Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2024, Volume 4, Page No: 59-73

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Hibiscus sabdariffa Leaf Extract Enhances Molecular Gene Expression of Insulin and GLP-1 Receptors in Streptozotocin-Induced Rats

Basiru Olaitan Ajiboye[1]*, Courage Dele Famusiwa[1], Damilola Ifeoluwa Oyedare[1], Biola Paul Julius[1], Zainab Odunola Adewole[1], Oluwafemi Adeleke Ojo[2], Ajoke Fehintola Idayat Akindele[3], Hossein Hosseinzadeh[4,5], Bartholomew I.C. Brai[6], Babatunji Emmanuel Oyinloye[7-9], Sara Vitalini[10], Marcello Iriti[10]

[1]Phytomedicine and Molecular Toxicology Research Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Oye-Ekiti, Ekiti, Nigeria.
[2]Phytomedicine, Molecular Toxicology, and Computational Biochemistry Research Laboratory (PMTCB-RL), Department of Biochemistry, Bowen University, Iwo, 232101, Osun, Nigeria.
[3]Department of Biosciences and Biotechnology, Environmental Management and Toxicology Unit, Faculty of Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria.
[4]Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
[5]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
[6]Nutritional Biochemistry and Membrane Biochemistry, and Toxicology, Federal University Oye-Ekiti, Oye-Ekiti, Ekiti, Nigeria.
[7]Institute of Drug Research and Development, SE Bogoro Center, Afe Babalola University, Ado-Ekiti, Nigeria.
[8]Phytomedicine, Biochemical Toxicology and Biotechnology Research Laboratories, Department of Biochemistry, College of Sciences, Afe Babalola University, Ado-Ekiti, Ekiti, Nigeria.
[9]Biotechnology and Structural Biology (BSB) Group, Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa, South Africa.
[10]Department of Biomedical, Surgical and Dental Sciences, Università Degli Studi di Milano, Via G. Celoria 2, 20133, Milan, Italy.

***E-mail** ✉ bash1428@yahoo.co.uk
Received: 16 September 2024; **Revised:** 16 November 2024; **Accepted:** 19 November 2024

ABSTRACT

This study investigated the therapeutic effects of flavonoid-rich *Hibiscus sabdariffa* L. leaf extracts in rats with streptozotocin-induced diabetes. The study evaluated various biochemical parameters, including markers of redox stress, inflammation, pancreatic tissue histology, insulin levels, purinergic enzyme functions, lipase activity, and homeostasis models for β-cell function and insulin resistance (HOMA-β, HOMA-IR). The results showed that *H. sabdariffa* extracts significantly ($P < 0.05$) alleviated redox imbalance and inflammation in the diabetic rats. This was evidenced by the reduction of malondialdehyde (MDA), interleukins (IL-1β, IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), alongside an increase in antioxidant enzymes such as reduced glutathione (GSH), glutathione-S-transferase (GST), catalase (CAT), and glutathione peroxidase (GPx). Histological evaluation of pancreatic tissue demonstrated protection against the degeneration of both acinar and β-cells. In addition, there was a significant increase in insulin concentration and a decrease in lipase activity. The extracts also modulated purinergic enzymes, including ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) and ATPase, while improving both HOMA-β and HOMA-IR indices. Notably, the extracts activated the gene expression of insulin and GLP-1 receptors in the treated diabetic rats. These findings highlight the potential of *H. sabdariffa* leaf extracts as a natural therapeutic approach for diabetes management.

Keywords: Diabetes, *Hibiscus sabdariffa*, ATPase, Asteraceae, GLP-1, Molecular mechanism

How to Cite This Article: Ajiboye BO, Famusiwa CD, Oyedare DI, Julius BP, Adewole ZO, Ojo OA, et al. *Hibiscus sabdariffa* Leaf Extract Enhances Molecular Gene Expression of Insulin and GLP-1 Receptors in Streptozotocin-Induced Rats. Spec J Pharmacogn Phytochem Biotechnol. 2024;4:59-73. https://doi.org/10.51847/9lS5Ohuhiv

Introduction

Excessive consumption of foods rich in fats and sugars heightens the likelihood of developing conditions such as dyslipidemia, diabetes, and obesity. Diabetes mellitus (DM), a prevalent global health issue affecting over 500 million individuals, is marked by abnormal blood glucose levels, which result from inadequate or dysfunctional insulin secretion. Factors like a sedentary lifestyle, genetics, and unhealthy eating habits contribute significantly to this disease. DM is one of the leading causes of early death, claiming over a million lives annually [1, 2]. The condition is primarily divided into type 1 (T1DM), which involves a loss of insulin-producing β-cells in the pancreas, and type 2 (T2DM), which is characterized by insulin resistance in the liver, fat, and muscle cells, hindering glucose absorption from the blood [1]. Both types are extensively studied to understand their progression and explore potential treatments, with hyperglycemia being a central factor in both. This condition contributes to processes like glucose autoxidation, protein glycation, and the breakdown of glycated proteins [2]. Insulin secretion from the pancreas is critical for regulating blood glucose levels. This process is meticulously controlled by dietary factors and hormones such as glucose-dependent insulinotropic peptide and glucagon-like peptide-1 (GLP-1) [3]. To counteract oxidative damage, mammals rely on a variety of enzymatic and non-enzymatic antioxidants, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) [4]. In diabetic models, variations in ROS levels are often observed, attributed to changes in antioxidant enzyme activity, which may either increase in an attempt to mitigate oxidative stress or be suppressed. Excessive oxidative stress, resulting from high ROS levels and insufficient neutralization, can lead to significant cellular damage and metabolic disruptions. In many cases, this leads to apoptosis, a form of cell death regulated by specific genes and proteins involved in the process [5-17].

Given the promising effects of bioactive plant compounds on cellular processes, insulin function, and resistance, certain plants have gained attention as potential therapeutic agents for diabetes. However, *Hibiscus sabdariffa* L., known for its bioactive properties, has yet to be fully explored in terms of its influence on insulin and GLP-1 receptor activation in diabetic rats induced by streptozotocin. Further investigation is needed to assess its viability for treating diabetes in humans.

This research explored the therapeutic effects of flavonoid-rich *Hibiscus sabdariffa* L. leaf extracts in rats with streptozotocin-induced diabetes.

Materials and Methods

Source and authentication of plant material

The leaves of *Hibiscus sabdariffa* were procured from Oja-Oba Market, Ado-Ekiti, Ekiti State, Nigeria. The plant was authenticated by Mr. S.A. Odewo, a senior taxonomist from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, who assigned voucher number FHI: FHI:113742.

Reagents and enzyme kits

The chemicals used in this research, including methanol, ammonium hydroxide, fructose, sulfuric acid, absolute ethanol, streptozotocin (STZ), formalin, citrate buffer, and phosphate buffer, were obtained from Sigma-Aldrich, Germany. The reagents employed were of analytical grade. The enzyme kits utilized in the assays were provided by Randox Laboratories, located in Crumlin, United Kingdom.

Preparation of H. sabdariffa and extraction process

The leaves of *H. sabdariffa* were air-dried for two weeks before being ground using an electric blender. A 1 g sample of the powder was macerated in 80% methanol for 72 hours, followed by filtration through muslin cloth. The filtrate was concentrated using a rotary evaporator. The residue was then treated with 200 mL of 10% sulfuric acid, heated for 30 minutes at 100 °C in a water bath. The solution was then cooled on ice for 15 minutes to facilitate the precipitation of flavonoid aglycones. These aglycones were dissolved in 50 mL of warm 95% ethanol and filtered into a 100 mL volumetric flask, which was topped up with 95% ethanol. After further concentration by rotary evaporation, ammonium hydroxide was added to precipitate the flavonoids. The precipitate was collected and rinsed with dilute ammonium hydroxide.

Induction of diabetes in experimental animals

The rats were housed in groups of five and allowed to acclimatize for one week. The animals were administered a 20% fructose solution for 2 weeks [6]. Afterward, they were injected with streptozotocin (forty mg/kg body

weight), while the control group was treated with an equal volume of citrate buffer (pH = 7.4). Three days after the injection, rats exhibiting fasting blood glucose levels exceeding 250 mg/dL were considered for the study [7].

Grouping and treatment of animals

The rats were divided into five groups of five rats each based on their weight:
Group I: Normal (non-diabetic) control
Group II: Diabetic control (no treatment)
Group III: Diabetic rats treated with a low dose of *H. sabdariffa* flavonoid-rich extract (150 mg/kg body weight)
Group IV: Diabetic rats treated with a high dose of *H. sabdariffa* flavonoid-rich extract (300 mg/kg body weight)
Group V: Diabetic rats treated with metformin (200 mg/kg body weight).

Tissue collection and sample processing

On day 22, the rats were euthanized using cervical dislocation, and blood was collected via cardiac puncture. The blood was placed into a plain tube, centrifuged at 1,500 rpm for 15 minutes, and the serum was stored at 4 °C. The pancreas was carefully harvested, homogenized in 0.1 M potassium phosphate buffer (pH = 6.5), and centrifuged at 4,000 rpm for fifteen minutes to prepare the pancreas homogenate [8].

Measurement of malondialdehyde (MDA) levels

MDA levels in the pancreas were assessed following the method of Oloyede *et al*. [9].

Assessment of superoxide dismutase (SOD) activity

SOD activity was measured using the protocol by Misra and Fridovich [10].

Glutathione-S-transferase (GST) activity

GST activity was evaluated by the method of Habig *et al*. [11] with slight modifications. The GST activity was measured using CDNB or DCNB as substrates, and the reaction was monitored spectrophotometrically at 340 nm (CDNB) and 345 nm (DCNB).

Catalase (CAT) activity

The method of Beers and Sizer [12] was used to determine catalase activity.

Glutathione peroxidase (GPx) activity

GPx activity was determined according to the method of Haque *et al*. [13].

Measurement of reduced glutathione (GSH) levels

The concentration of GSH in the pancreas homogenate was quantified using Ellman's method [14].

Protein carbonyl content

The protein carbonyl content was measured using the procedure described by Levine *et al*. [15].

Measurement of inflammatory cytokines (IL-1β, IL-6, TNF-α, and CRP)

The concentrations of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, and CRP, were quantified using commercial ELISA kits, following the procedure outlined by Bergqvist *et al*. [16].

Pancreatic lipase activity determination

Pancreatic lipase activity was analyzed by monitoring the hydrolysis of p-nitrophenol in a colorimetric assay. P-Nitrophenyl butyrate (PNPB), dissolved in acetonitrile, was used as the substrate at a final concentration of 100 µM, replacing the original 5 mM concentration. A volume of 0.10 mL of the pancreatic lipase solution was added to the reaction mixture, and the total volume was brought to 1 mL with tris-HCl buffer. The assay was carried out at 37 °C, and absorbance was measured at 410 nm across several time points (1–5 minutes) to track the release of p-nitrophenol.

Quantification of pancreatic insulin levels

Pancreatic insulin concentration was determined using an ELISA kit, following the methodology described by Thevis *et al*. [18].

Activity measurement of membrane ATPases

The activities of Na+/ K+-ATPase, Ca2+/Mg2+-ATPase, and Mg2+-ATPase were measured according to the protocol established by Bewaji *et al*. [19].

ENTPDase activity measurement

The activity of ecto-nucleoside triphosphate diphosphohydrolase (ENTPDase) was determined following the method described by Akomolafe *et al*. [20].

Assessment of Ecto-5'-nucleotidase activity

Ecto-5'-nucleotidase enzyme activity was measured using the procedure outlined by Freundlieb *et al*. [21].

The evaluation of insulin resistance (HOMA-IR) and beta cell function (HOMA-β) is based on the basal levels of glucose and insulin, as described by Matthews *et al*. [22]. Both HOMA-IR and HOMA-β are strongly correlated with diabetes risk across different ethnic groups. The following formulas are used to compute the HOMA-IR and HOMA-β values:

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu U/mL) \times \text{fasting plasma glucose } (mg/dl)] / [405] \quad (1)$$

$$\text{HOMA-}\beta = [20] \times [\text{fasting insulin } (\mu U/ml)] / [\text{fasting plasma glucose } (mg/dl) - 63] \quad (2)$$

RNA isolation

Tissue samples were used to extract total RNA using the Quick-RNA MiniPrep™ Kit (Zymo Research). The RNA was treated with DNase I (NEB, Cat: M0303S) to eliminate DNA contamination. RNA concentration was assessed at two hundred sixty nm, and its purity was determined by measuring the absorbance at two hundred sixty nm and two hundred eighty nm using an A&E Spectrophotometer (A&E Lab. UK).

cDNA synthesis

One microgram of purified RNA underwent reverse transcription to synthesize complementary DNA (cDNA) with the ProtoScript II first-strand cDNA synthesis kit (New England BioLabs). The reaction protocol included incubation at 65 °C for 5 minutes, followed by 42 °C for 1 hour, and a final 5-minute step at 80 °C [23].

PCR amplification and gel electrophoresis

PCR amplification of the gene of interest was carried out using OneTaqR2X Master Mix (NEB) and primers from Inqaba Biotec (Hatfield, South Africa). The PCR reaction was carried out in a total volume of 25 µL, containing cDNA, primers, and Ready Mix Taq PCR master mix. The thermal cycling conditions consisted of an initial denaturation at 95 °C for five minutes, followed by 30 amplification cycles (95 °C for 30 seconds, annealing for 30 seconds, and extension at 72 °C for 1 minute). A final extension at 72 °C for ten minutes concluded the process. The amplified products were then resolved on a 1.0% agarose gel, with GAPDH used as a reference gene for normalization, and the band intensities were quantified using “Image J” software [23].

Histopathology analysis

Histopathological analysis was conducted using hematoxylin and eosin (H&E) staining according to the method described by Blume *et al*. [24].

Data analysis

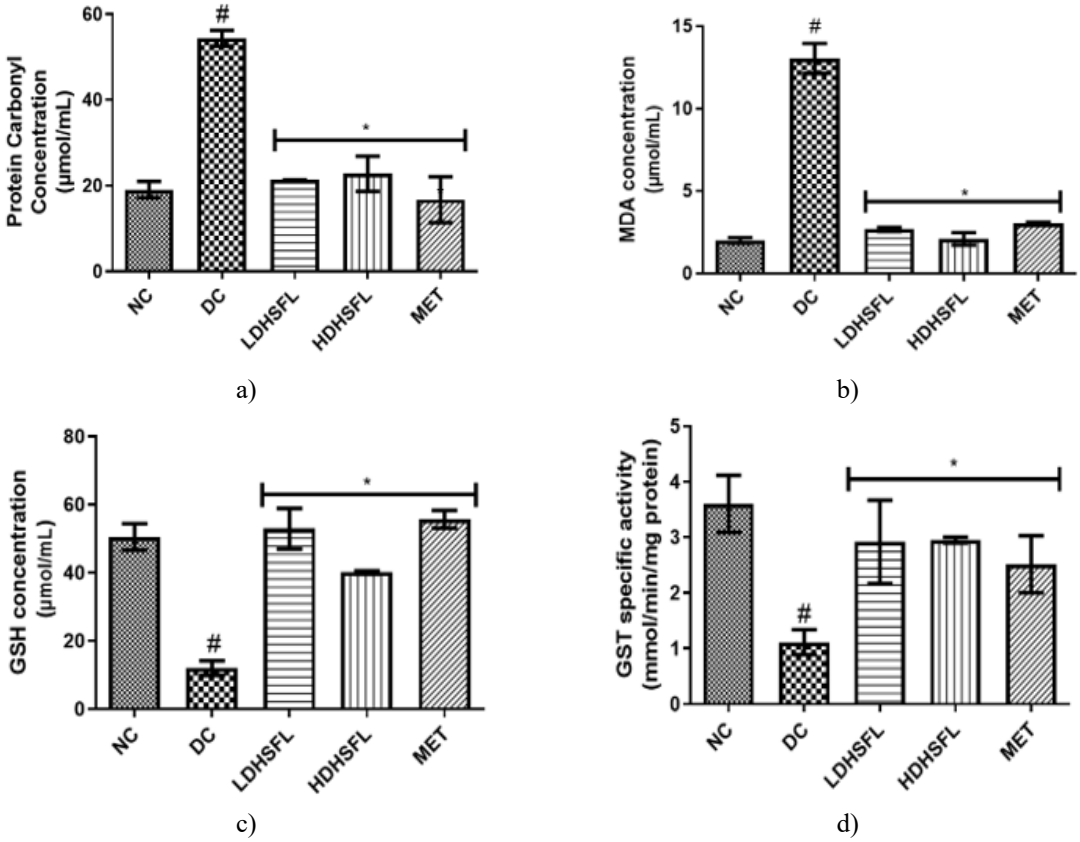
All results are presented as mean ± standard deviation (n = 5). For statistical evaluation, ANOVA was used, followed by Tukey’s post-hoc test for multiple comparisons, with significance defined at $P < 0.05$ using GraphPad Prism software (Version 5.0).

Results and Discussion

Effects of H. sabdariffa extract on redox stress biomarkers in the STZ-diabetic rat pancreas

The impact of the flavonoid-rich extract from *H. sabdariffa* leaves on pancreatic redox stress markers in STZ-diabetic rats is illustrated in **Figure 1**. The findings revealed a notable rise ($P < 0.05$) in both lipid peroxidation and protein degradation (assessed via MDA and protein carbonyl concentrations, respectively) alongside a significant drop ($P < 0.05$) in the activity of antioxidant enzymes, including glutathione-S-transferase (GST), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), as well as a reduction in the level of reduced glutathione (GSH) in the untreated diabetic group (DC) compared to the normal group. However, these disturbances were substantially mitigated ($P < 0.05$) in a dose-dependent manner following administration of both low and high doses of the extract, showing effects similar to the metformin-treated group. This highlights the antioxidant efficacy of *H. sabdariffa*, which aids in alleviating oxidative stress and the accumulation of free radicals linked to diabetes, corroborating earlier studies in the domain [25].

In this investigation, the normal control rats did not show elevated antioxidant enzyme activities since they didn't produce reactive oxygen species (ROS). On the other hand, the STZ-treated diabetic rats demonstrated significant pancreatic β-cell damage, resulting in insulin deficiency and an increase in ROS levels [26]. Enhancing the function and gene expression of antioxidant enzymes is vital for counteracting such oxidative stress. Previous studies have similarly shown an increase in MDA activity in the pancreas of STZ-induced diabetic rats, a process that damages several biological structures such as enzymes, proteins, lipids, cell membranes, and nucleic acids [27]. Diabetic patients experience a notable level of oxidative stress because of the accumulation of oxidation by-products and decreased clearance of free radicals. Reducing lipid peroxidation, preserving the integrity of cell membranes, and ensuring normal cellular activity are essential for safeguarding the organism against oxidative damage. Notably, maintaining the integrity of cell membrane structures is especially crucial for insulin receptor function, as these receptors are embedded in the membrane [28].



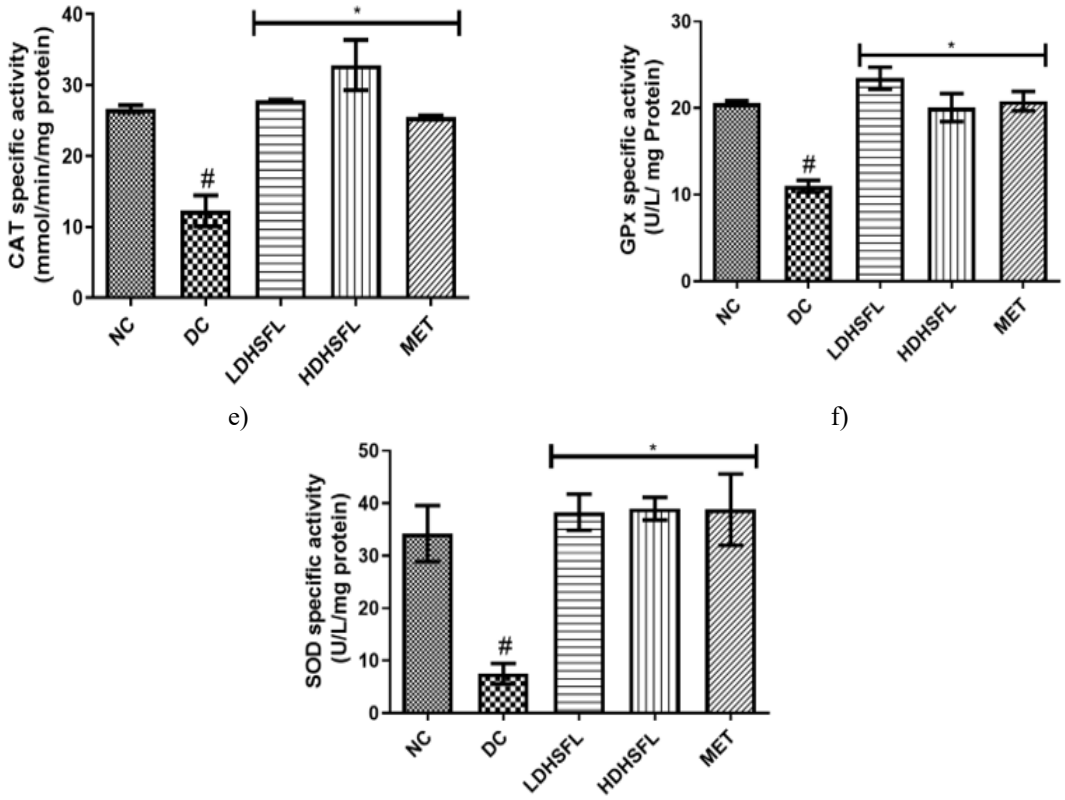


Figure 1. Redox stress biomarkers in STZ-diabetic rats after treatment with flavonoid-rich extract from *H. sabdariffa* leaves; each value is the mean of eight determinations ± SD; # $P < 0.05$ vs NC, * $P < 0.05$ vs DC; NC: normal control, DC: diabetic control, LDHSFL: STZ-diabetic rats treated with a low dose (150 mg/kg body weight) of extract, HDHSFL: STZ-diabetic rats treated with a high dose (300 mg/kg body weight) of extract, MET: STZ-diabetic rats treated with 200 mg/kg of metformin, MDA: malondialdehyde, GSH: reduced glutathione, GST: glutathione-*S*-transferase, CAT: catalase, GPx: glutathione peroxidase, and SOD: superoxide dismutase.

Effects of H. sabdariffa extract on pro-inflammatory markers in the pancreas of STZ-diabetic rats

The data presented in **Figure 2** highlights that treatment with STZ led to a marked ($P < 0.05$) increase in pro-inflammatory biomarkers such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) in the pancreas of untreated diabetic rats (DC) when compared to control rats (NC). These results are consistent with earlier studies [29] and further reinforce the well-established view that inflammatory cytokines like TNF-α contribute significantly to the development of insulin resistance. In obesity, where there is a decrease in adiponectin and an increase in fat tissue, cytokines such as TNF-α and IL-6 are released by adipocytes and macrophages, promoting a state of chronic low-grade inflammation that exacerbates insulin resistance and encourages the development of type 2 diabetes [30]. Recognizing these inflammatory pathways is vital to understanding the intricate relationship between obesity, inflammation, and insulin resistance. STZ-induced inflammation also induces pancreatic inflammation, which is a common consequence of diabetes. CRP, an important inflammation marker, has become more closely linked to T2DM due to its role in fostering insulin resistance through low-grade inflammation. Additionally, elevated CRP levels are commonly associated with an increased risk of cardiovascular diseases in diabetic patients, making it a crucial marker for assessing cardiovascular risk in individuals with diabetes [31].

The elevated levels of pro-inflammatory biomarkers were notably reduced ($P < 0.05$) by the treatment with both low and high doses of *H. sabdariffa* extract (150 mg/kg and 300 mg/kg body weight) and 200 mg/kg of metformin as the reference drug. The extract's administration suppressed the release of TNF-α, IL-1β, IL-6, and CRP, possibly influencing insulin secretion through direct or indirect modulation of hepatic enzymes involved in glucose regulation. This finding indicates that *H. sabdariffa* extract possesses anti-inflammatory effects, contributing to the reduction of inflammation in the pancreas associated with diabetes [32].

Effects of H. sabdariffa extract on histological changes in the pancreas of STZ-diabetic rats

Figure 3 illustrates the impact of *H. sabdariffa*'s flavonoid-rich extract on the pancreas of STZ-diabetic rats. As expected, the untreated diabetic rats (DC) displayed significant histological damage, such as the destruction of acidophilic pancreatic acinar cells and severe degeneration of β-cells in the islets of Langerhans, which led to a decrease in insulin secretion and elevated blood glucose levels. These findings align with other studies documenting similar pathological changes, which are important for understanding the cellular damage associated with diabetes and its complications. This damage is primarily due to sustained hyperglycemia and toxicity from STZ [33]. Histopathological alterations like inflammation, fibrosis, and vascular changes are commonly observed, which suggest that the pancreas is responding to STZ-induced injury. Vascular sclerosis might hinder blood flow, while amyloid deposits and ductal alterations affect pancreatic exocrine function [34].

However, upon examining the tissue sections, it was found that both low and high doses of *H. sabdariffa* extract, as well as the metformin treatment, led to a significant restoration of pancreatic architecture. The damage to pancreatic acinar cells and β-cells was notably reversed, suggesting that *H. sabdariffa* may aid in the partial recovery of pancreatic function in diabetic rats [25].

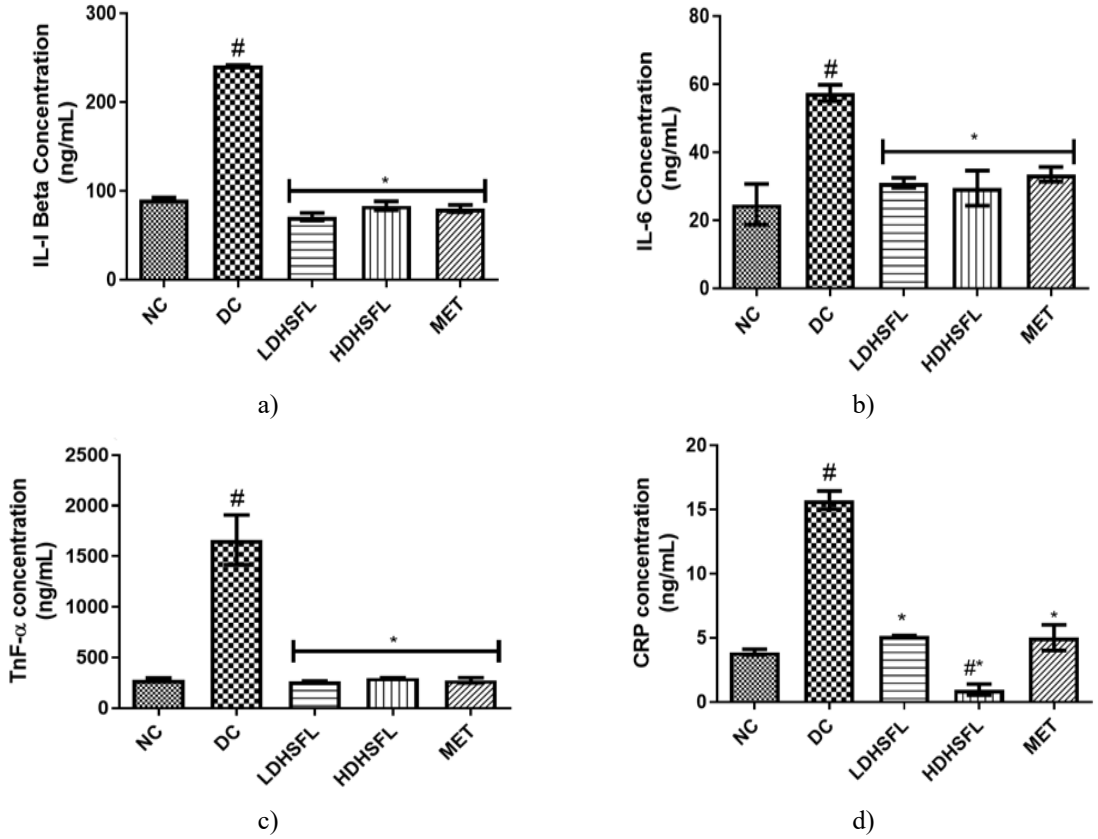


Figure 2. Levels of pro-inflammatory markers in STZ-induced diabetic rats following treatment with flavonoid-enriched extract from *H. sabdariffa* leaves; the data presented are the mean values of eight separate measurements ± SD; statistical significance is indicated by # $P < 0.05$ compared to NC and * $P < 0.05$ compared to DC; NC: normal control, DC: diabetic control, LDHSFL: STZ-diabetic rats treated with a low dose (150 mg/kg body weight) of extract, HDHSFL: STZ-diabetic rats treated with a high dose (300 mg/kg body weight) of extract, MET: STZ-diabetic rats treated with 200 mg/kg of metformin, IL-1β: interleukin-1 beta, IL-6: interleukin-6, TNF-α: tumor necrosis factor-α, CRP: C-reactive protein.

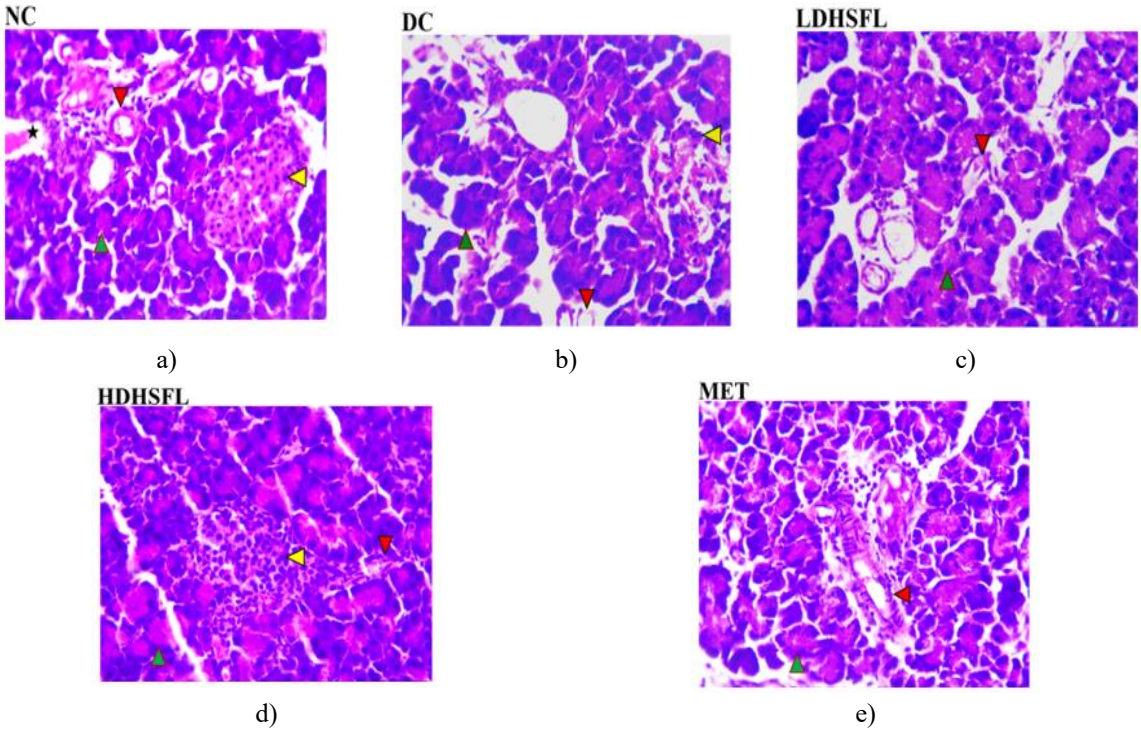


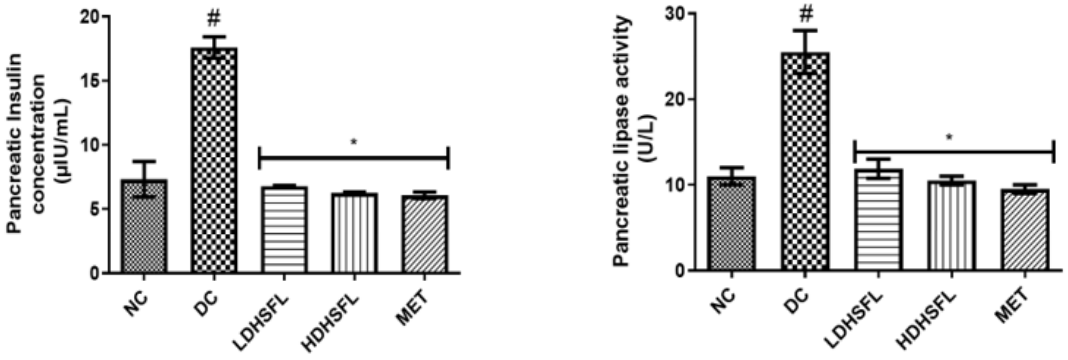
Figure 3. Histological evaluation of pancreatic tissue in STZ-induced diabetic rats post-treatment with the flavonoid-rich extract from *H. sabdariffa* leaves, using H&E staining at x800 magnification; NC: normal control, DC: diabetic control, LDHSFL: low-dose extract: 150 mg/kg of body weight, HDHSFL: high-dose extract: 300 mg/kg of body weight, MET: metformin: 200 mg/kg.

The NC group exhibited normal pancreatic acinar cells (indicated by a green arrowhead), healthy blood vessels (marked with a star), and evenly distributed β-cells in the endocrine regions. The DC group showed significant damage, including the loss of pancreatic acinar cells (green arrowhead) and the degeneration of β-cells in the islets. In the LDHSFL group, there was moderate damage to the exocrine pancreatic acinar cells (green arrowhead) and moderate degeneration of the endocrine region. The HDHSFL group demonstrated almost normal pancreatic acinar cells (green arrowhead) and a balanced distribution of β-cells within the endocrine region. In the MET group, there was moderate degeneration of the exocrine cells (green arrowhead) and some degeneration within the endocrine region.

Impact of H. sabdariffa extract on insulin and lipase activity in the pancreas of STZ-diabetic rats

In untreated diabetic rats, STZ administration led to a notable ($P < 0.05$) elevation in pancreatic lipase activity, alongside increased pancreatic insulin levels, which suggests enhanced lipolysis and impairment of insulin signaling pathways [35].

As depicted in **Figure 4**, treatment with both high and low doses of *H. sabdariffa* extracts significantly mitigated ($P < 0.05$) this abnormal trend in a dose-dependent manner, ultimately bringing the levels closer to normal, resembling the effects of metformin. These findings highlight the extract's protective effects on the pancreas and its potential in enhancing insulin sensitivity, supporting previous research [36].



a) b)

Figure 4. Measurement of pancreatic insulin levels and lipase activity in STZ-diabetic rats after administering the flavonoid-rich extract from *H. sabdariffa* leaves; values represent the average ± standard deviation of eight samples; statistical significance is indicated as # $P < 0.05$ when compared to NC, and * $P < 0.05$ when compared to DC; NC refers to the normal control group, DC refers to the diabetic control group, LDHSFL denotes STZ-diabetic rats receiving a low dose (150 mg/kg of body weight) of the extract, HDHSFL represents STZ-diabetic rats treated with a high dose (300 mg/kg of body weight) of extract; MET refers to STZ-diabetic rats treated with 200 mg/kg of metformin.

The management of diabetes requires a deep understanding of pancreatic insulin levels and lipase activity. In type 1 diabetes mellitus (T1DM), the immune system attacks and destroys insulin-producing cells, resulting in a severe insulin deficiency. In contrast, type 2 diabetes mellitus (T2DM) is characterized by initial insulin production, but insulin resistance in body cells leads to elevated blood sugar and associated complications [37]. Treatment for T1DM involves insulin replacement through injections or pumps, while managing T2DM focuses on improving insulin sensitivity, controlling blood glucose levels, and, when needed, using insulin-enhancing drugs, alongside lifestyle modifications such as a balanced diet and regular physical activity [38]. In diabetes, lipase, which is crucial for fat digestion, can be compromised due to related digestive issues. Adjusting the diet according to the specific type of diabetes can support lipase function, and medications might be prescribed to manage digestive symptoms [39].

Effect of H. sabdariffa extract on purinergic enzyme activities in STZ-diabetic rats' pancreas

Figure 5 reveals that administration of STZ caused a marked reduction ($P < 0.05$) in the activities of purinergic enzymes, including ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase), Na+/K+ATPase, Ca2+/Mg2+ATPase, and Mg2+ATPase, while a significant rise ($P < 0.05$) in 5'-nucleotidase activity was observed in diabetic rats compared to the normal control group. These enzymes are vital for regulating energy production and purinergic signaling processes [40]. E-NTPDase acts by breaking down ATP and ADP into AMP, thus influencing immune responses and neurotransmission, while ATPase catalyzes the conversion of ATP to ADP and inorganic phosphate, providing energy for metabolic functions. The altered activities of these enzymes contribute to a significant reduction in nucleotide levels and energy within the pancreas [41].

Notably, treatment with *H. sabdariffa* extract at both low (150 mg/kg body weight) and high (300 mg/kg body weight) doses significantly reversed ($P < 0.05$) these changes, restoring enzyme activities to near-normal levels, similar to the effect of metformin (200 mg/kg body weight). These results indicate that *H. sabdariffa* extract has the potential to improve purinergic signaling and may positively influence the neurological functions of diabetic rats [42].

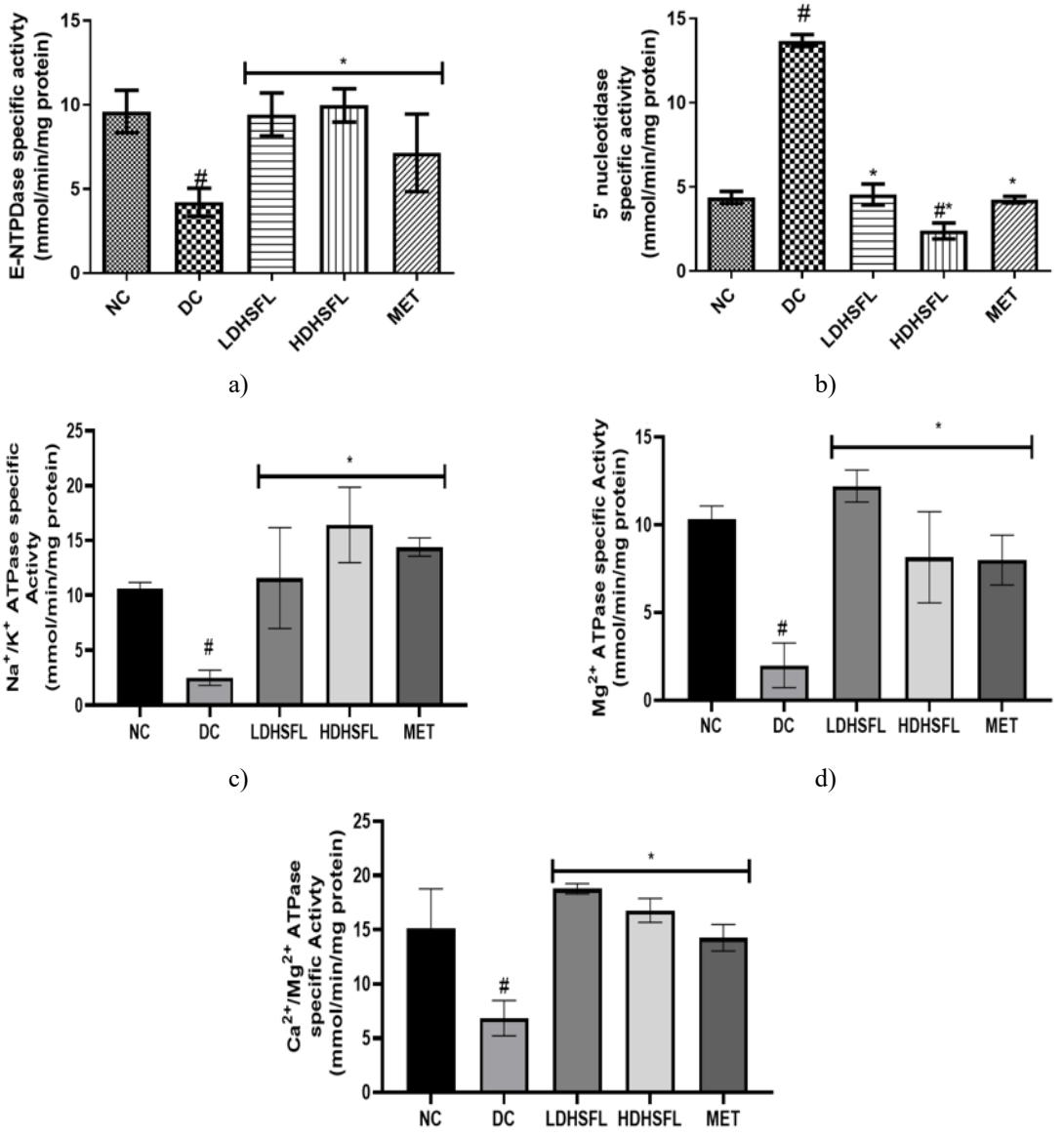


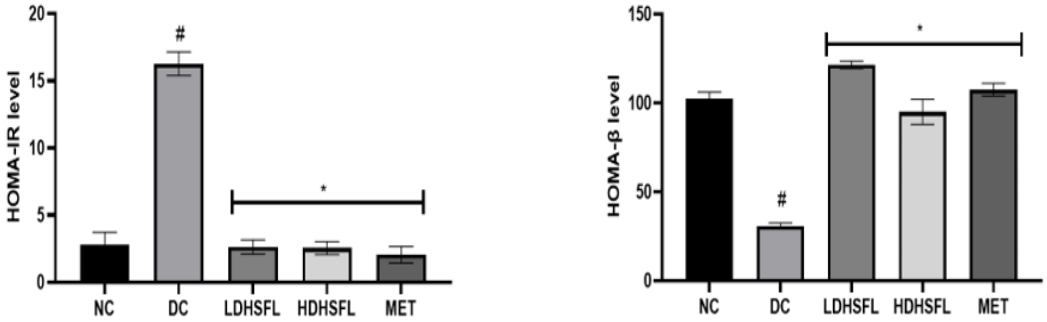
Figure 5. Enzyme activity in STZ-induced diabetic rats following treatment with *H. sabdariffa* extract; the data presented in **Figure 5** show the purinergic enzyme activities in the pancreas of STZ-induced diabetic rats treated with a flavonoid-rich extract from *H. sabdariffa* leaves; values are expressed as the mean ± SD of eight separate measurements. Statistically significant differences were observed at $P < 0.05$, comparing treated groups to the normal control (NC) and the untreated diabetic control (DC); the following groups are indicated: NC = normal control, DC = diabetic control, LDHSFL = low dose *H. sabdariffa* extract, 150 mg/kg body weight, HDHSFL = high dose *H. sabdariffa* extract, 300 mg/kg body weight, and MET = metformin-treated, 200 mg/kg body weight.

The enzymes analyzed include ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase), a key regulator in energy transfer, and other ATPases essential for cellular energy production. Results indicate that treatment with the extract in both doses resulted in significant alterations in enzyme activity, mirroring the effects of metformin treatment.

Effects of H. sabdariffa extract on insulin resistance and β-cell function in STZ-induced diabetic rats

The induction of diabetes through STZ administration in rats caused a significant rise ($P < 0.05$) in the homeostasis model assessment of insulin resistance (HOMA-IR), while HOMA-β values were significantly reduced ($P < 0.05$), and pancreatic insulin concentrations were elevated in the untreated diabetic group compared to the normal

controls **(Figure 6)**. However, treatment with *H. sabdariffa* extract at doses of 150 mg/kg and three hundred mg/kg body weight led to a notable improvement ($P < 0.05$) in both HOMA-IR and HOMA-β, with these improvements resembling those seen in rats treated with the standard drug metformin (200 mg/kg body weight) **(Figure 6)**. The extract-treated rats showed a marked reduction in HOMA-IR, confirming its potential to aid in β-cell function restoration and improving insulin resistance, which aligns with findings from prior research [43, 44].

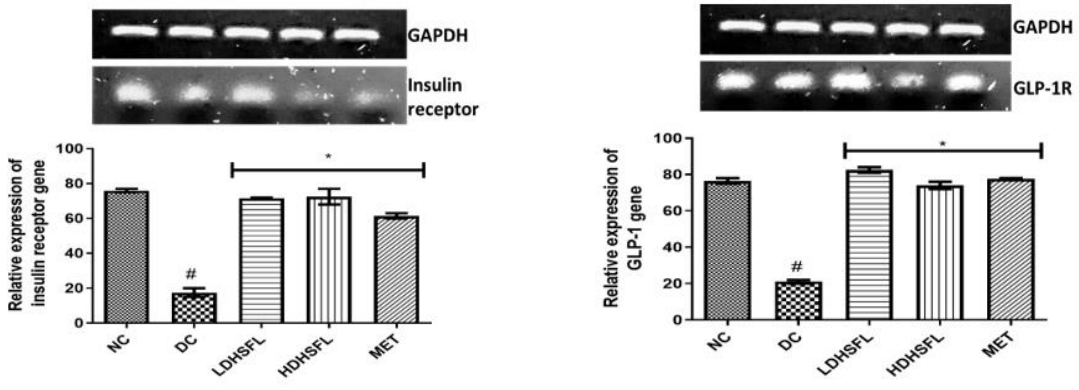


a) b)

Figure 6. Assessment of insulin resistance (HOMA-IR) and β-cell functionality (HOMA-β) in STZ-induced diabetic rats following treatment with flavonoid-rich extract from *H. sabdariffa* leaves; data represents the average of eight measurements ± standard deviation; # $P < 0.05$ compared to NC, * $P < 0.05$ compared to DC; NC: normal control, DC: diabetic control, LDHSFL: STZ-diabetic rats treated with a low dose (150 mg/kg of body weight) of extract, HDHSFL: STZ-diabetic rats treated with a high dose (300 mg/kg of body weight) of extract, MET: STZ-diabetic rats treated with 200 mg/kg of metformin.

Impact of H. sabdariffa extract on gene expression of IR and GLP-1 in the pancreas of STZ-induced diabetic rats

As illustrated in **Figure 7**, the gene expression levels of insulin receptor (IR) and glucagon-like peptide (GLP-1) were analyzed in the pancreases of rats with STZ-induced diabetes, revealing the beneficial effects of the flavonoid-rich extract from *H. sabdariffa*. Utilizing GAPDH as the reference gene, an important downregulation ($P < 0.05$) of both IR and GLP-1 was observed in the diabetic control (DC) group when compared to the normal control (NC) group. Following prior studies [36, 45], the administration of *H. sabdariffa* extract resulted in a substantial ($P < 0.05$) upregulation of both genes in a dose-dependent manner, bringing their expression back toward normal levels. This effect was similar to the one induced by metformin, with the extract likely exerting its influence by interacting with the promoter/enhancer regions of the IL and GLP-1 genes, thus inhibiting their repressor elements.



a) b)

Figure 7. Gene expression of insulin receptor and GLP-1 in STZ-induced diabetic rats following treatment with *H. sabdariffa* extract; each value is the mean of eight determinations ± SD; # $P < 0.05$ vs NC, * $P < 0.05$ vs DC; NC: normal control, DC: diabetic control, LDHSFL: STZ-diabetic rats treated with a low dose (150 mg/kg body weight) of extract, HDHSFL: STZ-diabetic rats treated with a high dose (300 mg/kg body weight) of extract, MET: STZ-diabetic rats treated with 200 mg/kg of metformin, GLP-1: glucagon-like peptide; note that the grouping of gels/blots was cropped from different gels.

Gene expression regulation plays a pivotal role in diabetes control by influencing the activation and suppression of genes involved in insulin secretion, glucose metabolism, and other vital processes. Through strategies such as lifestyle changes, pharmacological treatments, and novel therapies, gene expression modulation holds promise for enhancing blood sugar control, boosting insulin sensitivity, and lowering the risk of complications associated with diabetes [46]. In the case of STZ-induced diabetes in rats, drug treatments induce significant shifts in gene expression, particularly targeting insulin receptor (IR) and glucagon-like peptide 1 (GLP-1). The decrease in IR expression is closely linked to the toxic effects of STZ on the pancreatic β-cells, leading to impaired insulin production and reduced gene expression. Moreover, STZ disrupts pancreatic function overall, affecting the expression of GLP-1, a critical regulator of glucose homeostasis [47]. These changes in gene expression underscore the damage caused by STZ-induced pancreatic injury and insulin deficiency, emphasizing the need for strategies that restore normal insulin and GLP-1 activity to improve blood glucose control. Key molecules in liver glycogen synthesis and secretion regulation, which are typically expressed in hepatocytes and pancreatic cells, play an essential role in insulin signaling. The results from this study suggest that the flavonoid-rich extract from *H. sabdariffa* leaves can enhance insulin signaling in diabetic rats by modulating the expression of important insulin-related genes [48].

Conclusion

In conclusion, this study emphasizes the significant therapeutic potential of *H. sabdariffa* leaf extracts for managing streptozotocin-induced diabetes in Wistar rats. The results reveal a wide array of benefits, addressing both molecular and physiological aspects of diabetes control. The extracts exhibit a marked ability to reduce oxidative stress and inflammation, while supporting pancreatic function and improving β-cell distribution. These positive effects suggest potential improvements in diabetes management. Furthermore, the extracts influence insulin secretion, lipid metabolism, purinergic enzyme activity, and key markers of β-cell function and insulin resistance. Particularly important is their ability to regulate the gene expression of insulin receptor (IR) and glucagon-like peptide 1 (GLP-1), indicating their potential role in enhancing glycemic control. Overall, this research provides valuable insights and a solid foundation for further investigations into the use of *H. sabdariffa* as a multifaceted therapeutic approach to diabetes and related complications.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: All procedures followed ethical guidelines and were approved by the FUOYE Faculty of Science Ethics Committee, ethics approval number FUOYEFSC 201122-REC2022/008.

References

1. Halsey G. Global diabetes prevalence will double by 2050, affecting 1.3 billion people: New predictions. Patient Care, Gale OneFile: Health and Medicine. 2023. Available from: https://go.gale.com/ps/i.do?p=HRCA&u=anon~a374341f&id=GALE|A777403152&v=2.1&it=r&sid=googleScholar&asid=5b3f19ae
2. Arokiasamy P, Salvi S, Selvamani Y. Global burden of diabetes mellitus. In: Kickbusch I, Ganten D, Moeti M. (eds) Handbook of global health. New York: Springer; 2021. p. 1-39. doi:10.1007/978-3-030-05325-3_28-2
3. Veedfald S, Vedtofte L, Skov-Jeppesen K, Deacon CF, Hartmann B, Vilsbøll T, et al. Glucose-dependent insulinotropic polypeptide is a pancreatic polypeptide secretagogue in humans. J Clin Endocrinol Metab. 2020;105(3):e502-e10. Available from: https://academic.oup.com/jcem/article/105/3/e502/5607542 doi:10.1210/clinem/dgz097
4. Demirci-Cekic S, Özkan G, Avan AN, Uzunboy S, Çapanoğlu E, Apak R. Biomarkers of oxidative stress and antioxidant defense. J Pharm Biomed Anal. 2022;209:114477. doi:10.1016/j.jpba.2021.114477

5. Ott M, Gogvadze V, Orrenius S, Zhivotovsky B. Mitochondria, oxidative stress and cell death. Apoptosis. 2007;12:913-22. doi:10.1007/s10495-007-0756-2
6. Salau VF, Erukainure OL, Olofinsan KA, Ijomone OM, Msomi NZ, Islam MS. Vanillin modulates activities linked to dysmetabolism in psoas muscle of diabetic rats. Sci Rep. 2021;11(1):18724. doi:10.1038/s41598-021-98158-7
7. Ajiboye BO, Shonibare MT, Oyinloye BE. Antidiabetic activity of watermelon (Citrullus lanatus) juice in alloxan-induced diabetic rats. Diabetes Metab Dis. 2020;19:343-52. doi:10.1007/s40200-020-00515-2
8. Ojo OA, Ojo AB, Oyinloye BE, Ajiboye BO, Anifowose OO, Akawa A, et al. Ocimum gratissimum Linn. leaves reduce the key enzymes activities relevant to erectile dysfunction in isolated penile and testicular tissues of rats. BMC Complement Altern Med. 2019;19:71. doi:10.1186/s12906-019-2481-0
9. Oloyede HOB, Abdulkareem HO, Salawu MO, Ajiboye TO. Protective potentials of Vernonia amygdalina leaf extracts on cadmium induced hepatic damage. Al-Hikmah J Pure Appl Sci. 2019;7:9-21.
10. Misra HP, Fridovich I. The purification and properties of superoxide dismutase from Neurospora crassa. J Biol Chem. 1972;247(11):3410-4. doi:10.1016/S0021-9258(19)45155-7
11. Habig WH, Pabst MJ, Fleischner G, Gatmaitan Z, Arias IM, Jakoby WB. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proc Natl Acad Sci. 1974;71(10):3879-82. doi:10.1073/pnas.71.10.3879
12. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952;195(1):133-40.
13. Haque R, Bin-Hafeez B, Parvez S, Pandey S, Sayeed I, Ali M, et al. Aqueous extract of walnut (Juglans regia L.) protects mice against cyclophosphamide induced biochemical toxicity. Hum Exp Toxicol. 2003;22(9):473-80. doi:10.1191/0960327103ht388oa
14. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7. doi:10.1016/0003-9861(59)90090-6
15. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990;186:464-78. doi:10.1016/0076-6879(90)86141-h
16. Bergqvist A, Bruse C, Carlberg M, Carlström K. Interleukin 1β, interleukin-6, and tumor necrosis factor-α in endometriotic tissue and in endometrium. Fertil Steril. 2001;75(3):489-95. doi:10.1016/s0015-0282(00)01752-0
17. Bustanji Y, Issa A, Mohammad M, Hudaib M, Tawah K, Alkhatib H, et al. Inhibition of hormone sensitive lipase and pancreatic lipase by Rosmarinus officinalis extract and selected phenolic constituents. J Med Plants Res. 2010;4(21):2235-42. doi:10.5897/JMPR10.399
18. Thevis M, Thomas A, Schänzer W. Mass spectrometric determination of insulins and their degradation products in sports drug testing. Mass Spectrom Rev. 2008;27(1):35-50. doi:10.1002/mas.20154
19. Bewaji CO, Olorunsogo OO, Bababunmi EA. Comparison of the membrane-bound (Ca2+/Mg2+)-ATPase in erythrocyte ghosts from some mammalian species. Comp Biochem Physiol B. 1985;82(1):117-22. doi:10.1016/0305-0491(85)90138-5
20. Akomolafe SF, Akinyemi AJ, Ogunsuyi OB, Oyeleye SI, Oboh G, Adeoyo OO, et al. Effect of caffeine, caffeic acid and their various combinations on enzymes of cholinergic, monoaminergic and purinergic systems critical to neurodegeneration in rat brain-In vitro. Neurotoxicology. 2017;62:6-13. doi:10.1016/j.neuro.2017.04.008
21. Freundlieb M, Zimmermann H, Müller CE. A new, sensitive ecto-5′-nucleotidase assay for compound screening. Anal Biochem. 2014;446:53-8. doi:10.1016/j.ab.2013.10.012
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-9. doi:10.1007/BF00280883
23. Elekofehinti OO, Ayodele OC, Iwaloye O. Momordica charantia nanoparticles promote mitochondria biogenesis in the pancreas of diabetic-induced rats: gene expression study. Egypt J Med Hum Genet. 2021;22(1):1-11. doi:10.1186/s43042-021-00200-w
24. Blume G, Pestronk A, Frank B, Johns DR. Polymyositis with cytochrome oxidase negative muscle fibres. Early quadriceps weakness and poor response to immunosuppressive therapy. Brain 1997;120(1):39-45. doi:10.1093/brain/120.1.39

25. Adeyemi DO, Adewole OS. Hibiscus sabdariffa renews pancreatic β-cells in experimental type 1 diabetic model rats. Morphologie. 2019;103(341):80-93. doi:10.1016/j.morpho.2019.04.003
26. Seedevi P, Ramu Ganesan A, Moovendhan M, Mohan K, Sivasankar P, Loganathan S, et al. Anti-diabetic activity of crude polysaccharide and rhamnose-enriched polysaccharide from G. lithophila on streptozotocin (STZ)-induced in Wistar rats. Sci Rep. 2020;10(1):556. doi:10.1038/s41598-020-57486-w
27. Liu H, Li N, Jin M, Miao X, Zhang X, Zhong W. Magnesium supplementation enhances insulin sensitivity and decreases insulin resistance in diabetic rats. Iran J Basic Med Sci. 2020;23(8):990. doi:10.22038/ijbms.2020.40859.9650
28. Ammendolia DA, Bement WM, Brumell JH. Plasma membrane integrity: implications for health and disease. BMC Biol. 2021;19:71. doi:10.1186/s12915-021-00972-y
29. Dludla PV, Mabhida SE, Ziqubu K, Nkambule BB, Mazibuko-Mbeje SE, Hanser S, et al. Pancreatic β-cell dysfunction in type 2 diabetes: implications of inflammation and oxidative stress. World J Diabetes 2023;14(3):130-46. doi:10.4239/wjd.v14.i3.130
30. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. Immunity 2022;55(1):31-55. doi:10.1016/j.immuni.2021.12.013
31. Stanimirovic J, Radovanovic J, Banjac K, Obradovic M, Essack M, Zafirovic S, et al. Role of C-reactive protein in diabetic inflammation. Mediators Inflamm. 2022;2022:3706508. doi:10.1155/2022/3706508
32. Von Dentz KE, Silva BS, Queiroz EA, Bomfim GF, Nascimento AF, Sugizaki MM, et al. Hibiscus sabdariffa ethanolic extract modulates adipokine levels, decreases visceral fat and improves glycemic profile in high-fat/sugar diet-induced obese rats. Nutr Food Sci. 2021;51(2):222-33. doi:10.1108/NFS-03-2020-0092
33. Renner S, Blutke A, Clauss S, Deeg CA, Kemter E, Merkus D, et al. Porcine models for studying complications and organ crosstalk in diabetes mellitus. Cell Tissue Res. 2020;380:341-78. doi:10.1007/s00441-019-03158-9
34. Phuthego T. Analysing the Genetic, Immunological and Pancreatic Characteristics of Type 1 Diabetes to Gain New Insights Into the Aetiology of the Disease (Doctoral dissertation, University of Bristol); 2020.
35. Salau VF, Erukainure OL, Ijomone OM, Islam MS. Caffeic acid regulates glucose homeostasis and inhibits purinergic and cholinergic activities while abating oxidative stress and dyslipidaemia in fructose-streptozotocin-induced diabetic rats. J Pharm Pharmacol. 2022;74(7):973-84. doi:10.1093/jpp/rgac021
36. Janson B, Prasomthong J, Malakul W, Boonsong T, Tunsophon S. Hibiscus sabdariffa L. calyx extract prevents the adipogenesis of 3T3-L1 adipocytes, and obesity-related insulin resistance in high-fat diet-induced obese rats. Biomed Pharmacoth. 2021;138:111438. doi:10.1016/j.biopha.2021.111438
37. Abdou HM, Hamaad FA, Ali EY, Ghoneum MH. Antidiabetic efficacy of Trifolium alexandrinum extracts hesperetin and quercetin in ameliorating carbohydrate metabolism and activating IR and AMPK signaling in the pancreatic tissues of diabetic rats. Biomed Pharmacoth. 2022;149:112838. doi:10.1016/j.biopha.2022.112838
38. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?) Nat Rev Endocrinol. 2021;17(3):150-61. doi:10.1038/s41574-020-00443-4
39. Spínola V, Llorent-Martínez EJ, Castilho PC. Inhibition of α-amylase, α-glucosidase and pancreatic lipase by phenolic compounds of Rumex maderensis (Madeira sorrel). Influence of simulated gastrointestinal digestion on hyperglycaemia-related damage linked with aldose reductase activity and protein glycation. LWT. 2020;118:108727. doi:0.1016/j.lwt.2019.108727
40. Huang Z, Xie N, Illes P, Di Virgilio F, Ulrich H, Semyanov A, et al. From purines to purinergic signalling: molecular functions and human diseases. Signal Transduct Target Ther. 2021;6(1):162. doi:10.1038/s41392-021-00553-z
41. Zimmermann H. History of ectonucleotidases and their role in purinergic signaling. Biochem Pharmacol. 2021;187:114322. doi:10.1016/j.bcp.2020.114322
42. Olofinsan KA, Erukainure OL, Brian BK, Islam MS. Harpephyllum caffrum stimulates glucose uptake, abates redox imbalance and modulates purinergic and glucogenic enzyme activities in oxidative hepatic injury. Asian Pac J Trop Biomed. 2022;12(1):9-19. doi:10.4103/2221-1691.333209
43. Nadig P, Asanaliyar M, Salis KM. Establishment of long-term high-fat diet and low dose streptozotocin-induced experimental type-2 diabetes mellitus model of insulin resistance and evaluation of seed extracts of Syzygium cumini. J Herbmed Pharmacol. 2021;10(3):331-8. doi:10.34172/jhp.2021.38

44. Al-Snafi AE. The efficacy and safety of medicinal plants documented by clinical trials (part 1). World. 2022;3(1):30-77. doi:10.53346/wjapmr.2022.3.1.0036
45. Jamrozik D, Borymska W, Kaczmarczyk-Żebrowska I. Hibiscus sabdariffa in diabetes prevention and treatment—Does it work? An evidence-based review. Foods. 2022;11(14):2134. doi:10.3390/foods11142134
46. Chang Y, Dong M, Wang Y, Yu H, Sun C, Jiang X, et al. GLP-1 gene-modified human umbilical cord mesenchymal stem cell line improves blood glucose level in type 2 diabetic mice. Stem Cells Int. 2019;2019(1):4961865. doi:10.1155/2019/4961865
47. Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, et al. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: current therapeutics strategies and future perspectives. Free Rad Biol Med. 2022;184:114-34. doi:10.1016/j.freeradbiomed.2022.03.019
48. Kartinah NT, Fadilah F, Ibrahim EI, Suryati Y. The potential of Hibiscus sabdariffa Linn. in inducing glucagon-like peptide-1 via SGLT-1 and GLPR in DM rats. BioMed Res Int. 2019;2019(1):1-8. doi:10.1155/2019/872482