the diabetic state, lipoprotein lipase, which hydrolyzes triglycerides, does not function properly due to the shortage of insulin. As a result, hypertriglyceridemia occurs [43]. The lowering of serum lipid levels through diet or drug supplementation seems to be associated with a decreased risk of cardiovascular disease and its associated complications [44]. In this experimental study, control rats with STZ-induced diabetes showed significantly elevated levels of TG and cholesterol. In contrast, treatment with GP extract significantly decreased TC, TG, and LDL-C and increased HDL-C levels in the diabetic animals. Sharma et al. reported a similar

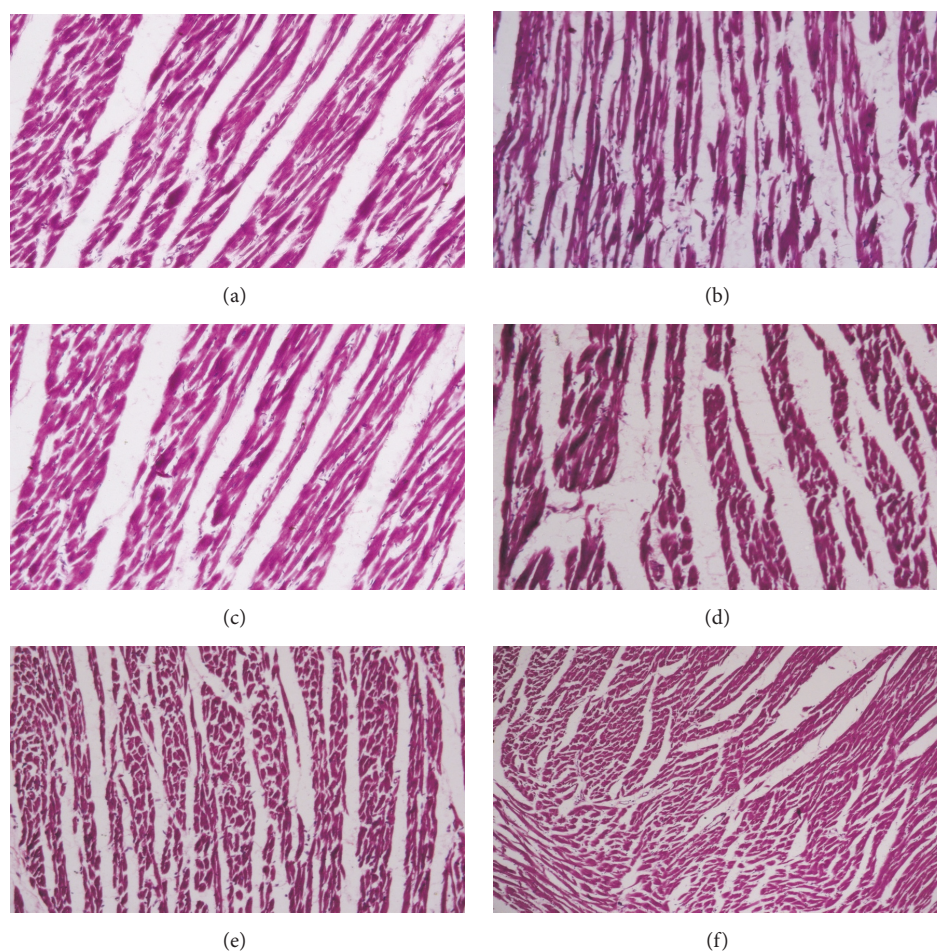


FIGURE 11: Histopathological photomicrographs of heart tissue (6100x magnification; scale bar: 20 μ m). (a) Normal control rats showed normal cardiac muscle fibers. (b) Diabetic control rats showed pathological changes including marked separation of cardiac muscle fibers and myocardial necrosis. (c–f) Glibenclamide- and GP-treated rats showed cardioprotection, with markedly increased areas of normal muscle fibers.

hypolipidemic potential of polyphenol-rich GP extract in high-fat diet (HFD) rats [14]. Altogether, the methanolic extract of GP seems to possess insulin-stimulating activity, which would be helpful to reduce the incidence of lipid-related complications.

Diabetic nephropathy is one of the major complications of DM. High blood sugar from DM destroys the tiny blood vessels in the kidneys, leading to impaired renal function and diabetic nephropathy [45]. In our present study, STZ caused increased levels of renal function markers that indicate renal damage in these animals. However, the daily administration of GP, especially the 1000 mg/kg dose, reduced these markers, indicating a nephroprotective effect in these diabetic animals. Garcinol, one of the major phytoconstituents of GP, has been reported to show significant nephroprotective effects in rats with STZ-induced diabetes.

During DM, insulin deficiency contributes to increased serum levels of transaminase enzymes because sufficient amounts of amino acids stimulate the occurrence of gluconeogenesis and ketogenesis [46]. Increased levels of liver enzymes such as ALT, AST, ALP, GGT, and LDH and

metabolites such as TB, TP, ALB, and GLB in the serum are markers of liver dysfunction and/or liver mitochondrial injury in diabetes [47]. In this study, the elevated levels of these enzymes confirmed the existence of liver dysfunction in the diabetic control group. Treatment with GP significantly reversed the increased levels of liver marker enzymes such as ALT, AST, ALP, GGT, and LDH and metabolites such as TB and TP and restored the levels to normal values, indicating stimulation of insulin secretion into the circulation and a resulting hepatoprotective effect. A similar hepatoprotective effect of GP extract was also found in HFD rats.

Oxidative stress is considered a potential contributor to the development of DM as well as its complications. Oxidative stress develops in DM through increased generation of reactive oxygen species (ROS) and a decrease in ROS scavenging capacity [48]. The excessive levels of MDA are attributed to increased production of ROS (O_2^- , H_2O_2 , and $^{\cdot}OH$). Excessive lipid peroxidation causes lipid degradation that impairs cell membrane function, resulting in tissue damage and leading to many pathological conditions and cytotoxicity in the body [49, 50]. In this study, lipid peroxidation was

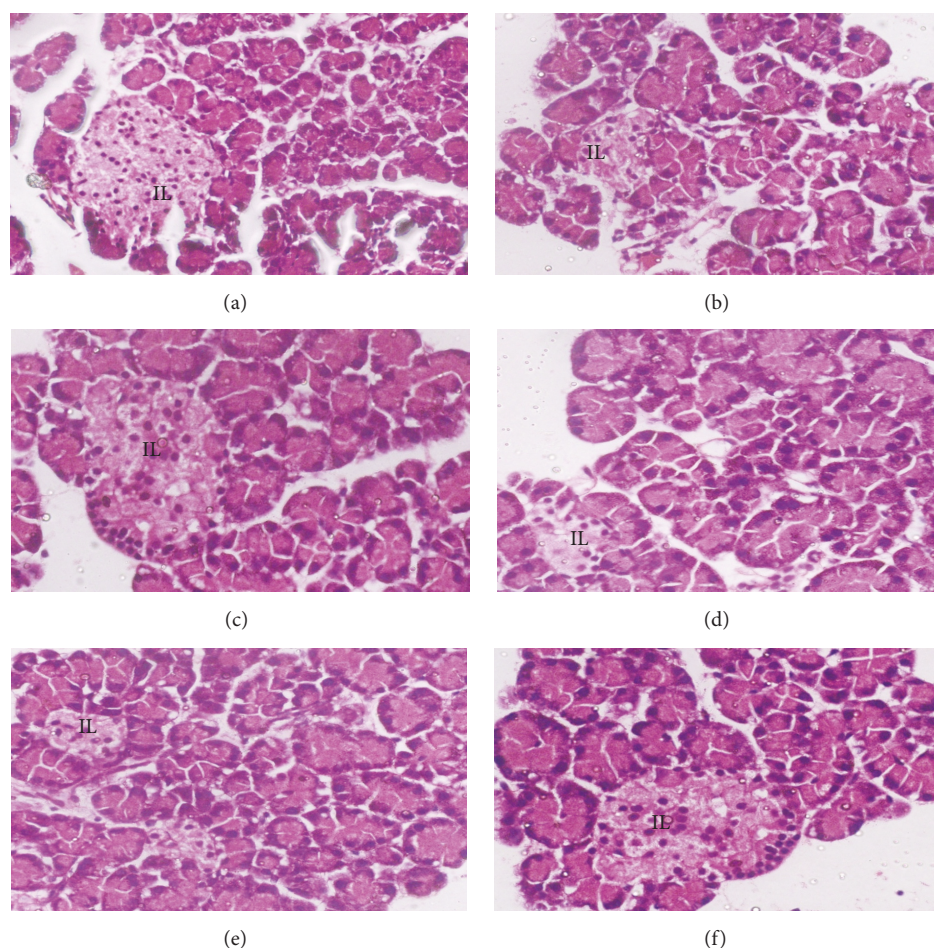


FIGURE 12: Histopathological photomicrographs of pancreas tissue (6100x magnification; scale bar: 20 μ m). (a) Normal control rats had normal islets of Langerhans (IL). (b) The rats from the diabetic control group showed disruption of the IL with degenerative as well as necrotic changes. (c–f) Glibenclamide- and GP-treated rats showed a notable degree of protection, with markedly decreased necrosis of IL cells.

estimated in the tissues of four different organs: liver, kidney, heart, and pancreas. As expected, STZ caused a significant increase in lipid peroxidation as indicated by the level of MDA (a secondary product of LPO). Treatment with a 1000 mg/kg dose of GP significantly decreased the LPO levels in all tissues. This suggests the antilipid peroxidation capacity of GP in all of these important tissues.

Cellular antioxidant enzymes are considered the first line of defense against free radicals [51]. Free radicals are constantly formed in the living cells and removed by antioxidant defense mechanisms. Uncontrolled generation of ROS is involved in a number of human diseases including DM. SOD and GPx are two important antioxidant enzymes. SOD catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide, whereas GPx reduces free hydrogen peroxide to water. Reductions in the activity of these antioxidant enzymes can lead to harmful effects via the accumulation of free radicals. Our findings revealed that STZ administration significantly reduced the levels of SOD and GPx activity in the liver, kidney, heart, and pancreas in the diabetic control rats. However, treatment with GP fruit extract significantly increased the levels of these antioxidant enzymes

in all the tissues compared with the diabetic control group. This finding might result from the scavenging activity of GP, which suppresses oxidative stress. A study has also reported the capacity of GP fruit extract to remediate the alterations in GSH, SOD, CAT, and MDA levels caused by HFD.

Histopathological examination of different organs showed that STZ causes degenerative necrosis of hepatocytes, vacuolization of the hepatic lobules, degeneration of the kidney glomeruli with inflammatory infiltrates, massive separation of cardiac muscle, and necrotic damage to the myocytes of the heart tissue and islets of Langerhans of the pancreas in diabetic control group rats. Administration of different doses of GP extract restored the morphology of these tissues to normalcy. These histopathological findings further confirmed the potential of GP fruit extract against STZ-induced tissue damage.

5. Conclusions

The findings of this study indicated that oral administration of GP in Wistar rats can ameliorate hyperglycemia, diabetes, and diabetic complications and protect against oxidative

stress-induced damage. Although establishing the mechanisms behind these effects is far beyond the scope of this study, we can certainly mention that the phytoconstituents of GP might be responsible for these pharmacological properties. We can conclude that GP is a nontoxic source of natural antioxidants that could be used to treat diabetes and its complications. However, more detailed biochemical and molecular studies should be conducted to identify the main active ingredients in GP and their mechanistic roles as well.

Disclosure

Md. Yousuf Ali and Sudip Paul are joint first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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