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RESEARCH

Nrf-2/HO-1 modulatory effects on *Ocimum gratissimum* flavonoid-rich leaf extract in the livers of streptozotocin-induced diabetic rats

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Abstract

The present study examined the modulatory effects of *Ocimum gratissimum* leaf flavonoid-rich extracts on the Nrf-2 and HO-1 pathways in the livers of streptozotocin-induced diabetic rats. The animals were divided into five groups ($n=8$). These included a normal control, a diabetic control, diabetic rats administered low (LDOGFL) or high (HDOGFL) doses of *Ocimum gratissimum* leaf flavonoid-rich extracts at 150 and 300 mg/kg, respectively, and diabetic rats administered 200 mg/kg metformin. The animals were sacrificed on the 22nd day of the study, the liver was excised, and different biochemical parameters were evaluated. At the end of this study, diabetic rats administered LDOGFL and HDOGFL presented significant ($p<0.05$) decreases in fragmented DNA, protein carbonyl and lipid peroxidation levels, as well as glucose-6-phosphatase, and fructose 1,6 bisphosphatase activities. However, there was a significant ($p<0.05$) increase in the levels of antioxidant biomarkers; phosphatase and transaminase activities; GLUT 2 and glycogen levels; glycogen synthase and phosphorylase; hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase activities; and serum albumin and insulin in diabetic rats treated with extracts. Furthermore, there was a substantial increase in the relative gene expression of Nrf2 and HO-1, especially in diabetic rats administered LDOGFL. Hence, these findings suggest that these extracts might be helpful in managing hepatopathy in patients with diabetes mellitus.

Keywords Nrf2/HO-1 · Hepatopathy · Flavonoids · Antioxidant markers · Insulin regulation

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from either inadequate insulin production or impaired insulin utilization, ultimately leading to defective glucose metabolism and serious health complications [72]. Globally, diabetes has emerged as one of the leading causes of death and remains a major public health challenge [47, 48]. Its long-term consequences include an increased risk of cardiovascular disease, cerebrovascular disease, peripheral vascular disease, blindness, and end-stage renal failure. Alarmingly, the prevalence of diabetes continues to rise across nations, posing a global threat to human health, with projections indicating an exponential increase in incidence by 2025 [47, 48]. This reality underscores the urgent need for continuous research into alternative strategies for prevention and management.

Accumulating evidence suggests that oxidative stress plays a central role in the onset and progression of diabetes [72, 3, 50]. Oxidative stress results from an imbalance between the generation and elimination of reactive oxygen species (ROS), as well as the impaired activity of cellular redox regulators. A pivotal component of this defense system is the transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2), which regulates genes involved in detoxification and responses to oxidative stress, thereby protecting cells from toxic insults [72]. Through its interaction with the antioxidant response element (ARE), Nrf2 drives the expression of several antioxidant proteins, including heme oxygenase-1 (HO-1), which are crucial

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in counteracting oxidative damage [62]. However, when inactive, Nrf2 is degraded via the ubiquitin–proteasome pathway, allowing ROS to accumulate and exacerbating oxidative stress [70]. Notably, Nrf-2 dysregulation in diabetic conditions mechanistically contributes to hepatic damage through several interconnected pathways, primarily involving an impaired antioxidant response, exacerbated oxidative stress, inflammation, altered lipid metabolism, and increased apoptosis.

Given these mechanisms, medicinal plants have attracted considerable attention for their antioxidant and therapeutic potential in the management of diabetes and related complications. Globally, it is estimated that up to 80% of the population relies on ethnomedicine for primary healthcare needs [71]. This widespread use is attributed to the accessibility, affordability, and perceived safety of herbal medicines, as well as the rich phytochemicals they contain, which exert therapeutic effects with fewer side effects than synthetic drugs do [54, 71]. Indeed, many conventional drugs, including aspirin, morphine, and quinine, originate from medicinal plants [42].

Among such plants, *Ocimum gratissimum* (commonly known as scent leaf) stands out for its wide application in traditional and modern medicine. O. gratissimum, which belongs to the Lamiaceae family, is native to Asia, South America, and Africa and is widely used in Nigeria both as a culinary herb, notably in "pepper soup," and as a medicinal plant [19]. In addition to its use as a flavoring agent, the plant is endowed with diverse pharmacological properties. It has been traditionally employed in the treatment of ailments such as fever, diarrhea, anemia, pain, cough, fungal infections, and diabetes [4]. Experimental studies further highlight its broad therapeutic potential, demonstrating antimicrobial, anti-inflammatory, immunomodulatory, antioxidant, antimycotoxigenic, and vasorelaxant properties in both animal models and in vitro systems [2, 11].

Taken together, several methods are available for inducing diabetes mellitus in rat models. However, streptozotocin (STZ) is often preferred over other compounds, particularly alloxan, because it provides more stable and reproducible results. Its well-defined mechanism of action, greater stability, and lower off-target toxicity make it a more convenient and predictable choice for establishing experimental diabetes in rats [25]. In addition, it is faster than high-fat diet is. Streptozotocin induces diabetic pathophysiology by triggering systemic apoptosis within the pancreatic tissue, ultimately resulting in diabetes characterized by dysregulated glucose and lipid metabolism [32, 33].

In light of these findings, the present study investigated the modulatory effects of flavonoid-rich extracts of Ocimum gratissimum leaves on the Nrf2/HO-1 signaling pathway in the livers of streptozotocin-induced diabetic rats, with the aim of exploring its potential as an effective phytomedicine for diabetes management.

Materials and methods

Plant material sources and authentication

Ocimum gratissimum leaves were obtained from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves were air-dried for two weeks at 25 °C; thereafter, they were turned into a powder via an electrical blender.

Chemicals, reagents and enzyme kits

Methanol, sulfuric acid, absolute ethanol, fructose, concentrated ammonium hydroxide, dilute ammonium hydroxide, streptozotocin (STZ), 10% formalin, sodium citrate buffer, and phosphate buffer, among others, were obtained from Signal Aldrich, Germany. All the reagents used in this study were of analytical grade. Additionally, the enzyme kits used in this research were all products of Randox Laboratory (Crumlin, United Kingdom).

Preparation of Ocimum gratissimum flavonoid-rich extracts

An 80% methanol mixture was used to defat *Ocimum gratissimum* leaves (in powder form) with intermittent shaking for 72 h. This mixture was filtered to obtain a filtrate, which was concentrated via a rotary evaporator. A known gram of the residue was dissolved in a specific volume (20 mL) of 10% H_2SO_4 and hydrolyzed. The mixture was placed on ice for 15 min for the precipitation of the flavonoid aglycones. The cooled solution was filtered, and the filtrate (flavonoid aglycone mixture) was dissolved in 50 mL of warm 95% ethanol (50 °C). The resulting solution was again filtered into a 100 mL volumetric flask, which was filled with 95% ethanol. The methods described by Chu et al. [15] and Obafemi et al. [45] were followed. The obtained flavonoid extract was stored in a refrigerator at 4 °C.

Experimental animals and induction of diabetes

Forty male Wistar rats weighing 150 ± 20 g were obtained from Show-Gold Animal House Idofin, Oye-Ekiti, Ekiti State, Nigeria. They were kept in a conventional laboratory setting and acclimatized for one week.

The animals were fed adequately, and prior to induction, the rats to be induced were given 20% (w/v) fructose

solution [64]. In addition, twelve hours before the induction of diabetes with streptozotocin (STZ), the feed was withdrawn, and only water was left. A dose of 40 mg/kg body weight STZ was administered to the animals. Hence, only animals with fasting blood glucose levels ≥ 250 mg/dL at 72 h after STZ injection were used in this study [3].

Animal grouping

The animals were grouped into five groups with eight animals per group as follows:

Group 1: normal control (NC);
Group 2: diabetic control (DC);
Group 3: Diabetic rats were administered a low dose (150 mg/kg body weight) of *Ocimum gratissimum* flavonoid-rich extract leaf (OGHDFL);
Group 4: Diabetic rats were administered a high dose (300 mg/kg body weight) of *Ocimum gratissimum* flavonoid-rich extract leaf (OGHDFL).
Group 5 included diabetic rats that were administered 200 mg/kg metformin (MET) (reported by [30]).

The treatment lasted for 21 days.

Tissue collection

On the twenty-second day after oral administration, the rats were sacrificed via cervical dislocation. Blood samples were immediately withdrawn from each rat via cardiac puncture into a plain bottle (no anticoagulant bottle) and centrifuged for 5 min at 1,500 × *g*. The obtained serum was stored in a refrigerator. Each animal's liver was collected, washed in normal saline, cleaned with filter paper, and homogenized in 0.1 M potassium phosphate buffer at pH 6.5. The samples were subsequently centrifuged at 4000 rpm for 15 min. The obtained filtrate was stored in a freezer for further analysis [50].

Biomarker assays studied

DNA fragmentation was measured via the diphenylamine (DPA) spectrophotometric method as described by Wolozin et al. [78]. The protein carbonyl content was determined according to the method of Levine et al. [36]. Albumin levels and the activities of oxidative stress biomarkers (i.e., MDA, SOD, CAT, GST, GPx, and GSH), phosphatases (ALP and ACP) and transaminases (ALT, AST and GGT) were determined via appropriate commercial kits produced by Randox.

Table 1 Primer sequences

Gene	Forward primer	Reverse primer
Nrf-2	5'- CAGCGACGGAAAGAGT ATGA-3'	5'- TGGGCAACCT GGGAGTAG-3'
HO-1	5'- CAACATCCAGCTCTTTG AGG-3'	5'- GGCAGAATCT TGCACTTTG-3'
GAPDH	5'-GCAAGGATACTGAGAGC AAGAG-3'	5'-CATCTCCCTCA CAATTCCATCC-3'

Relative gene expression of Nrf-2 and HO-1

The total RNA of the liver was isolated via a Quick-RNA Mini-Prep™ Kit (Zymo Research). This product was then converted into cDNA via a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs). Finally, the obtained mixture was subjected to real-time polymerase chain reaction (RT–PCR) amplification (30 cycles). Each target was normalized against houskeeping GAPDH. The sequences of the primers used are listed in Table 1. The quantification of band intensity was performed via ImageJ software [20].

Carbohydrate metabolic enzymes studied

Serum insulin was determined via a commercial ELISA kit. GLUT2 and hepatic glycogen levels, as well as glycogen synthase, glycogen phosphorylase, hexokinase, pyruvate kinase, glucose-6- phosphatase, glucose-6-dehydrogenase, and fructose-1,6-bisphosphatase activities, were determined via commercial Randox kits, and their respective procedures were followed.

Histopathological examination

This was carried out via hematoxylin and eosin (H&E) staining as described by Drury and Wellington [17].

Statistical analysis

All the experimental results are shown as the mean ± S.D. ($n = 8$). Statistical significance was investigated by ANOVA followed by Tukey's multiple comparison test (post hoc test) via software (GraphPad Prism, Version 5.0). $p < 0.05$ was considered statistically significant.

Results

Effects of flavonoid-rich extracts from Ocimum gratissimum leaves on hepatic DNA fragmentation and protein carbonyl levels in streptozotocin-induced diabetic rats

Streptozotocin induction caused DNA fragmentation in the livers of diabetic rats, but treatment with flavonoid-rich

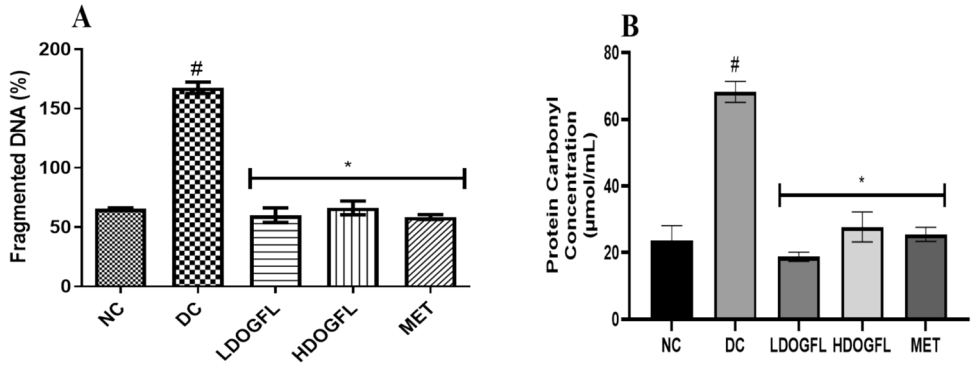

Fig. 1 Percentage of liver fragmented DNA and protein carbonyl concentration of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats. Each value is the mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum, HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin

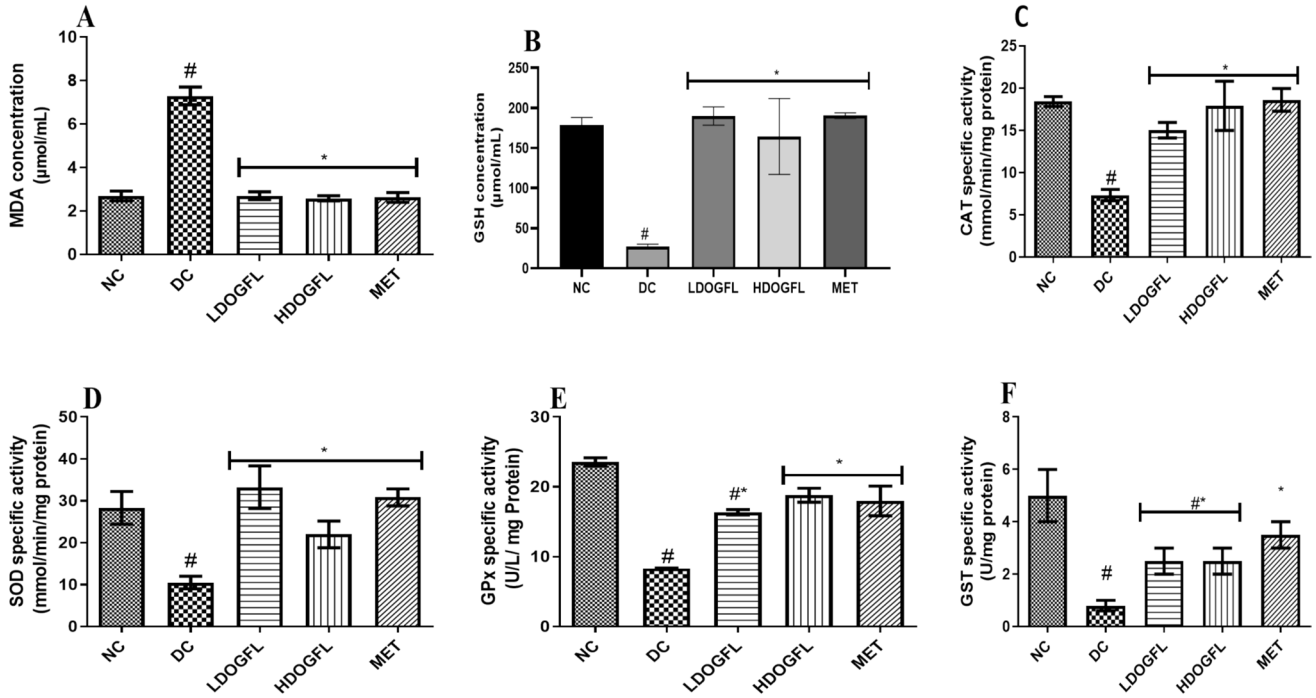

Fig. 2 Oxidative stress biomarkers of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats. Each value is the mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum, HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin, *MDA malondialdehyde, GSH* reduced glutathione, *CAT* catalase, *SOD* superoxide dismutase, *GPx* glutathione peroxidase and *GST* glutathione-S-transferase

extracts from *Ocimum gratissimum* leaves decreased the degree of STZ-induced DNA fragmentation. In addition, STZ intoxication increased the level of protein carbonylation in the liver tissue of the experimental animals (Fig. 1); however, treatment with flavonoid-rich extracts from *Ocimum gratissimum* leaves effectively reduced the level of hepatoxicity under hyperglycemic conditions, with a low dose (LDOGFL) being more effective than a high dose (HDOGFL).

Effects of flavonoid-rich extracts from Ocimum gratissimum leaves on oxidative stress biomarkers in streptozotocin-induced diabetic rats

Figure 2 (a-f) shows that streptozotocin-induced diabetic rat livers were in a state of oxidative stress, as revealed by the results of the biochemical tests. The administration of STZ to experimental rats caused an increase in lipid peroxidation

and hence in MDA production compared with those in the normal control group. The groups treated with flavonoid-rich extracts from *Ocimum gratissimum* leaves presented a significant reduction in elevated MDA levels. In contrast, there was a substantial decrease in the levels of reduced glutathione (GSH), *catalase* (CAT), *superoxide* dismutase (SOD), *glutathione* peroxidase (GPx) and *glutathione*-S-transferase (GST) in the *livers* of diabetic rats treated with flavonoid-rich extracts from *Ocimum gratissimum leaves* compared *with those in* diabetic control *rats*. The antioxidant effects of *the* flavonoid-rich extracts from *Ocimum gratissimum* leaves, as presented in Fig. 2 (b-e), revealed that the activities of GSH, CAT, SOD and GPx were significantly different from those of the normal control.

Effects of flavonoid-rich extracts from Ocimum gratissimum leaves on the hepatic tissue histology of streptozotocin-induced diabetic rats

Photomicrographs (Fig. 3) obtained from H&E staining revealed the hepatocytes (yellow arrowhead) and portal triad (bile duct, hepatic artery, portal vein) to reveal the effects of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rat livers. The normal control group presented normal pyknotic hepatocyte nuclei and a normal portal area. The livers of the diabetic control group exhibited dilation of the blood vessels and vacuolations of the hepatocytes, whereas the low- and high-dose *Ocimum gratissimum* leaves (LDOGFL and HDOGFL, respectively) presented normal pyknotic hepatocyte nuclei and normal portal areas, indicating that flavonoid-rich extracts of *Ocimum gratissimum* leaves ameliorated the effects of STZ.

Effects of flavonoid-rich extracts from Ocimum gratissimum leaves on hepatic phosphatase and transaminase activities as well as serum ALB levels in streptozotocin-induced diabetic rats

The activity of alkaline phosphatase (ALP) significantly decreased with the induction of streptozotocin; however, treatment with flavonoid-rich extracts from *Ocimum gratissimum* leaves reversed the effect of streptozotocin, and the activity of ALP increased in a dose-dependent manner, with the activity of ALP in the high-dose HDOGFL group being significantly different from that in the normal control group. Similarly, the activity of ACP (acid phosphatase), which was decreased by streptozotocin induction, increased in response to treatment with flavonoid-rich extracts from *Ocimum gratissimum* leaves, and both the low and high doses (LDOGFL and HDOGFL) did not significantly differ from that of the normal control, as shown in Fig. 4.

Additionally, Fig. 4 shows the effects of flavonoid-rich extracts from *Ocimum gratissimum* leaves on liver function indices (GGT: gamma-glutamyl transferase; AST: aspartate transaminase; ALT: alanine aminotransferase)

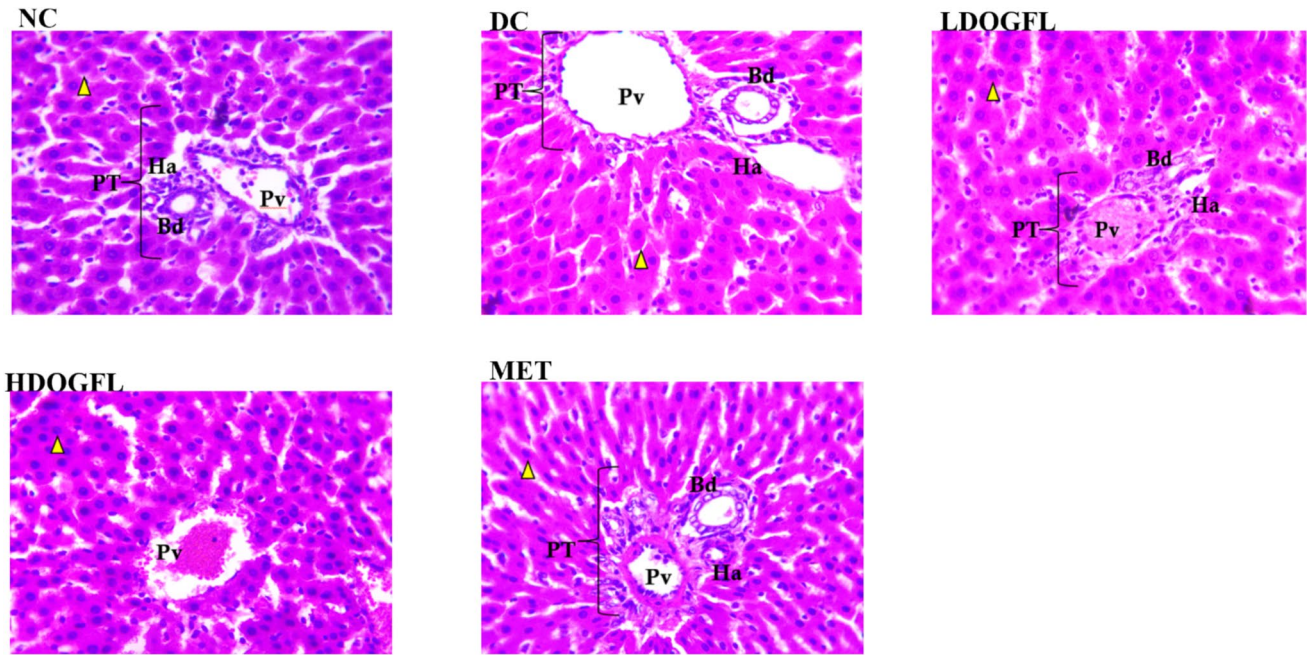

Fig. 3 Liver histoarchitecture examination of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum, HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin

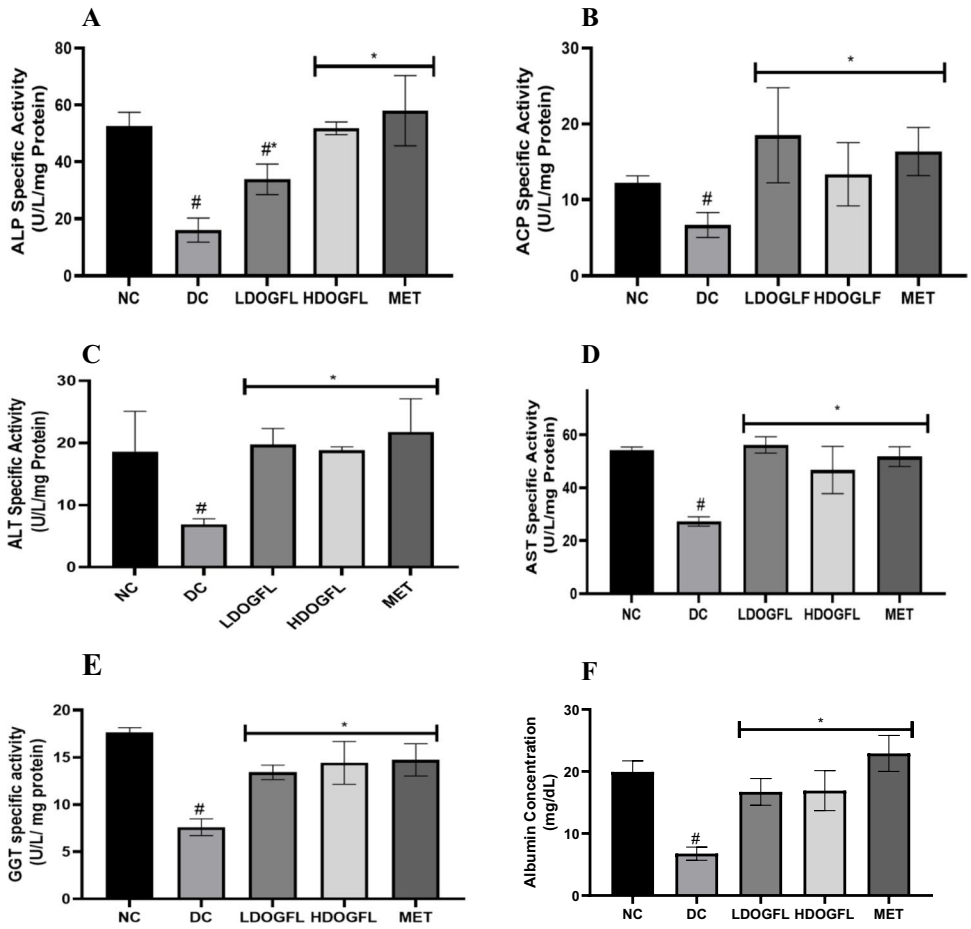

Fig. 4 Hepatic phosphatase and transaminase activities as well as the serum ALB levels of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats

in streptozotocin-induced diabetic rats. Streptozotocin induced a decrease in the levels of ALT, AST and GGT in the liver compared with those in the normal control. However, flavonoid-rich extracts from *Ocimum gratissimum* leaves reversed the effects of STZ.

Compared with the normal control, STZ induced a significant decrease ($p<0.05$) in the serum ALB level in diabetic rats. Moreover, the effect of streptozotocin was reversed, and an increased serum ALB concentration was observed when both low (LDOGFL) and high (HDOGFL) doses of flavonoid-rich extracts from *Ocimum gratissimum* leaves were administered, as depicted in Fig. 4.

Each value is the mean of eight determinations ± SD. # $p<0.05$ vs. NC, * $p<0.05$ vs. DC. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin, *ALP* alkaline phosphatase, *ACP* acid phosphatase, *ALT* alanine transferase, *AST* aspartate transaminase, *GGT* gamma-glutamyltransferase.

Effects of flavonoid-rich extracts from Ocimum gratissimum leaves on the relative gene expression of Nrf2 and OH-1 in streptozotocin-induced diabetic rats

The expression of the Nrf-2 gene and OH-1 gene was suppressed by STZ induction; however, these effects were reversed by flavonoid-rich extracts from *Ocimum gratissimum* leaves, with the low-dose extract being more effective than the normal control extract (Fig. 5).

Effects of flavonoid-rich extracts from Ocimum gratissimum leaves on the serum insulin concentration and GLUT 2 and hepatic glucose levels in streptozotocin-induced diabetic rats

Compared with normal control rats, streptozotocin-induced diabetic rats presented decreased serum insulin and glucose transporter 2 (GLUT2) concentrations, as depicted in Fig. 6. However, flavonoid-rich extracts from *Ocimum gratissimum* leaves reversed the effects of STZ, and the levels of serum insulin and GLUT2 were significantly different from those in the normal control group. Hepatic glucose was high in

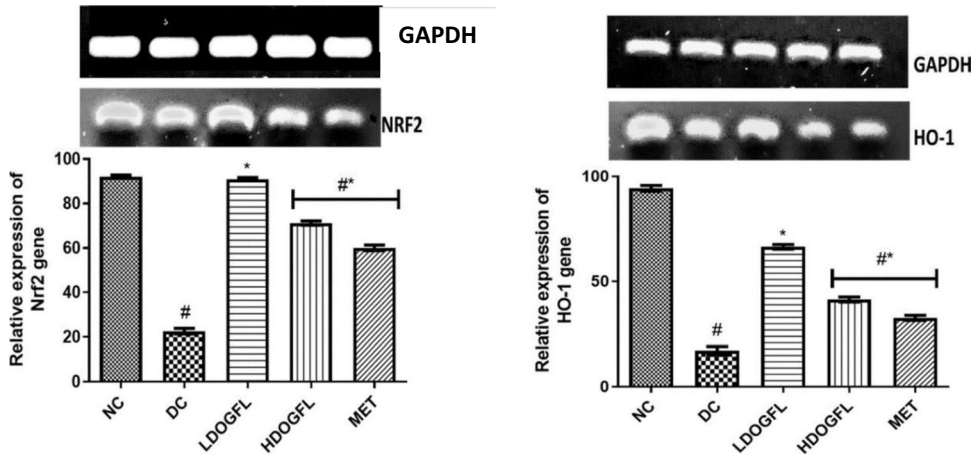

Fig. 5 Relative gene expression of Nrf2 and OH-1 in flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats. Each value is the mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin. GAPDH was used as the loading control. The GAPDH bands shown correspond to the same membranes and sample lanes as the respective target proteins, although presented above the target blots due to figure layout constraints

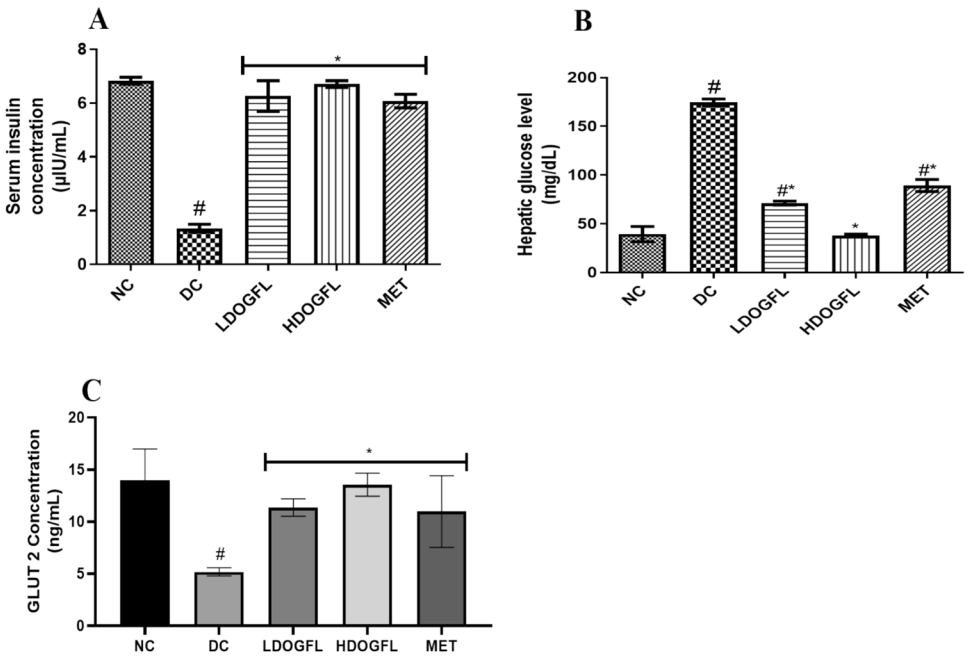

Fig. 6 Serum insulin concentration and GLUT2 and hepatic glucose levels of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats. Each value is the mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin, *GLUT2* glucose transporter 2

the diabetic groups and low in the treated groups, which was significantly similar to the normal control group (Fig. 6).

The concentration of glycogen in the livers of streptozotocin-induced diabetic rats was lower than that in the livers of normal control rats that were administered saline. Moreover, the hepatic glycogen concentrations in the groups treated with flavonoid-rich extracts from *Ocimum gratissimum* leaves and the standard antidiabetic drug metformin were significantly similar to those in the normal control group. Streptozotocin induction decreased the specific activity of glycogen synthase but increased the specific activity of glycogen phosphorylase, as shown in Fig. 7. However, flavonoid-rich extracts from *Ocimum gratissimum* leaves reversed the effects of STZ.

Furthermore, the effects of the administration of flavonoid-rich extracts from *Ocimum gratissimum* leaves on carbohydrate-metabolizing enzyme levels in streptozotocin-induced diabetic rats, as shown in Fig. 8, were compared among the normal control group, diabetic control group and treated groups. The induction by STZ significantly ($P < 0.05$) increased glucose-6-phosphatase and fructose-1,6-bisphosphatase activities. In contrast, STZ induction significantly

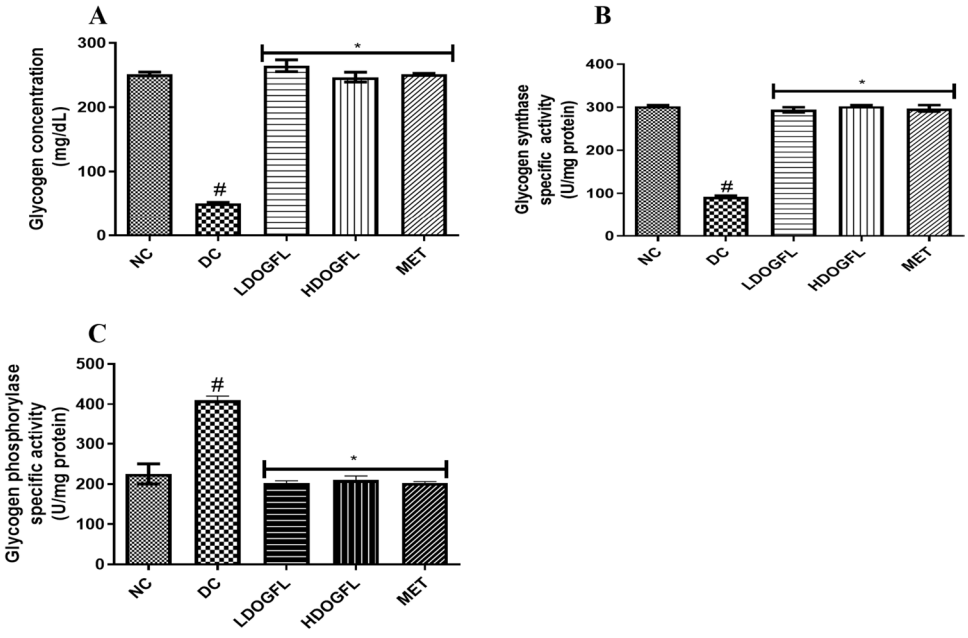

Fig. 7 Serum glycogen levels and glycogen synthase and glycogen phosphorylase enzyme activities of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats. Each value is the mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin

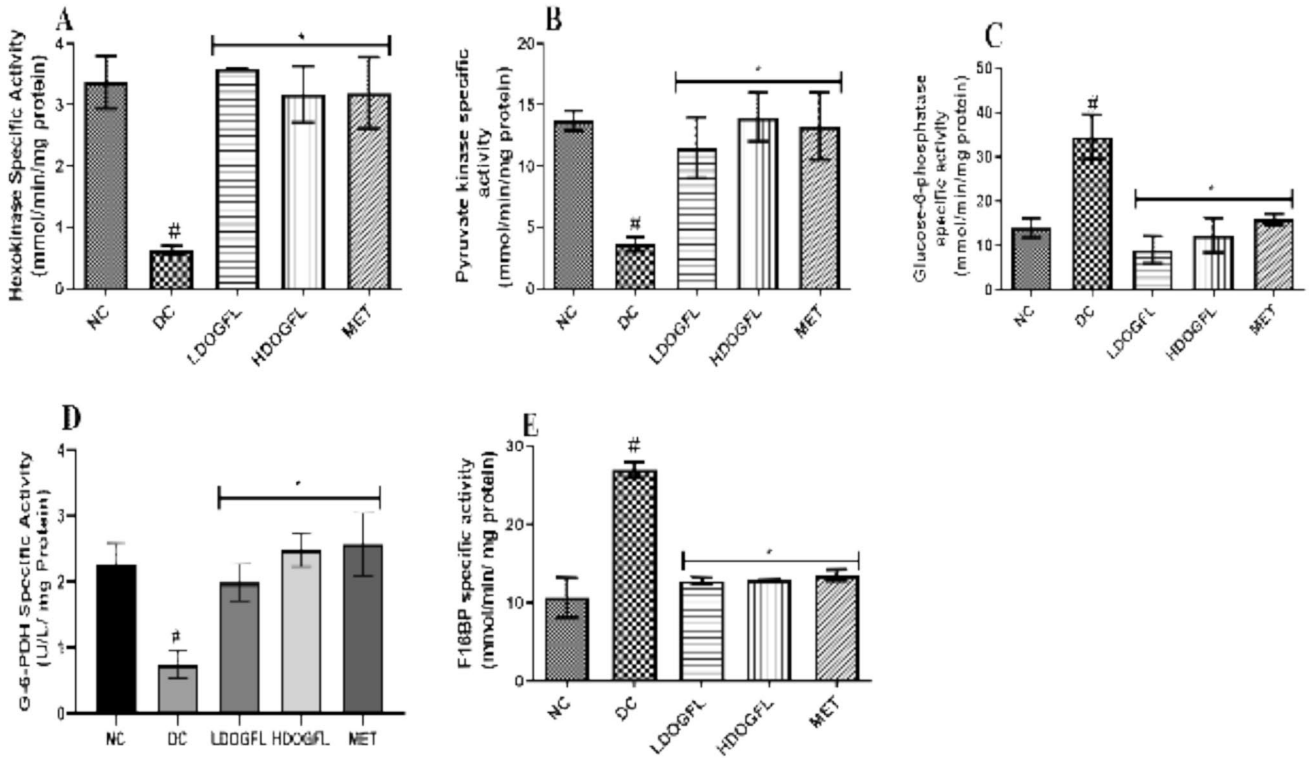

Fig. 8 Selected carbohydrate-metabolizing enzyme activities of flavonoid-rich extracts from *Ocimum gratissimum* leaves in streptozotocin-induced diabetic rats. Each value is the mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. *Legend*: *NC* normal control, *DC* diabetic control, *LDOGFL* diabetic rats administered a low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *HDOGFL* diabetic rats administered a high dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, *MET* diabetic rats administered 200 mg/kg metformin, *G-6-PDH* glucose-6-phosphate dehydrogenase, *F1,6BP* fructose-1,6-bisphosphatase-rich extract of Ocimum gratissimum

($P<0.05$) decreased the activities of hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase. The effects of the administration of flavonoid-rich extracts from *Ocimum gratissimum* leaves on all carbohydrate-metabolizing enzymes were similar to those of the normal control.

Discussion

Diabetes mellitus is the most prevalent metabolic disorder worldwide, and its global burden continues to rise. Consequently, there has been a significant increase in scientific investigations focused on the use of medicinal plants as potential therapeutic or management options for this disease [35, 58]. In addition to metabolic disturbances, diabetes is also associated with elevated oxidative stress, which contributes significantly to cellular and molecular damage.

DNA fragmentation refers to the breakdown of DNA strands into smaller fragments that progressively accumulate within cells. Owing to the ability of reactive oxygen species (ROS) to directly oxidize and damage DNA, proteins, and lipids, free radicals are considered major contributors to the development of diabetic complications [27]. The persistent hyperglycemic state in diabetes further elevates oxidative stress, resulting in increased levels of oxidative DNA damage markers and protein oxidation products such as carbonyls [67]. In the present study, streptozotocin induction markedly increased hepatic DNA fragmentation and protein carbonylation compared with those in the normal control group. However, treatment with flavonoid-rich extracts from *Ocimum gratissimum* leaves significantly reversed these alterations. Similar ameliorative effects on streptozotocin-induced hepatic DNA fragmentation have been reported for arjulonic acid [40], *Clerodendrum volubile* flowers [22], and stevioside [63].

Oxidative stress refers to a pathological condition in which the generation of free radicals, particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS), exceeds the capacity of the body's enzymatic and nonenzymatic antioxidant defense systems. This imbalance leads to cellular and tissue injury and has been strongly implicated in the initiation and progression of diabetes mellitus and its associated complications [1]. Excessive ROS can damage cellular macromolecules, including lipids, proteins, and nucleic acids, thereby impairing normal metabolic functions [31].

The body is equipped with a sophisticated antioxidant defense network consisting of enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), as well as nonenzymatic antioxidants such as reduced glutathione (GSH), vitamins C and E, carotenoids, and flavonoids. Among these, enzymatic antioxidants play central roles in neutralizing free radicals. SOD catalyzes the dismutation of superoxide anions, one of the most reactive ROS, into hydrogen peroxide. Although less reactive than superoxide, hydrogen peroxide can still be harmful if it is not promptly removed. CAT subsequently decomposes hydrogen peroxide into water and molecular oxygen, thereby preventing the formation of highly toxic hydroxyl radicals [52]. In addition, glutathione peroxidase (GPx) utilizes reduced glutathione (GSH) as a cofactor to convert hydrogen peroxide and lipid hydroperoxides into water and corresponding alcohols, protecting cellular membranes and organelles from oxidative injury [34]. Glutathione-S-transferase (GST) further contributes by detoxifying electrophilic compounds and products of lipid peroxidation through conjugation with GSH, thereby assisting in the elimination of toxic metabolites. Because these enzymes are essential for maintaining redox homeostasis, their activities are widely used as biomarkers of oxidative stress in experimental models of diabetes [73]. In diabetes, persistent hyperglycemia enhances mitochondrial ROS generation, depletes antioxidant reserves, and downregulates the activities of these protective enzymes, leading to oxidative damage in vital organs, particularly the liver and pancreas.

In this study, treatment with flavonoid-rich extracts from *Ocimum gratissimum* leaves significantly increased the activities of hepatic antioxidant enzymes, including SOD, CAT, GPx, and GST, compared with those in diabetic controls. These findings suggest that the extract restored the endogenous antioxidant defense system, thereby offering increased protection against oxidative damage. The observed increase in antioxidant enzyme activity is consistent with the well-documented antioxidant potential of *O. gratissimum*, which is attributed to its high content of bioactive flavonoids, phenolic acids, and essential oils. These phytochemicals act both as direct free radical scavengers and as modulators of antioxidant gene expression [5, 51, 66]. Collectively, these findings indicate that *O. gratissimum* leaf extract may mitigate oxidative stress not only by providing exogenous antioxidants but also by increasing endogenous enzymatic defenses, thereby preserving the cellular redox balance and reducing the risk of oxidative stress–mediated diabetic complications. This could be one of the mechanisms of this extract in the current study.

Histology is the microscopic study of animal and plant cells and tissues, providing vital insights into normal structural organization as well as pathological alterations. The histological process generally comprises five key stages: fixation, embedding, sectioning, staining, and microscopic examination [7]. Fixation preserves cellular morphology and prevents autolysis or microbial degradation, embedding stabilizes the tissue for cutting, sectioning produces

thin slices suitable for microscopy, staining enhances tissue contrast, and light microscopy allows the visualization and interpretation of these structures [9].

In this study, hepatic tissues were processed via hematoxylin and eosin (H&E) staining, the gold standard technique for general histopathological evaluation [24]. Hematoxylin, a basic dye, stains cell nuclei blue to purple, highlighting nuclear details such as chromatin and nucleoli, whereas eosin, an acidic dye, stains the cytoplasm, connective tissue, and extracellular matrix shades from pink to red, thereby providing an overview of tissue architecture. This dual staining allows clear differentiation between cellular and stromal components, making it particularly suitable for evaluating liver structure.

The sections examined focused on hepatocytes, which are the primary parenchymal cells of the liver, and the portal triad, which consists of the hepatic artery, portal vein, and bile duct. The arrangement of hepatocytes in cords or plates, which are separated by sinusoidal spaces, is critical for assessing hepatic function and pathology (Kumar et al., 2020). Examination of the portal triad is equally important, as alterations such as vascular congestion, bile duct proliferation, or inflammatory infiltrates often reflect hepatic injury or repair processes [59].

Previous studies have reported that streptozotocin (STZ)-induced diabetes is associated with hepatocellular degeneration, sinusoidal dilatation, inflammatory cell infiltration, and necrosis in liver tissue [79]. Conversely, treatment with flavonoid-rich plant extracts, including *Ocimum gratissimum*, has been shown to restore hepatic architecture, reduce degenerative changes, and enhance regenerative features such as intact hepatocyte plates and normal portal triad morphology [5, 66]. Therefore, the histological examination in this study not only provided structural confirmation of the biochemical findings but also highlighted the hepatoprotective potential of *Ocimum gratissimum* extract in mitigating diabetes-induced liver injury.

Ocimum gratissimum leaves have been demonstrated to enhance the activity of phosphatase enzymes, an effect attributed to thebioactive flavonoid constituents present in this plant [46]. Phosphatases are critical regulatory enzymes that modulate cellular processes through the dephosphorylation of proteins, thereby influencing signal transduction pathways, energy metabolism, and stress responses. Several studies have reported that flavonoids, such as quercetin, luteolin, and apigenin, can modulate phosphatase and kinase activities, restoring cellular homeostasis under oxidative and metabolic stress [43]. In diabetic models, increased phosphatase activity has been linked with improved regulation of carbohydrate metabolism and attenuation of hyperglycemia [6]. The observed increase in phosphatase activity following *O. gratissimum* treatment may therefore represent a mechanistic pathway through which its flavonoid-rich extract exerts hepatoprotective, antioxidant, and antihyperglycemic effects. This finding aligns with earlier findings on the ability of dietary flavonoids to support enzymatic defense systems and maintain redox balance, ultimately contributing to the prevention of oxidative stress–induced tissue damage [53].

Aminotransferases (formerly called transaminases) are liver enzymes that catalyze the transfer of the α-amino group of amino acids to the α-keto group of ketoglutarate [10]. Streptozotocin toxicity is characterized by permeability changes in the liver membrane and hence, cellular leakage of liver enzymes from hepatocytes into the blood stream, which leads to an elevation of liver enzymes (aminotransferase) in the serum [49] and a significant reduction in the liver, thereby acting as indicators of liver injury. An increase in the activities of the serum aminotransferases gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is frequently observed in individuals with diabetes [65]. ALT and AST are the most specific markers of hepatic injury and are located in the hepatocellular cytosol and mitochondria, respectively, where they play a central role in amino acid metabolism, whereas GGT is located on the external surface of most cells and plays a central role in the uptake of glutathione (an antioxidant) [41, 49]. Mansour et al. [41] reported that increased levels of ALT and AST are associated with insulin resistance, type 2 diabetes mellitus and metabolic syndrome. In addition, the increased activity of AST and ALT in liver tissue suggests the defective utilization of glucose as a result of insulin deficiency, which leads to the breakdown of protein and increased amino acid catabolism to provide substrates for gluconeogenesis [61]. This study revealed that STZ caused a change in liver permeability that led to the leakage of liver enzymes. The results for aminotransferases compared well with the findings of Rodríguez et al. [61] in the treatment of STZ-induced liver damage via the use of plant flavonoids from naringin.

It has been established that insulin controls the protein expression of albumin; however, insulin is deficient in diabetes; hence, the hepatic production of serum albumin decreases [14]. Albumin is the most abundant protein in the circulation,it is synthesized in the liver and accounts for approximately sixty percent (60%) of total serum proteins, functioning as the carrier for several endogenous and exogenous compounds [23]. Majorly lapses in amino acid/protein metabolism as a result of a deficiency in insulin secretion and/or inadequate insulin in STZ-induced diabetes are more critical factors than high blood glucose in diabetic complications [55],therefore, the protein ALB is a crucial biomarker used to assess liver function. In this study, a significant reduction in the total protein concentration of

albumin was observed in streptozotocin-induced diabetic rats. Moreover, the reduction was significantly reversed after treatment with a flavonoid-rich extract from *Ocimum gratissimum* leaves. This finding is in agreement with similar studies reported in the literature [21].

The Nrf2/HO-1 pathway is a vital cellular defense mechanism against oxidative stress, controlling enzymes that combat reactive oxygen species (ROS) and promote the cellular redox balance [26]. The upregulation of Nrf2/HO-1 plays a role in cell survival and antiapoptotic effects [44]. In diabetic hepatopathy, the downregulation of Nrf-2 and HO-1 has been observed, contributing to liver tissue injury through increased ROS levels [77]. The translocation of Nrf-2 to the nucleus under oxidative stress triggers the upregulation of antioxidant enzymes [13]. Thus, enhancing Nrf-2/HO-1 activation has emerged as a therapeutic strategy to alleviate diabetic conditions as well as their complications [37]. This study is in line with the reports of Janson et al. [28] and Malar et al. [39]. The flavonoid-rich extract from *Ocimum gratissimum* leaves was able to upregulate these key genes, which may be responsible for protecting the liver against various insults, thereby maintaining glucose homeostasis, ameliorating redox status and increasing insulin sensitivity in STZ-induced rats. This may be related to the flavonoid compounds (rutin, ellagic acid, myricetin, rosmarinic acid, methyl eugenol, luteolin, apigenin, nepetoidin A, etc.) present in this extract, as reported by Venuprasad et al. [74], Ajayi et al. [2], etc. These observations are in line with the reports of Vomund et al. [75] and Kartinah et al. [29], among others. This observation suggests an additional potential mechanism of action of the plant in the present study.

Streptozotocin results in abnormalities in β-cell functions by impairing the oxidation of glucose and reducing the biosynthesis and secretion of insulin [69]. A decrease in serum insulin is usually a sign of successful diabetes induction [16]. In addition, streptozotocin disrupts normal glucose homeostasis [56]. Physiologically, the glucose level is regulated by the equilibrium between the production of glucose in the liver (gluconeogenesis and glycogenolysis) and the utilization of glucose by other tissues [12]. However, a lack of sufficient secretion or action of insulin leads to increased hepatic glucose production. Glucose transporter 2 (GLUT 2 is involved in the transport of glucose in the liver and in the secretion of glucose-stimulated insulin from the pancreas. GLUT2 acts as a glucose sensor that detects small changes in glucose levels, leading to increased insulin secretion,however, in this study, STZ induction led to abnormalities in the functions of beta cells, which resulted in a reduction in insulin secretion and activity, the expression of GLUT2 and hence high hepatic glucose.

In healthy individuals, the pancreas responds to high levels of blood glucose and releases insulin to lower blood glucose by stimulating the liver and muscle to take up glucose and store it as glycogen [60]. Conversely, under diabetic conditions, glycogen does not accumulate because of insulin abnormalities. Hence, a lower concentration of glycogen is observed in diabetic rats. Since the conversion of glucose to glycogen is impaired in diabetes, the activity of glycogen synthase, a key enzyme in glycogenesis that catalyzes the conversion of glucose to glycogen, is reduced. Another important metabolizing enzyme of glycogen, glycogen phosphorylase, takes part in the first step of glycogenolysis, that is, the breakdown of glycogen to release glucose-1-phosphate [38]. The activity of hepatic glycogen phosphorylase, which is high in individuals with diabetes, can be used as an important therapeutic agent, as its inhibition is a treatment strategy for attenuating hyperglycemia in individuals with type 2 diabetes [8].

Carbohydrate metabolism occurs primarily in the liver to regulate glucose levels through a closely regulated series of enzymes. The activity of these enzymes is altered in diabetic conditions [57]. Glucose-6-phosphatase and fructose-1, 6-biphosphatase are glucose homeostasis enzymes found in the liver that play major roles in gluconeogenesis, however, insulin inhibits the activity of these gluconeogenic enzymes. Hence, the increase in gluconeogenic enzymes could be attributed to insulin insufficiency. Glucose uptake requires glucose to be first phosphorylated, and this phosphorylation is performed by hexokinase to produce a phosphorylated glucose that is a substrate for metabolism [76]. Hepatic pyruvate kinase converts ADP and phosphoenolpyruvate into ATP and pyruvate, which is the last step in glycolysis. Another carbohydrate-metabolizing enzyme, glucose-6-phosphate dehydrogenase, is the first and rate-limiting enzyme of the pentose phosphate pathway,it speeds up the oxidation of glucose-6-phosphate (produced by insulin-stimulated hexokinase) to 6-phospho gluconate and simultaneously reduces $NADP^+$ to NADPH [18]. These enzymes, hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase, are stimulated by insulin, hence reducing their activities in diabetes. In contrast, the antihyperglycemic effect in the treatment groups might have been due to extrapancreatic activity, including increased glucose utilization by the liver and muscle (glycolysis), increased glucose oxidation through the shunt pathway via the activation of G6PDH, and decreased glucose production via the depression of glycogenolytic enzymes [68].

Conclusion

This study demonstrated that flavonoid-rich extracts from *Ocimum gratissimum* leaves can modulate the levels of fragmented DNA and protein carbonyls; oxidative stress

biomarkers; liver histology; phosphatase and transaminase activities; albumin levels; relative gene expression levels of Nrf2/OH-1; insulin, hepatic glucose, and GLUT2 levels; hepatic glycogen- and glycogen-metabolizing enzymes; and carbohydrate-metabolizing enzyme activities. This research suggests that flavonoid-rich extracts from *Ocimum gratissimum* leaves could be helpful in managing the hepatopathy associated with diabetes-related complications.

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Data Availability This information is available upon special request from the corresponding author.

Declarations

Ethical Approval All experimental protocols in this study were approved by the FUOYE Faculty of Science Ethics Committee (ethics number FUOYEFSC 201122-REC2022/008).

Consent to participate All the authors agreed to this submission.

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Competing interests The authors declare no competing interests.

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