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Research Article

Green Coffee Bean Extract Potentially Ameliorates Liver Injury due to HFD/STZ-Induced Diabetes in Rats

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The goal of the current study was to examine the therapeutic potential of green coffee bean extract (GCBE) in the treatment of diabetic hepatic damage induced by high-fat diet (HFD) and streptozotocin (STZ) administration. The novelty of this study lies in constructing a newly stabilized *in vivo* obese diabetic animal model in rats using HFD/STZ for investigating the dose-dependent effect of two commonly used doses of GCBE in hepatoprotection against oxidative stress-induced hepatic damage by measuring many parameters that have not been carried out previously in other studies. GCBE that was used in this study was a hot water extract of green coffee beans with a concentration of 0.1 g ml^{-1}. Male albino rats were given a single dose of STZ (35 mg kg^{-1}), and HFD to induce diabetes mellitus (DM). For 28 days, two separate doses of GCBE 50 mg kg^{-1} and 100 mg kg^{-1} were administered orally to diabetic animals. Leptin, liver enzymes, oxidative stress parameters, inflammatory parameters, fasting plasma glucose (FPG), fasting plasma insulin (FPI), and lipid profile levels were examined. Real-time PCR and ELISA were used to quantitatively detect the mRNAs of the genes involved in the insulin signaling pathway, the genes involved in glucose metabolism, and the amounts of proteins. The levels of FPG, lipid profile, liver enzymes, inflammatory markers, and leptin in the HFD/STZ diabetic group revealed a considerable spike, while they considerably decreased after GCBE treatment in a dose-dependent manner. After GCBE treatment, the diabetic group showed a significant rise in the antioxidant markers glutathione, superoxide dismutase, and catalase, as well as a decrease in malondialdehyde and nitric oxide levels. The liver changes caused by HFD/STZ were entirely reversed by GCBE, and most intriguingly, in a dose-dependent manner. We concluded that GCBE can repair the hepatic oxidative damage caused by HFD and STZ by reversing all the previously measured parameters and improving the insulin signaling pathways. GCBE demonstrated strong antifree radical activity and significantly protected cells from oxidative damage caused by HFD/STZ.

1. Introduction

Due to its enormous incidence and ongoing problems, diabetes mellitus (DM) is regarded as a worldwide health issue [1, 2]. According to recent data, the middle east is an area where diabetes is quickly expanding. Egypt is listed as one of the top ten nations in terms of diabetic patients (16.7% of the adult population in Egypt) [3, 4]. It is well acknowledged that the main symptoms of diabetes are hyperglycemia, hyperlipidemia, insulin resistance (IR), and inflammation [5]. Together, these symptoms can trigger the production of reactive oxygen species (ROS), which lead to a number of diabetes problems [5]. Multiple issues in numerous organs, including the brain, eyes, kidneys, heart, and testicular tissues, are caused by hyperglycemia [6–9]. In addition to the well-known diabetic consequences, liver damage frequently

happens due to a variety of processes. *In vitro* and *in vivo* models have both been used to study the effects of hyperglycemia on the cellular environment of the liver [10]; however, more research is needed to fully understand the underlying mechanisms.

Obesity is considered the most frequent cause of IR [11]. Excess adiposity and adipocyte dysfunction result in impaired regulation of a wide range of adipokines. These may contribute to the development of various metabolic diseases through alterations in glucose and lipid homeostasis along with inflammatory responses [12]. An important adipokine, leptin, plays a critical role in the regulation of body weight and fat mass. Downregulation of fat accumulation is a good strategy for the treatment of obesity and so, decreasing diabetic complications affecting hepatocytes [13]. Unfortunately, diabetes induces alterations in the expression of genes associated with glycolysis and gluconeogenesis such as (pyruvate kinase, G6PD, IRS-1, hexokinase, and glucokinase), so any strategy to improve these altered proteins and/or genes can help in delaying diabetes-associated hepatocyte damage [14]. The oxidative stress indicators nitric oxide (NO) and malondialdehyde (MDA), as well as inflammatory cytokines (TNF-α and IL-1β), play a central role in liver disease's pathogenesis and progression, through the inhibition of insulin signals [15]. The varied positive impacts that antioxidants from food or medicinal plants have on chronic diseases have attracted a lot of attention [16].

Natural products have been recognized to be valuable in the management of diabetes [6]. Coffee that is green, or unroasted and raw, is one of these naturally occurring goods [17]. Diterpenes, trigonelline, and chlorogenic acid (CGA), among other bioactive substances with antioxidant capabilities, are abundant in green coffee bean water extract (GCBE) [18, 19]. CGA, the primary phenolic component in GCBE, has been linked to numerous advantageous benefits [19, 20].

Despite GCBE's preventive benefits against diabetes, nothing is known about the molecular mechanisms behind the substance's hepatoprotective properties. Therefore, the current study aimed to assess the benefits of GCBE in postponing the development of diabetes complications, as well as its function in modulating oxidative stress and the inflammatory immune response to lessen hepatic damage. This study also attempted to identify the signaling pathways involved in GCBE-offered hepatoprotection in an animal model of type II diabetes.

2. Materials and Methods

2.1. Plant Material and Animals. Green coffee beans were acquired from an area trader in East Cairo, Egypt, recognized and verified by an expert (Botany Department, Faculty of Science) (HCH no. s.n. 2018), and then ground into a fine powder using an electric mixer. Similar to how coffee is traditionally made in Saudi Arabia, the resultant powder was used for GCBE extraction with a concentration of 0.1 mg ml^{-1} and was extracted with distilled water (70°C) and covered for 20 min. These conditions have just resembled the most common human method for coffee drink preparation and conditions. After that, the extract; defined as a GCBE (green coffee bean extract) was filtered and dissolved in water to a concentration of 0.1 mg ml^{-1} (10% w/v). The extract was freshly prepared daily and administered orally by gavage.

Furthermore, a high-performance liquid chromatography analysis of GCBE was performed. 21 peaks with retention times ranging from 0.135 min to 19.893 min were detected. Based on the UV-Visible spectral data and their retention times, GCBE has a UV band at 254 nm characteristic for polyphenols and flavonoids compounds, possibly caffeine and chlorogenic, gallic, protocatechuic, hydroxybenzoic, caffeic, ferulic, sinapic, and vanillic acids (Figure 1).

All rats were kept in the institutional animal home in a standardized setting (12 h light/dark cycle, 60% humidity, 24°C ± 2°C temperature). Four rats at most were kept in each cage, and they had complete access to food and water from the tap. Before the experiments started, rats were given at least 14 days to get used to the lab environment. They were handled in accordance with the National Institutes of Health's (8th Edition) guidelines for the care and management of laboratory animals (NIH Publication No. 85–23, updated 1985). The Faculty of Pharmacy at Helwan University in Egypt's ethical committee gave the experiments their blessing (approval no. 019A2018).

2.2. Experimental Design. Two weeks of acclimatization afterward, seven rats from each of the six groups were randomly assigned to the following categories: the group of healthy controls (CNTR), received a typical diet and distilled water; the diabetic group (HFD/STZ) was given a high-fat diet (HFD) orally for four days as a regular diet before receiving an immediate injection of STZ (35 mg kg^{-1}) intraperitoneal injection (I.P.); normal rats were given 100 mg kg^{-1} day^{-1} of the extract as part of the green coffee group GCBE. Diabetes + low dosage GCBE (STZ + GCBE50), in which diabetic rats received 50 mg kg^{-1} day^{-1}; diabetes + high dose GCBE (STZ + GCBE100), in which diabetic rats received the extract orally at a dose of 100 mg kg^{-1} day^{-1}. Standard group (STZ/HFD + Met): diabetic rats treated with metformin (200 mg kg^{-1} day^{-1}) (Table 1).

2.3. Induction of Diabetes in Rats. Rats received 35 mg kg^{-1} I.P. dose of STZ (Sigma–Aldrich, St. Louis, MO, USA) after fasting for 12 h [21]. The STZ solution was freshly prepared in 0.1 M citrate buffer (pH = 4.6) [22] and was injected once after feeding with HFD (total energy 25.07 KJ g^{-1}, including 60% fat, 20% protein, and 20% carbohydrates) for four weeks. At the studied concentrations, neither a fatality nor any other negative signs were noticed. An Accu-Chek® blood glucose meter was used to check the blood sugar level after three days (Roche Diagnostics, Basel, Switzerland). Diabetic rats were those with blood glucose levels above 200 mg/dl [23]. The experimental layout is depicted in Figure 2.

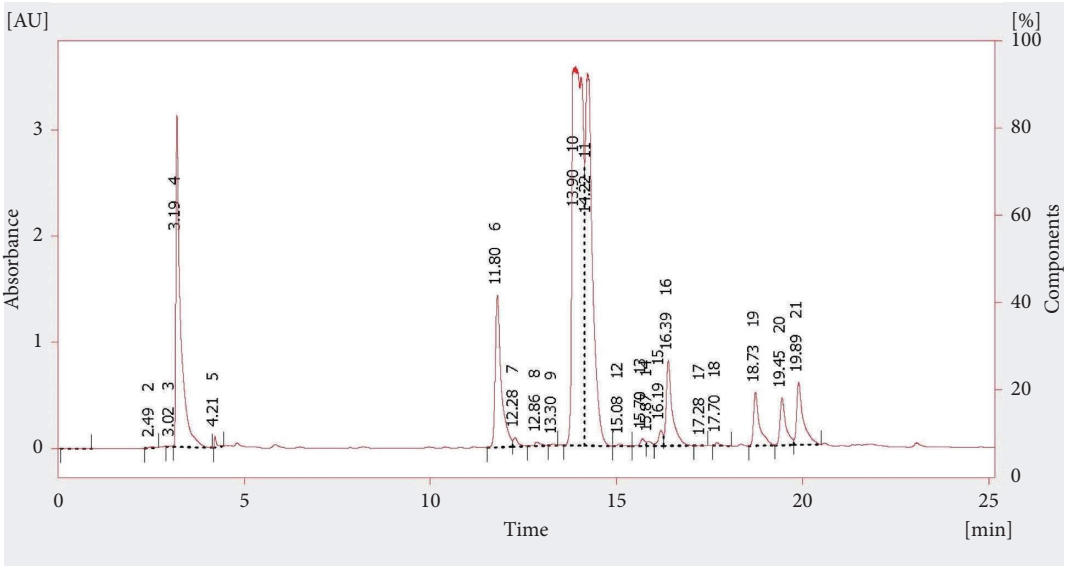

Figure 1: High-performance liquid chromatography fingerprint of green coffee bean water extract revealed the presence of 30 peaks with retention times ranging from 0.135 min to 19.893 min.

Table 1: Showed the classification of study groups.

Groups	Experimental design (diet and treatment)
Group 1	Normal control group (CNTR): rats received a normal diet with distilled water
Group 2	Diabetic group: received high-fat diet (HFD) (orally as a normal diet) for 4 weeks and injected with STZ (35 mg kg^{-1}) (I.P) once
Group 3	Green coffee group (normal + GCBE): rats received GCBE (100 mg kg^{-1} day^{-1}) orally
Group 4	Standard group: diabetic rats treated with metformin (200 mg kg^{-1} day^{-1}) orally
Group 5	Diabetic + low dose GCBE: diabetic rats received GCBE (50 mg kg^{-1} day^{-1}) orally
Group 6	Diabetic + high dose GCBE: diabetic rats received GCBE (100 mg kg^{-1} day^{-1}) orally

2.4. Collection of Blood Samples and Rat Tissues. To assess each rat's glucose tolerance and validate the diabetic model, an oral glucose tolerance test (OGTT) was administered after ten weeks. They were then put to death by cervical dislocation while under anesthesia, and blood was drawn, incubated for 30 minutes at 37°C, and centrifuged for 10 minutes. To prepare the collected sera for additional biochemical tests, they were kept at −80°C. The liver was removed, weighed, and then cut into three pieces. One piece was preserved in buffered formalin at a concentration of 10% for histopathological studies, while the other two pieces were kept at −80°C for biochemical and molecular analysis.

2.5. Liver Function and Lipid Profile Analysis. Using commercial kits from Bio Diagnostic Reagents (USA), the manufacturer's methods were followed to assess the liver functional biomarkers (ALT and AST) in sera. Whereas, total cholesterol (TC), total triglycerides (TG), low-density lipoprotein (LDL-c), and high-density lipoprotein (HDL-c) were assessed using commercial kits obtained from RANDOX Reagents (USA).

2.6. Determination of Cytokines Levels (TNF-α and IL-1β). According to the manufacturer's instructions, a specific ELISA kit (bought from the R&D system) was used to measure the levels of inflammatory cytokines in sera.

2.7. Determination of Insulin Levels. According to the manufacturer's instructions, commercial ELISA kits (CUSABIO Life Sciences, Wuhan, China) were used to measure the insulin level. A spectrophotometer was used to determine the optical density (OD) at 450 nm using a reference wavelength of 620–630 nm (BioTek Inc., Germany). A homeostasis model of insulin resistance was used to analyze the IR that developed in the experimental animals (HOMA-IR).

2.8. Oral Glucose Tolerance Test. In the final week of the trial, overnight-fasted rats from each group underwent an OGTT. Following oral administration of (1 g kg^{-1} body weight) glucose as an aqueous solution, the blood glucose levels were obtained from the tail vein by tail milking and measured at 0, 30, 60, 90, and 120 min using an Accu-Chek® blood glucose meter (Roche Diagnostics, Basel, Switzerland).

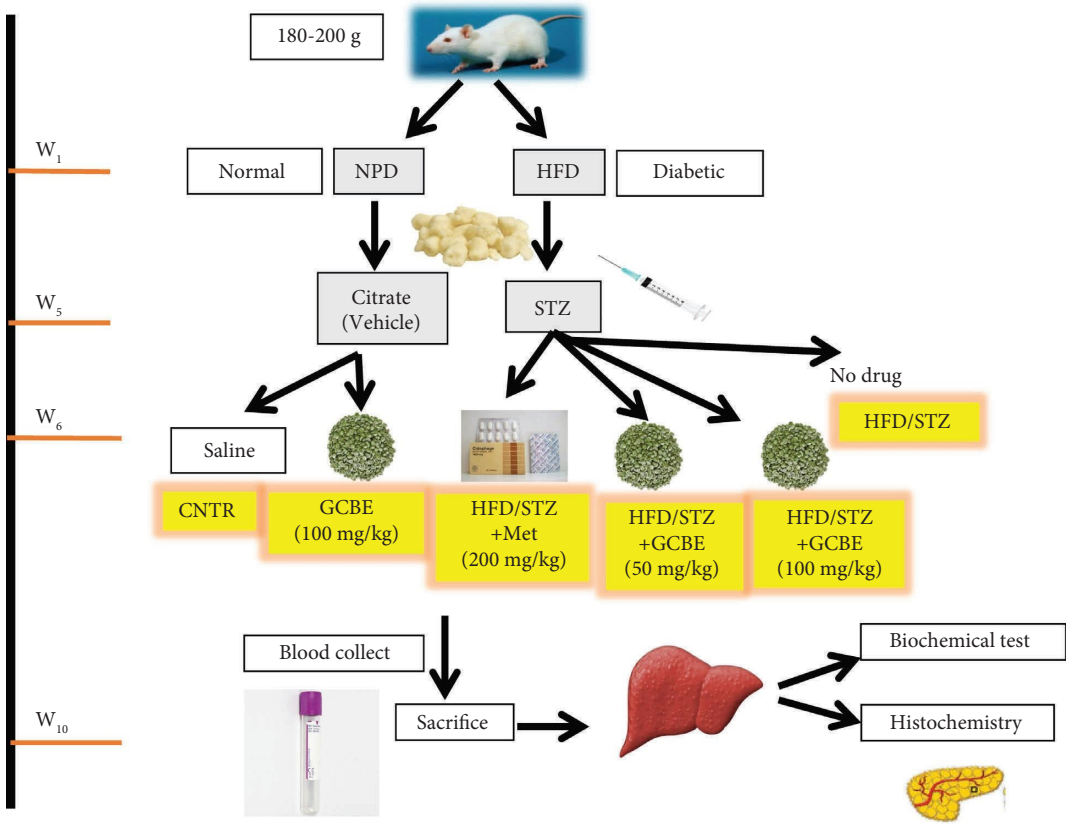

Figure 2: Graphical abstract of experimental design. The abbreviations of the figure: NPD: normal pellet diet, HFD: high-fat diet, STZ: streptozotocin, CNTR: control group, and GCBE: green coffee bean water extract.

2.9. Determination of Leptin. A commercial ELISA kit (Abcam's Leptin Rat ELISA kit) was used to measure the leptin level in accordance with the manufacturer's recommendations.

2.10. Antioxidant Parameters. Liver tissues have undergone homogenization in 10 mM phosphate buffer (pH 7.4) and centrifugation for 10 min (3000 × *g*) at 4°C. Using the Lowry technique, the protein concentration in the supernatants was measured [24]. According to a prior description, the supernatant was used to calculate the amount of malondialdehyde (MDA) generated as a result of lipid peroxidation (LPO) based on the thiobarbituric acid reaction assay [25]. Nitric oxide (NO) level was measured by using the Griess reagent [26]. The method described by Ellman was used to quantify the amount of reduced glutathione (GSH) Ellman [27]. Superoxide dismutase (SOD) activity was estimated according to the described method by Nishikimi et al. [28]. Catalase (CAT) activity was assayed according to the described method by Aebi [29].

2.11. Histological Procedures. Rat liver was divided into smaller pieces and left to fix for 24 h at room temperature in 10% neutral phosphate-buffered formalin. The obtained paraffin blocks were chopped into 5 m thick portions, which were subsequently deparaffinized in xylene. To identify any histopathological changes, sections were counterstained with hematoxylin and eosin and viewed under a Nikon Eclipse E200-LED microscope (Tokyo, Japan) at various magnifications.

2.12. Quantitative Real-Time PCR Analysis. Following the manufacturer's instructions, total RNA was extracted from freshly isolated liver tissues using the QIAzol reagent (Qiagen, Germantown, MD, USA). Thermo Fisher Scientific Inc., Canada's Fermentas brand of RevertAidTM H Minus Reverse Transcriptase kit was used to reverse transcribe RNA into cDNA in accordance with the manufacturer's instructions. SYBR Green PCR kit (Qiagen, Germany) was used to determine mRNA levels of glucose uptake-related genes (glucokinase (GK), hexokinase (HK), pyruvate kinase (PK), and glucose-6-phosphate dehydrogenase (G6PD)), insulin signaling pathway-related genes (insulin receptor substrate 1 (IRS1), phosphoinositide-3-kinase, regulatory subunit 2 (beta) (PIK3R2), and insulin receptors (IR)), and lipogenesis-related genes (fatty acid synthase (FASN) and peroxisome proliferator-activated receptor α (PPARα)).

Using the Rotor gene PCR system, a triplicate run of quantitative PCR was carried out (Qiagen, Germany). The Primer-Blast tool from the National Center for Biotechnology Information was used to build the PCR primers for the genes below, which were then manufactured by Jena Bioscience GmbH (Jena, Germany). The following were the PCR cycling conditions: 10 min at 95°C for polymerase

activation and 40 cycles at 95°C for 10 s, 58°C for 15 s, and 72°C for 15 s. The relative expression of mRNA was calculated by the $2^{-\Delta\Delta Ct}$ method, which was standardized to the mRNA level of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene. Primer sequences were shown in Table 2.

2.13. Statistical Analysis. Results were given as means SE for all statistical tests using SPSS (IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY, IBM Corp). Multiple group differences were assessed using one-way ANOVA, and differences were deemed statistically significant at $p < 0.05$. Drawing histograms with GraphPad Prism (version 6.01 for Windows, GraphPad Software, La Jolla, CA, USA). The Kruskal–Wallis test was used to compare differences between non-normally distributed data (percent of change in body weight), and the Dunn multiple comparison test was used to assess those differences.

3. Results

3.1. The Effect of GCBE on Body Weight. As shown in Table 3, at the beginning of the experiment, there were no significant differences between groups regarding body weight ($p > 0.05$). At week 10 of the experiment, the diabetic group showed a significant increase in body weight compared to the control group. Whereas groups treated with either GCBE (50 or 100 mg kg^{-1}) or metformin showed a significant decrease to the level of the control group ($p < 0.05$).

3.2. The Effect of GCBE on the Concentration of Fasting Plasma Glucose (FPG), Fasting Plasma Insulin (FPI), and Oral Glucose Tolerance. FPG was significantly elevated in HFD/STZ group compared to the control group ($p < 0.05$). Furthermore, treatment of diabetic rats with metformin significantly improved the elevated FPG in comparison with the diabetic group ($p < 0.05$). The same effect was observed in groups treated with GCBE in a dose-dependent manner. FPI was significantly decreased in the diabetic group compared to the control group. Although metformin failed to reverse this situation ($p > 0.05$), a dose-dependent elevation in insulin level was seen in groups receiving GCBE (50 and 100 mg kg^{-1}) ($p < 0.05$). In the same line, GCBE and metformin ameliorated insulin sensitivity ($p < 0.05$) as indicated by a significant reduction in the HOMA-IR index (Table 4).

Regarding the OGTT and in the control group, the blood glucose level attained the maximum peak at 60 min after an oral glucose load, and it progressively reverted to the physiological level at 120 min, indicating the maintenance of normal glucose homeostasis. On the other side, the blood glucose levels in diabetic rats reached the maximum peak at 60 min, but remained unsubsidized over the next 60 min. Oral treatment with GCBE as well as metformin resulted in a significant decrease in fasting glucose at 30, as well as 60 min, compared with untreated diabetic rats, suggesting the efficacy of GCBE in the maintenance of blood glucose homeostasis which owed to either insulin stimulatory and/or insulin-mimetic properties of the extract (Figure 3).

3.3. Effect of GCBE on Serum Lipid Profile. TC, TG, and LDL-c levels in diabetic rats showed a significant rise ($p < 0.05$) in comparison to those of the control group, while the level of HDL-c in the HFD/STZ group was significantly ($p < 0.05$) lowered than those of the control group. GCBE (50 and 100 mg kg^{-1}) markedly reduced TC, TG, and LDL-c levels, while raising the HDL-c concentration restoring them near to normal levels of control rats. Notably, metformin improved the levels of TC, TG, LDL-c, and HDL-c in the same manner (Table 4).

3.4. Effect of GCBE on Liver Function. Serum ALT and AST activities increased significantly in the diabetic group compared to controls ($p < 0.05$). Administration of metformin or GCBE at either dose reversed this condition in a dose-response manner as shown in (Table 4).

3.5. Effect of Green Coffee on Liver Oxidative Stress Markers. To assess the effects of GCBE or metformin on enzymatic oxidative stress markers in the liver tissue of diabetic rats, the activities of SOD, and CAT were measured. Compared to the control rats, the diabetic group showed significantly elevated levels of hepatic oxidative stress markers (MDA and NO) concomitantly with a significant ($p < 0.05$) decrease in antioxidant GSH levels. However, the co-administration of metformin or GCBE at either dose reversed this condition in a dose-response manner. Regarding the tested antioxidant enzymes (SOD and CAT), it was found that diabetes caused a significant ($p < 0.05$) decrease in their activities compared to those in the control group, but significant elevations ($p < 0.05$) were noted in metformin and GCBE at either dose in a dose-response manner (Table 5).

3.6. Effect of Green Coffee on Hepatic Inflammatory Markers. IL-1β and TNF-α levels showed a significant increase in the diabetic group compared to the control group ($p < 0.05$). Even so, treatment with metformin or GCBE at either dose showed a significant decrease compared to the diabetic group in a dose-dependent manner ($p < 0.05$) as shown in Table 5.

3.7. Effect of Green Coffee on Leptin. Compared to the control group, diabetes caused profound elevation ($p < 0.05$) in leptin levels. On the contrary, the administration of metformin or GCBE with either dose showed a significant decrease compared to the diabetic group in a dose-dependent manner ($p < 0.05$) as shown in Table 5.

3.8. The Effect of GCBE on mRNA Expression of Genes of Glucose and Lipid Metabolism. As shown in Figures 4–6, the hepatic mRNA expression levels of *GK, HK, PK,* and *G6PDH* genes had been significantly downregulated in the diabetic

Table 2: The primers' sequences.

Gene	Primer sequence (5′–3′)	Reference	Gene accession number
GAPDH reference gene	Sense: 5′GCATCTTCTTGTGCAGTGCC-3′ Antisense: 5′ GATGGTGATGGGTTTCCCGT-3′	GenBank primer-blast program, NCBI	NM_017008.4
Glucokinase (GK)	Sense: 5′GTGGTGCTTTTGAGACCCGTT-3′ Antisense: 5′-TTCGATGAAGGTGATTTC GCA-3′	GenBank primer-blast program, NCBI	NM_001270850.1
Hexokinase (HK)	Sense: 5′-AGGGGGATTTCATTGCCCTG-3′ Antisense: 5′-TCTTCTCGTGGTTCACCTGC-3′	GenBank primer-blast program, NCBI	NM_012734.1
Glucose-6- phosphate dehydrogenase (G6PD)	Sense: 5′-GTATGTGGGGAACCCCAGTG-3′ Antisense: 5′-AGGGACAGACTGATAGGCGT-3′	GenBank primer-blast program, NCBI	NM_017006.2
Pyruvate kinase (PK)	Sense: 5′-CCTCTGCCTTCTGGATACTGACT-3′ Antisense: 5′-GCAAGACTCCGGTTCGTA TCT-3′	GenBank primer-blast program, NCBI	NM_012624.3
Fatty acid synthase (FASN)	Sense: 5′-GCTTGGTGAACTGTCTCCGA-3′ Antisense: 5′-GGGTCCAGCTAGAGGGTACA-3′	GenBank primer-blast program, NCBI	NM_017332.1
Phosphoinositide-3-kinase, regulatory subunit 2 (beta) (PIK3R2)	Sense: 5′-TACCAGCACGCATCACTTGT-3′; Antisense: 5′-ATGGCTTGAAGAACTCGGGG-3′	GenBank primer-blast program, NCBI	NM_022185.2;
Insulin receptor (INSR)	Sense: 5′-GTTTTTGTTCCCAGGCCATCC3′; Antisense: 5′AATGCTTCCGGGAGACACAG-3′	GenBank primer-blast program, NCBI	NM_006248753.2
Insulin receptor substrate 1 (IRS1)	Sense: 5′CTGCATAATCGGGCAAAGGC3′ Antisense: 5′CATCGCTAGGAGAACCGGAC3′	GenBank primer-blast program, NCBI	NM_012969.1
Peroxisome proliferator-activated receptor α (PPARα)	Sense: 5′TATTTATTGCATCCAGTGGG3′ Antisense: 5′ATGTTTCCCATCTCTTGTAA3′	GenBank primer-blast program, NCBI	NM_013196.2

Table 3: The effect of GCBE on body weight.

Experimental groups	CNTR	HFD/STZ	HFD/STZ + Met	HFD/STZ + GCBE (50 mg kg^{-1})	HFD/STZ + GCBE (100 mg kg^{-1})	GCBE
Body weight at beginning (g)	192.2 ± 1.6	193.3 ± 3.1	196.8 ± 3.41	187.0 ± 2.7	192.8 ± 2.9	186.3 ± 1.4
Body weight at end (g)	221.3 ± 1.2	373.5 ± 11.5^{a}	217.8 ± 7.8^{b}	278.2 ± 4.1^{bc}	204.7 ± 14.9^{bd}	198.2 ± 2.5^{abcde}

Data are represented as the mean ± SE ($n = 7$). ^{a}Represents the statistical significance relative to that of the CNTR group at $p < 0.05$. ^{b}Represents the statistical significance relative to that of the HDF/STZ group at $p < 0.05$. ^{c}Represents the statistical significance relative to that of the HDF/STZ-Met group at $p < 0.05$. ^{d}Represents the statistical significance relative to that of the HDF/STZ-GCBE (50 mg kg^{-1}) group. ^{e}Represents the statistical significance relative to that of a group HDF/STZ-GCBE (100 mg kg^{-1}) group.

Table 4: The effect of green coffee bean water extract on biochemical parameters.

Experimental groups	CNTR	HFD/STZ	HFD/STZ + Met (200 mg kg^{-1})	HFD/STZ + GCBE (50 mg kg^{-1})	HFD/STZ + GCBE (100 mg kg^{-1})	GCBE (100 mg kg^{-1})
Glucose level (mg/dL)	106.2 ± 3	313.7 ± 3^{a}	172.8 ± 12^{ab}	219.8 ± 8^{abc}	129.8 ± 4.3^{bcd}	98.8 ± 3.7
Insulin level (ng/ml)	0.6 ± 0.03	0.25 ± 0.02^{a}	0.29 ± 0.01^{a}	0.39 ± 0.02^{ab}	0.5 ± 0.02^{bcd}	0.6 ± 0.03
HOMA-IR	17.05 ± 0.6	52.57 ± 0.6^{a}	18.52 ± 1.6^{b}	27.62 ± 1.2^{abc}	16.74 ± 0.6^{bd}	14.6 ± 0.7
Cholesterol conc. (mg/dL)	66 ± 2.4	131 ± 2.7^{a}	84 ± 2^{ab}	115.0 ± 3.7^{abc}	92.9 ± 3^{abd}	55.8 ± 0.6
Triglycerides conc. (mg/dL)	39.7 ± 2.6	162.1 ± 6.2^{a}	70.50 ± 2.6^{ab}	115.5 ± 0.9^{abc}	79 ± 1.7^{abd}	37.4 ± 2.4
LDL conc. (mg/dL)	4.0 ± 0.1	14.49 ± 0.2^{a}	4.503 ± 0.4^{b}	8.802 ± 0.3^{abc}	5.223 ± 0.3^{bd}	3.2 ± 0.4
HDL conc. (mg/dL)	29.55 ± 0.9	13.06 ± 0.4^{a}	28.47 ± 0.9^{b}	17.91 ± 0.6^{abc}	28.28 ± 1.3^{bd}	31.4 ± 0.5
ALT activity (U/L)	32.5 ± 3.5	53.80±4^{a}	33.05 ± 1.7^{b}	39.53 ± 1.5^{b}	32.75 ± 1.5^{b}	33 ± 2.7
AST activity (U/L)	34.96 ± 0.9	69.77 ± 1^{a}	40.71 ± 3^{b}	59.14 ± 1.5^{abc}	40.89 ± 1.9^{bd}	34.5 ± 1.2
TNF-α (pg/mg protein)	0.26 ± 0.01	0.64 ± 0.02^{a}	0.45 ± 0.01^{ab}	0.52 ± 0.01^{ab}	0.28 ± 0.02^{bcd}	0.24 ± 0.02
IL-1β (pg/mg protein)	0.27 ± 0.01	0.58 ± 0.02^{a}	0.51 ± 0.01^{a}	0.43 ± 0.02^{abc}	0.33 ± 0.03^{bcd}	0.25 ± 0.02
Leptin level (ng/ml)	852.5 ± 8.7	1808 ± 20.6^{a}	1628 ± 19.34^{ab}	1538 ± 13.5^{abc}	1107 ± 8.6^{abcd}	808.3 ± 14.6

FPG: fasting plasma glucose, FPI: fasting plasma insulin, HOMA-IR: homeostatic model assessment for insulin resistance TC: total cholesterol, LDL: low-density lipoprotein, TG: triglyceride, HDL: high-density lipoprotein, ALT: alanine transaminase, and AST: aspartate transaminase. Data are represented as the mean ± SE ($n = 7$). ^{a}Represents the statistical significance relative to that of the CNTR group at $p < 0.05$. ^{b}Represents the statistical significance relative to that of the HDF/STZ group at $p < 0.05$. ^{c}Represents the statistical significance relative to that of the HDF/STZ-Met group at $p < 0.05$. ^{d}Represents the statistical significance relative to that of the HDF/STZ-GCBE (50 mg kg^{-1}) group. ^{e}Represents the statistical significance relative to that of a group HDF/STZ-GCBE (100 mg kg^{-1}) group.

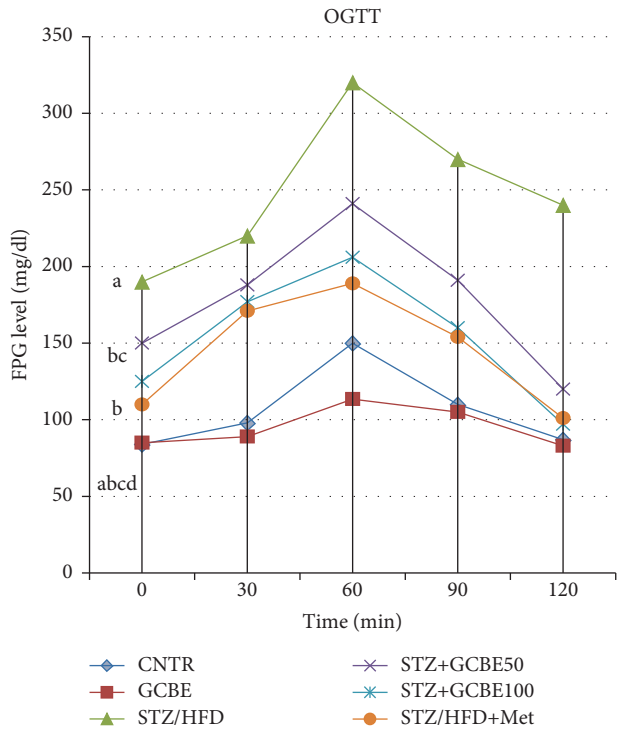

Figure 3: Effect of green coffee water extract (50 and 100 mg kg^{-1}) or metformin (200 mg kg^{-1}) on oral glucose tolerance test (OGTT) following high-fat diet-streptozotocin-induced diabetes in male rats. Data are represented as the mean ± SE ($n = 7$). ^{a}Represents the statistical significance relative to that of the CNTR group at $p < 0.05$. ^{b}Represents the statistical significance relative to that of the HDF/STZ group at $p < 0.05$. ^{c}Represents the statistical significance relative to that of the HDF/STZ-Met group at $p < 0.05$. ^{d}Represents the statistical significance relative to that the of HDF/STZ-GCBE (50 mg kg^{-1}) group. ^{e}Represents the statistical significance relative to that of a group HDF/STZ-GCBE (100 mg kg^{-1}) group.

Table 5: The effect of GCBE on oxidative stress.

Experimental groups	CNTR	HFD/STZ	HFD/STZ + Met (200 mg kg^{-1})	HFD/STZ + GCBE (50 mg kg^{-1})	HFD/STZ + GCBE (100 mg kg^{-1})	GCBE (100 mg kg^{-1})
MDA level (nmol/mg protein)	10.1 ± 0.3	17.6 ± 0.4^{a}	11.5 ± 0.6^{b}	12.2 ± 0.6^{b}	12.3 ± 0.6^{b}	10.8 ± 0.4
NO level (mmol/mg protein)	6.5 ± 0.4	17 ± 0.5^{a}	10.6 ± 0.2^{b}	11.8 ± 0.5^{b}	7.5 ± 0.4^{bcd}	9 ± 0.5^{a}
GSH level (mmol/mg protein)	31.1 ± 0.3	15.6 ± 0.8^{a}	26.61 ± 0.7^{b}	18.71 ± 1.1	30.1 ± 0.8^{bcd}	30.2 ± 0.6
SOD activity (U/mg protein)	215.6 ± 1.4	160.2 ± 3^{a}	181.8 ± 2.7^{b}	204.8 ± 1.5^{b}	215.6±2^{bcd}	227.3 ± 0.8^{a}
CAT activity (U/mg protein)	96.36 ± 0.8	72.07 ± 1^{a}	86.04 ± 1.5^{b}	88.1 ± 2.6^{b}	93.4 ± 2.7^{b}	96.6 ± 1^{bcd}

MDA: malondialdehyde, NO: nitric oxide, GSH: reduced glutathione, SOD: superoxide dismutase, and CAT: catalase. Data are represented as the mean ± SE ($n = 7$). [a]Represents the statistical significance relative to that of the CNTR group at $p < 0.05$. [b]Represents the statistical significance relative to that of the HDF/STZ group at $p < 0.05$. [c]Represents the statistical significance relative to that of the HDF/STZ-Met group at $p < 0.05$. [d]Represents the statistical significance relative to that of the HDF/STZ-GCBE (50 mg kg^{-1}) group. [e]Represents the statistical significance relative to that of a group HDF/STZ-GCBE (100 mg kg^{-1}) group.

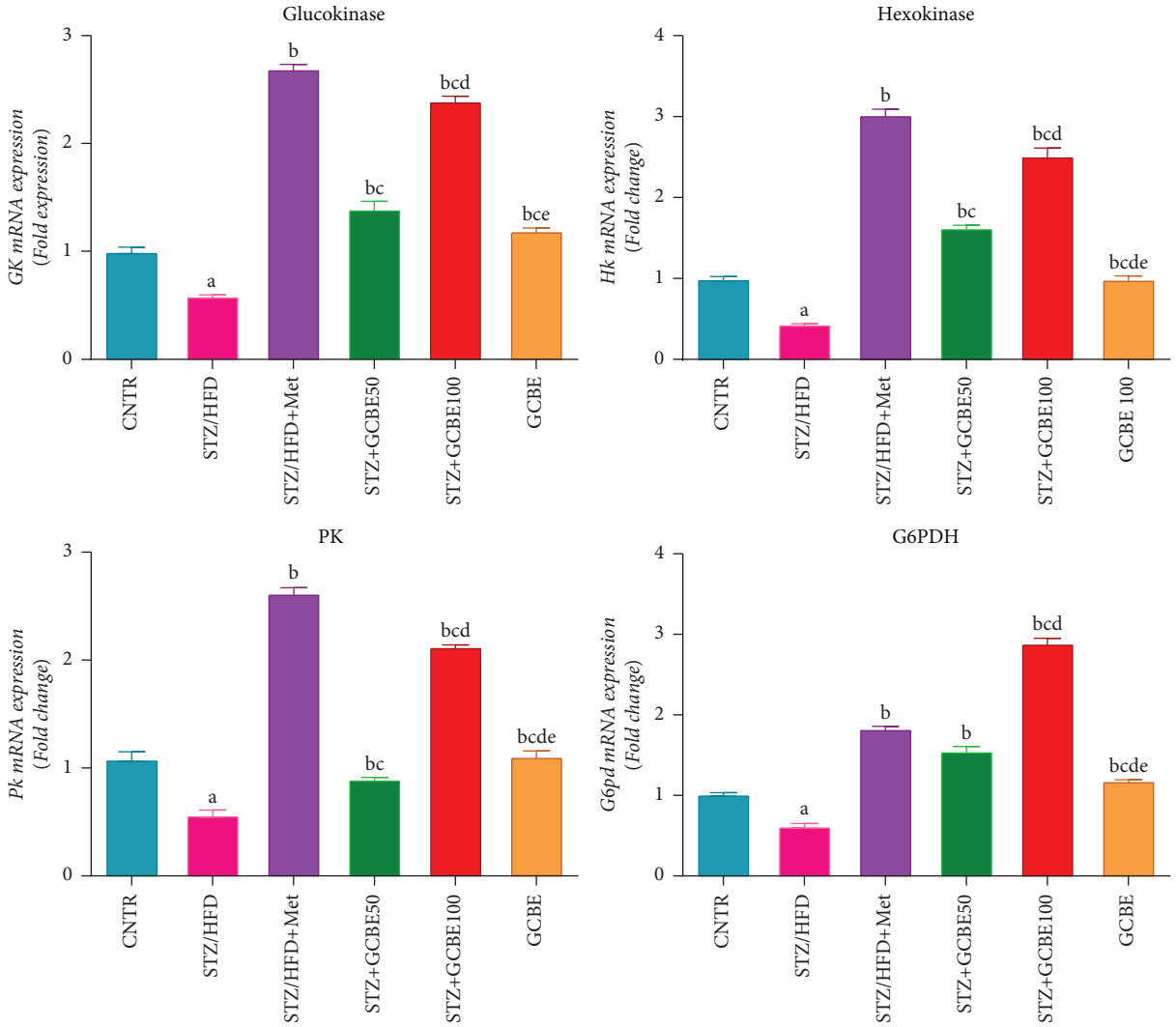

Figure 4: Effect of green coffee water extract (50 and 100 mg kg^{-1}) or metformin (200 mg kg^{-1}) on mRNA expression of glucose uptake-related genes following high-fat diet-streptozotocin-induced diabetes in male rats. mRNA expression data are recorded as the mean ± SE of three assays in duplicate referenced to GAPDH and represented as fold changes (log2 scale) as compared with the mRNA levels of the control group. [a]Represents the statistical significance relative to that of the CNTR group at $p < 0.05$. [b]Represents the statistical significance relative to that of the HDF/STZ group at $p < 0.05$. [c]Represents the statistical significance relative to that of the HDF/STZ-Met group at $p < 0.05$. [d]Represents the statistical significance relative to that of the HDF/STZ-GCBE (50 mg kg^{-1}) group. [e]Represents the statistical significance relative to that of a group HDF/STZ-GCBE (100 mg kg^{-1}) group.

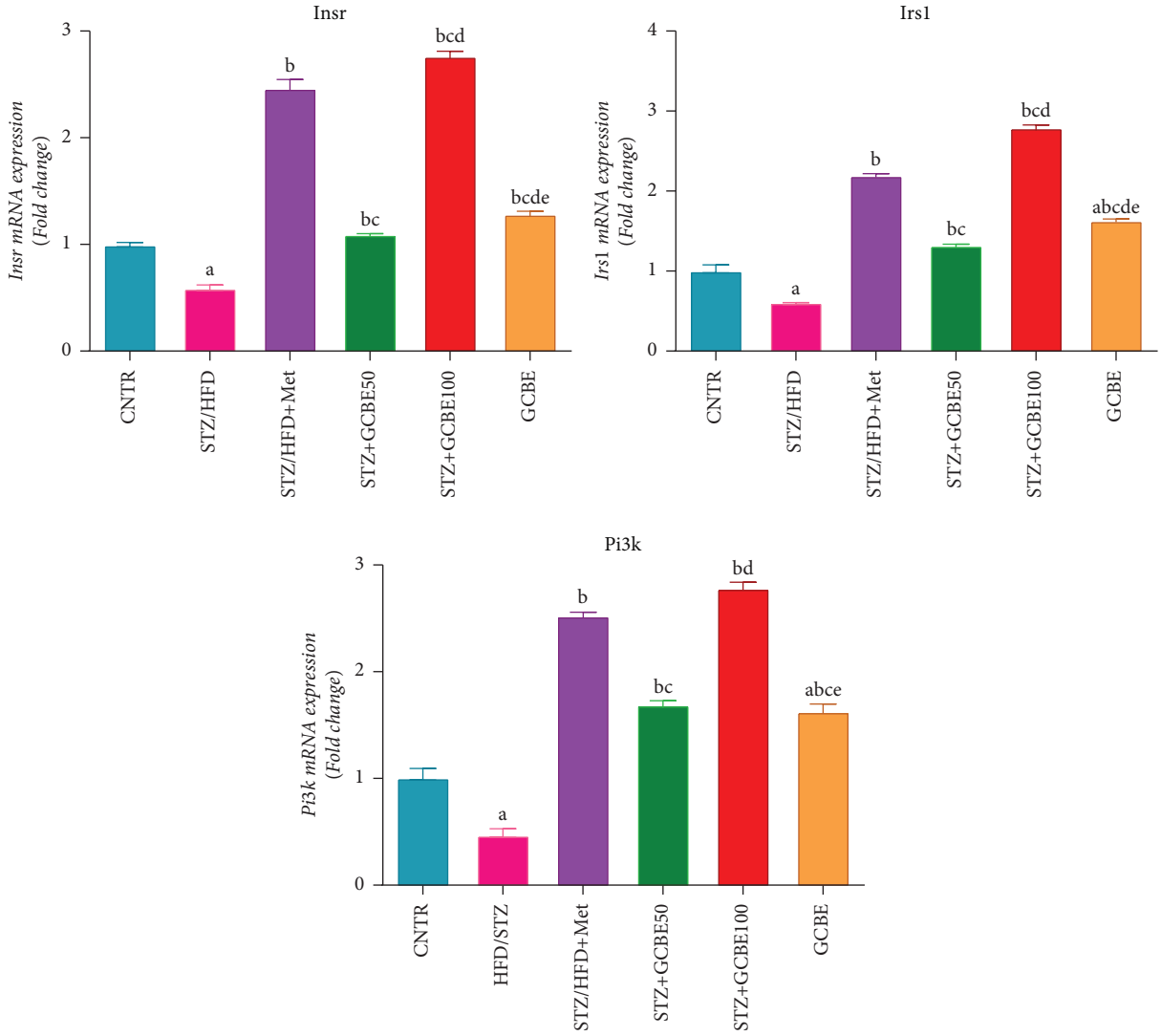

Figure 5: Effect of green coffee water extract (50 and 100 mg kg^{-1}) or metformin (200 mg kg^{-1}) on mRNA expression of insulin signaling-related genes following high-fat diet-streptozotocin-induced diabetes in male rats. mRNA expression data are recorded as the mean ± SE of three assays in duplicate referenced to GAPDH and represented as fold changes (log2 scale) as compared with the mRNA levels of the control group. [a]Represents the statistical significance relative to that of the CNTR group at $p < 0.05$. [b]Represents the statistical significance relative to that of the HDF/STZ group at $p < 0.05$. [c]Represents the statistical significance relative to that of the HDF/STZ-Met group at $p < 0.05$. [d]Represents the statistical significance relative to that of the HDF/STZ-GCBE (50 mg kg^{-1}) group. [e]Represents the statistical significance relative to that of a group HDF/STZ-GCBE (100 mg kg^{-1}) group.

group. Whereas treatment with metformin or GCBE (50 and 100 mg kg^{-1}) showed a significant up-regulation in these genes.

Besides, the hepatic mRNA expression levels of INSR, IRS-1, and PI3K genes had been significantly downregulated in the diabetic group. Fortunately, treatment with metformin or GCBE (50 and 100 mg kg^{-1}) showed a significant up-regulation in these genes.

Furthermore, the hepatic mRNA expression levels of the PPARα gene had been significantly downregulated in the diabetic group. In addition, treatment with metformin or GCBE (50 and 100 mg kg^{-1}) showed significant upregulation of their levels in a dose-response manner. Unsurprisingly, this affects FASN gene expression by way of up-regulation of their levels significantly in the diabetic group compared to CNTR. Likewise, treatment with GCBE (50 and 100 mg kg^{-1}) showed significant downregulation of FASN compared to diabetes ($p < 0.05$).

3.9. Histopathology of Liver and Pancreas following GCBE Treatment. Histological examination of the liver in the HFD/STZ group showed lipid accumulation in the vesicles of hepatocytes and eventually fatty degeneration of hepatocytes (Figure 7). Treatment with GCBE or metformin largely attenuated the vacuolar degeneration, as shown by the tremendous decrease in the formation of fat vacuoles in

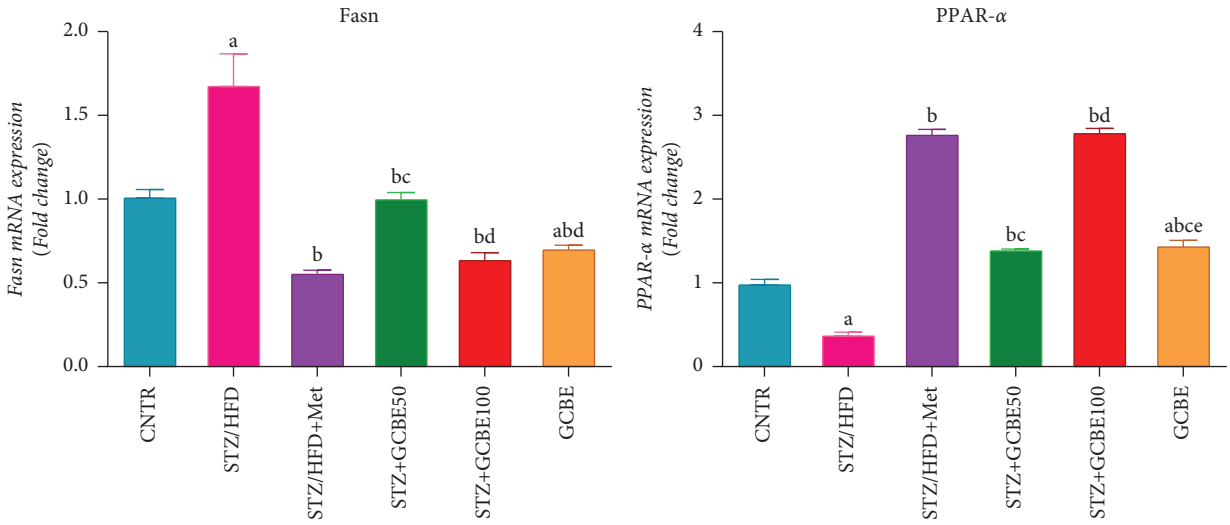

FIGURE 6: Effect of green coffee water extract (50 and 100 mg kg^{-1}) or metformin (200 mg kg^{-1}) on mRNA expression of lipogenesis-related genes following high-fat diet-streptozotocin-induced diabetes in male rats. mRNA expression data are recorded as the mean ± SE of three assays in duplicate referenced to *GAPDH* and represented as fold changes (log2 scale) as compared with the mRNA levels of the control group. [a]Represents the statistical significance relative to that of the CNTR group at $p < 0.05$. [b]Represents the statistical significance relative to that of the HDF/STZ group at $p < 0.05$. [c]Represents the statistical significance relative to that of the HDF/STZ-Met group at $p < 0.05$. [d]Represents the statistical significance relative to that of the HDF/STZ-GCBE (50 mg kg^{-1}) group. [e]Represents the statistical significance relative to that of a group HDF/STZ-GCBE (100 mg kg^{-1}) group.

the liver sections. In line with this finding, GCBE or metformin reduced the formation of oil droplets in the HFD/STZ group. In the HFD/STZ group, the fat droplets were big and obvious, while in GCBE or metformin-treated groups, the fat droplets were smaller and less dense.

4. Discussion

Diabetes has a significant impact on a variety of endogenous organs, with the liver being one of the most crucial [30–32]. A healthy diet high in fruits and vegetables protects the liver against various ailments, according to epidemiological research, and this is mostly because these foods contain antioxidant molecules such as polyphenols [33, 34]. One of the polyphenols present in GCBE in significant amounts is CGA [35, 36].

This research sought to determine if GCBE might mitigate the liver damage brought on by the HFD/STZ rat model [22]. To show how GCBE affects the course of diabetes and to highlight a potential underlying mechanism, the measurement of FPG, FPI, lipid profile, oxidative stress parameters, enzymatic and nonenzymatic antioxidant molecules, liver enzymes, inflammatory cytokines, adipokines, and gene expression of certain genes involved in glucose and lipid metabolism were the main objectives of this study. High FPG, FPI, and HOMA-IR values in the STZ/HFD animal model suggested that the STZ/HFD group had hyperglycemia, whereas hyperinsulinemia and insulin resistance showed that the diabetic group had serious cell failure and was in the late stages of type 2 diabetes mellitus (T2DM). As a result, the STZ/HFD model accurately represented the development of T2DM. These findings are consistent with prior research [37].

Treatment with GCBE at low and high dosages improved OGTT and increased FPI levels by maintaining healthy glucose homeostasis. These findings were consistent with another study's findings that GCBE improved OGTT by maintaining normal glucose homeostasis and lowered FPG, improved FPI, and HOMA-IR levels and its effects on lowering FPG [38]. Improving insulin sensitivity may be mediated by up-regulating the expression of glucose transporter type 4 (GLUT4), which can ultimately improve insulin activity and enhance the uptake and utilization of glucose in peripheral tissues [37, 39, 40]. It should be noted that not all naturally occurring antidiabetics function similarly. For instance, valproic acid was discovered to have no impact on FPI despite being a well-known naturally antidiabetic drug. According to the findings of this study, GCBE may be exerting its antidiabetic impact by boosting insulin sensitivity and secretion [41]. The findings obtained showed that GCBE had a substantial antidiabetic function and that its glucose-lowering impact was equivalent to that of metformin. Its high CGA content may be the cause of this. This result is consistent with findings from prior research indicating CGAs had a decreasing effect on glucose levels [6, 42–45].

The recent investigation also found dyslipidemia in diabetic rats in addition to hyperglycemia. The levels of TG, TC, and LDL-c were noticeably decreased, while HDL-c levels rose after treatment with GCBE (low and high) doses. It is interesting to note that the variables listed above, which were dysregulated in response to the production of diabetes, returned to their baseline values. This result was consistent with Ong et al. [46]. Besides dyslipidemia and hyperglycemia, oxidative stress is considered a significant risk factor for the development of T2DM and its complications [47].

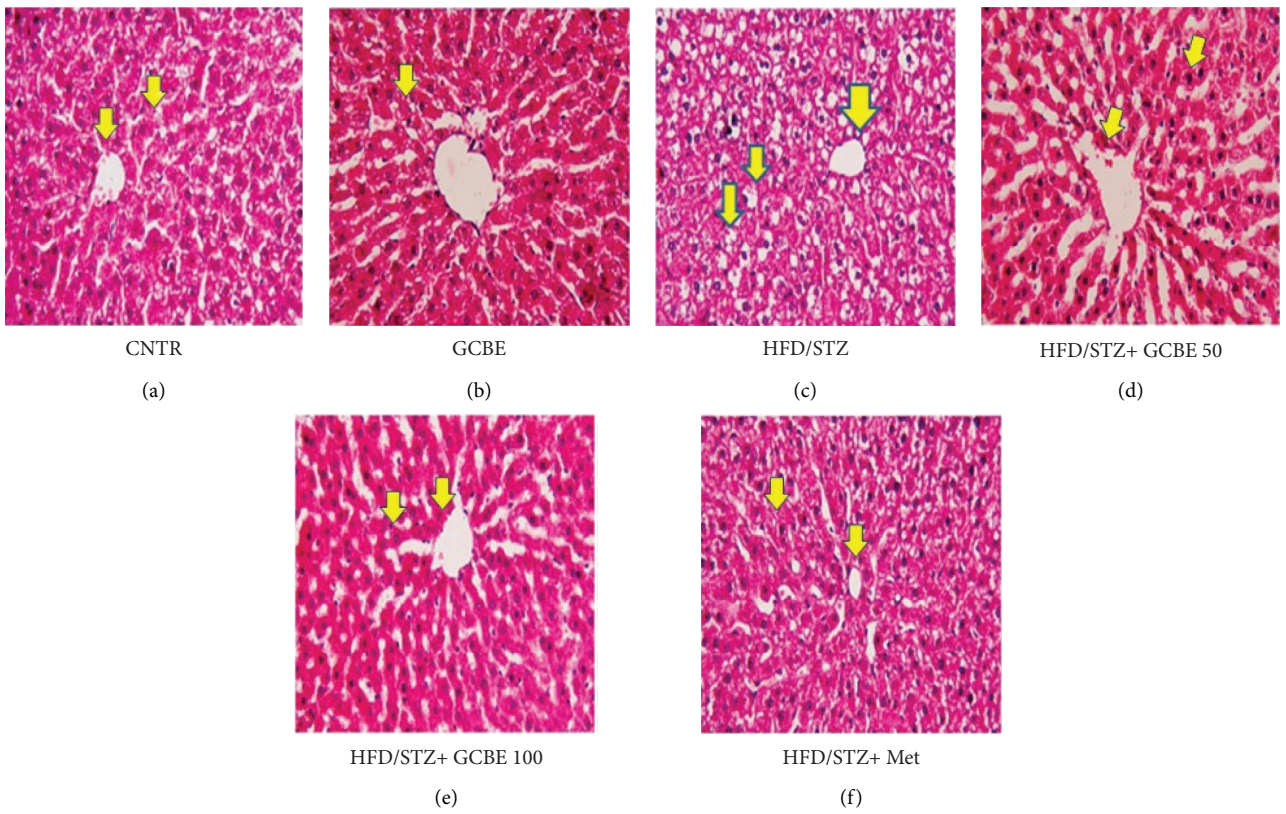

Figure 7: Histopathological changes of hepatic tissue following GCBE (50 and 100 mg kg^{-1}) or metformin (200 mg kg^{-1}) in high-fat diet-streptozotocin-induced diabetes in male rats stained with H&E. (a-b) Normal liver structure characterized by the normal portal veins and central veins were observed in the control and green coffee water extract-treated alone groups, respectively. (c) Focal infiltration of inflammatory cells and aggregation in between the damaged cells were observed in the HFD and STZ groups. (d-f) Metformin or green coffee showed improvement in the pathological changes in the liver of rats evidenced by the normal structure of the liver.

Conversely, increases in glucose and perhaps free fatty acid (FFA) levels, which are IR processes, cause oxidative stress by increasing the production of ROS and/or altering the redox balance, which can activate stress-sensitive signaling pathways [15, 48]. SOD and CAT are the major antioxidant enzymes involved in the direct scavenging of oxygen free radicals [35]. According to reports, the most significant factors contributing to liver damage in diabetes individuals are hyperglycemia-induced oxidative stress and the resultant disruption of the metabolism of carbohydrates, proteins, and lipids. In our investigation, we found that diabetic rats had decreased SOD, CAT, and GSH content. According to Matough et al., the enhancement of hepatic oxidative damage may be caused by this alteration in antioxidant capability [49].

The onset of nitrogenous stress and lipid peroxidation (NO and MDA), which came after the destruction of cellular compartments and function, was previously demonstrated to be primarily caused by high glucose levels and ROS formation [50]. According to the current data, the elevated MDA level found in the diabetic group was consistent with previously published findings [41, 51–53]. By administering GCBE (low and high dosages) in the current investigation, the increased production of MDA and decreased content of GSH, as well as the activities of SOD and CAT, were recovered, indicating a clear antioxidant activity of the GCBE that was consistent with that shown in previous studies [6, 7, 10, 54, 55].

Transaminases (ALT and AST) are extremely sensitive biomarkers that have been directly associated with toxicity and liver impairment [56]. According to Ghosh et al., significant elevations in ALT and AST activity in the serum of diabetic rats may be primarily caused by the leakage of these enzymes from the liver cytosol into the circulation as a result of hepatic damage linked to STZ [57]. The results of the HFD/STZ group in the present study were matched with the previous studies [56, 58, 59]. However, this work has demonstrated that administration of GCBE (in low or high dosages) returned the ALT and AST activity to normal levels. These results corroborated those of Xu et al., who found that CGA treatment decreased serum AST and ALT in a mouse model of tetrachloro-1,4-benzoquinone-induced acute liver damage [60].

On the other hand, our results are at odds with more recent research, which showed that GCBE did not significantly alter the activity of hepatic enzymes during the study in any of the study groups [51]. Through managing glucose levels, the liver performs a special function in regulating carbohydrate metabolism. This is accomplished by a complex network of enzymes that is closely controlled.

Insulin is one of the primary glucoregulatory mediators that regulate this process. A crucial enzyme for glycolysis, hexokinase, catalyzes the conversion of glucose to glucose-6-phosphate [61]. Hepatic hexokinase activity in diabetic rats is nearly completely suppressed or inactivated since it is an insulin-dependent enzyme [62]. In diabetic rats, decreased insulin levels cause hexokinase expression to be down-regulated, which eventually causes the establishment of chronic hyperglycemia. Hexokinase gene mRNA expression was significantly upregulated after oral administration of GCBE (low and high dosages) [63, 64].

All living things express the essential glycolytic enzyme pyruvate kinase, which catalyzes the irreversible phosphoryl group transfer from phosphoenolpyruvate to adenosine diphosphate to produce pyruvate and ATP. One may anticipate that its altered activity under diabetes circumstances will reduce glycolysis. Pk mRNA expression increased significantly after diabetic rats were treated with GCBE (low and high dosages), which may be related to the drug's ability to boost insulin activity. Additionally, hyperglycemia may cause G6PDH activity to decline. A risk factor for the etiology of diabetes may be a G6PDH deficiency. The oral delivery of GCBE significantly boosted the activity of G6PDH in the current investigation, demonstrating the regulatory role of GCBE in preserving normoglycemia in experimental diabetes [65–67].

The current investigation confirmed earlier findings by demonstrating that liver concentrations of inflammatory markers (IL-1β and TNF-α) were considerably greater in the STZ/HFD group than in the control group [6, 68, 69]. Additionally, we demonstrated that in diabetic rats, GCBE dramatically reduces the inflammatory cytokines (TNF-α and IL-1β) [70]. As a result, oral treatment of GCBE may also slow the progression of T2DM through anti-inflammation effects, matching the findings of Farhood et al., who showed that the antioxidant and anti-inflammatory properties of CGA are responsible for its hepatoprotective benefits [42].

Obesity and improved lipid metabolism are crucial for slowing the course of T2DM. An important organ in this problem is the liver. As a result, T2DM should be influenced by hepatic gene expression linked to lipid catabolism and lipogenesis. There are several important genes of lipid metabolism in the liver, such as PPAR-α and FASN. PPAR-α is a transcription factor that exerts notable effects on lipid homeostasis by regulating the expression of genes involved in lipid metabolism and the major regulator of lipid metabolism in the liver, where it enhances the uptake and catabolism of fatty acids since FFAs enhance IR and T2DM.

Increasing PPAR-α expression could decrease blood fatty acids and decrease ROS. Thus, it is expected that PPAR-α agonists are suggested as potential anti-T2DM drugs [51]. Interestingly, in this study, treatment with GCBE is associated with the induction of hepatic PPAR-α and inhibition of FASN expression. These results were the same as observed by Ismail et al. who reported that metformin reduced the mRNA and protein expression levels of several lipogenic enzymes, such as FASN, which is consistent with the results of the present study [71].

Adipokines are crucial in the development of diabetes. An adipokine called leptin has a link to insulin sensitivity. Leptin plays an important function in giving the central nervous system a signal of energy (adipose) storage in the body so that the brain can make the changes necessary to balance energy intake and expenditure. Leptin can correct either T1DM or T2DM in animal models [72]. Subcutaneous fat has been a primary driver of circulating leptin levels, and leptin levels rise with obesity.

Most obese people and rodents do not have low levels of leptin in their blood. They often have very high levels of plasma leptin, in contrast, but this endogenous hyperleptinemia may neither boost energy expenditure nor decrease appetite, a condition known as "leptin resistance" [73]. The decreased blood leptin concentrations in the low and high GCBE dosage groups in the current investigation suggest that GCBE may treat STZ/HFD-induced diabetes by reducing leptin production in rats and enhancing leptin resistance. This finding was consistent with that of Zheng et al. [73].

Although the insulin receptor (IR), phosphoinositide-3-kinase (PI3K), and AKT signaling pathway are well recognized as the major mechanism involved in glucose absorption, further research is needed to understand how STZ and HFD affect these proteins. A T2DM rat model was created to research the pathophysiology of T2DM and possible treatment benefits to evaluate this impact [69].

The standard treatment for T2DM is metformin, which has been shown to reduce the symptoms of IR by restoring normal serum lipid profiles in diabetic rats. It has also been shown to cause degenerative changes in the cytoplasm and parenchyma of hepatocytes and to increase the expression of insulin receptors [71]. It was reported that insulin signaling pathway genes were altered in diabetes [74] and these results are the same as our results. GCBE treatment increased the expression levels of Ir, Pi3k, and Pk genes [75–77].

This suggests that GCBE stimulates these genes' overexpression through the insulin signaling system. Following metformin therapy, the T2DM-induced changes in the expression levels of genes linked to lipid metabolism were restored. After binding to insulin, the insulin receptor is activated by autophosphorylation in the PI3K pathway, which controls how insulin affects liver tissue metabolism [78].

Following the phosphorylation of certain tyrosine residues on the IRS, the active insulin receptor causes IRS-1 and IRS-2 to bind to PI3K, phosphorylate serine, and activate protein kinase B (PKB) [79]. The connection between the insulin receptor and IRS is disrupted when IRS-1 is phosphorylated by serine kinases, which prevents IRS from being tyrosine phosphorylated [52, 74]. These previously observed mechanisms are consistent with the results of the present study.

5. Conclusions

The current study has shown that GCBE reversed significantly all HFD/STZ-induced liver alteration; most interestingly, in a dose-dependent manner by increasing the

expression of genes related to glucose uptake, increasing insulin secretion, improving glucose tolerance, improving insulin sensitivity through modulation of (IRS-1/PI3K pathways), improving leptin sensitivity (increasing E expenditure and decreasing weight gain), improving lipid profile, decreasing lipogenesis, decreasing inflammatory markers, decreasing oxidative stress, and improving antioxidant capacity. This finding also showed that metformin, which was provided substantially at a greater dose (200 mg kg^{-1} b.w.), was less effective than GCBE at concentrations of 50 mg kg^{-1} b.w. and 100 mg kg^{-1} b.w. So, we conclude that hepatic oxidative impairment induced by HFD/STZ can be reversed by GCBE in a dose-dependent manner. GCBE displayed robust free radical scavenging activity and dramatically rescued cells from the HFD/STZ-mediated oxidative insults. Thus, GCBE is recommended to be practically used as a natural safeguard food additive. As opposed to conventional medications, antioxidants may be an alternative and beneficial way to prevent and treat this life-threatening disease.

Data Availability

The data supporting the current study are given in the article.

Ethical Approval

Animals were handled under the guidelines of the National Institutes of Health (NIH), 8th edition (NIH Publication no. 85–23, revised 1985), for the care and use of laboratory animals. The experiments were approved by the ethical committee of the Faculty of Pharmacy, Helwan University, Egypt (approval no. 019A2018).

Conflicts of Interest

The authors declare that they have no conflicts of interest in this research.

Authors' Contributions

A. E. Abdel Moneim and H. K. Amin designed the project. D. M. Abdel-Mohsen and A. M. A. Akabawy performed the experiments. All the authors analyzed the data, interpreted the data, and drafted and edited the manuscript. M. F. El-Khadragy supplied the chemicals and reagents. All authors have read and approved the final draft. Ahmed E. Abdel Moneim and Hatem K. Amin contributed equally to this work.

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