Article's History: Received: 14.08.2024; Revised: 27.11.2024; Accepted: 25.02.2025

Bulletin of Medical and Biological Research

Vol. 7, No. 1
2025

DOI: 10.63341/bmbr/1.2025.33

Journal homepage: https://bmbr.com.ua/en

UDC 615.322.015:582.685.233:616.379-008.64-092.9

Evaluating the hypoglycaemic, anti-inflammatory, and antioxidant effects of *Hibiscus sabdariffa* in alloxan-induced diabetic rats

Lekan Sheriff Ojulari*
PhD in Physiology, Associate Professor
University of Ilorin
Ilorin, Nigeria
https://orcid.org/0000-0002-3405-0116

Ngaitad Stanislaus Njinga
PhD in Pharmacology, Associate Professor
University of Ilorin
Ilorin, Nigeria
https://orcid.org/0000-0001-5566-5905

Ridwan Abiodun Ganiyu
Bachelor of Physiology
University of Ilorin
Ilorin, Nigeria
https://orcid.org/0009-0009-8563-7652

Taofeek Olarewaju Ayinde
PhD in Physiology, Associate Professor
University of Ilorin
Ilorin, Nigeria
https://orcid.org/0000-0003-2092-0026

Eniola Riskat Kadir
PhD in Anatomy, Associate Professor
University of Ilorin
Ilorin, Nigeria
https://orcid.org/0000-0002-0097-4783

Abstract. *Hibiscus sabdariffa* is beneficial in treating diabetes mellitus. This study investigated the hypoglycaemic, anti-inflammatory, and antioxidant effects of *Hibiscus sabdariffa* in alloxan-induced diabetic rats. Thirty Wistar rats were divided into six groups of five and acclimatised for two weeks before the experiment commenced. Group I: non-diabetic control; Group II: diabetic control; Group III: non-diabetic with 200 mg/kg of *Hibiscus sabdariffa*; Group IV: non-diabetes with 300 mg/kg of *Hibiscus sabdariffa*; Group V: diabetic with 200 mg/kg of *Hibiscus sabdariffa*; Group VI: diabetic with 300 mg/kg of *Hibiscus sabdariffa*. The rats received a single intraperitoneal injection of alloxan (150 mg/kg of body weight), and diabetic rats were treated with *Hibiscus sabdariffa* for 21 days. Fasting blood glucose levels, insulin levels, superoxide dismutase, catalase, malondialdehyde, interleukin-6, and tumour necrosis factor-alpha were measured, and organ and blood samples were collected. The results were analysed using analysis of variance with $p < 0.05$ considered significant, and data were visualised using GraphPad. This study demonstrated that *Hibiscus sabdariffa* exerts significant effects on diabetic parameters, pro-inflammatory cytokines, and antioxidant enzymes. Daily oral treatment for 21 days lowered fasting blood glucose, interleukin-6, tumour necrosis factor-alpha, and malondialdehyde levels. It also enhanced insulin production, superoxide di smutase, and catalase activity in the skeletal muscle, liver, pancreas, and kidney. It can be concluded that

Suggested Citation:
Ojulari LSh, Njinga NS, Ganiyu RA, Ganiyu TO, Kadir ER. Evaluating the hypoglycaemic, anti-inflammatory, and antioxidant effects of *Hibiscus sabdariffa* in alloxan-induced diabetic rats. Bull Med Biol Res. 2025;7(1):33–42. DOI: 10.63341/bmbr/1.2025.33

*Corresponding author

Hibiscus sabdariffa has the potential to manage hyperglycaemia and inflammation while improving antioxidant enzyme activity. Furthermore, it may serve as a natural source or agent for the treatment or prevention of diabetes

Keywords: oxidative stress biomarkers; pro-inflammatory cytokines; bioactive compounds; pancreatic β-cells

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterised by elevated blood glucose levels and is associated with complications affecting the heart, blood vessels, eyes, kidneys, and nerves. DM is primarily classified into two types: type 1 diabetes mellitus (T1DM), which typically results from the destruction of insulin-producing β-cells, and type 2 diabetes mellitus (T2DM), which is characterised by insulin resistance and accounts for over 90% of all diabetes cases [1]. According to the International Diabetes Federation (IDF), diabetes affects approximately 537 million people aged 20-79 years, with this number projected to rise to 643 million by 2030 and 783 million by 2045 [2]. As of 2025, conventional antidiabetic medications do not always provide optimal treatment outcomes. For instance, D.T. Liss *et al*. [3] reported that nearly 40% of patients with T2DM discontinued their second-line medication within a year, highlighting challenges related to the effectiveness and tolerability of current pharmacotherapies.

Oxidative stress disrupts cellular signalling and contributes to endothelial dysfunction by damaging DNA, proteins, cell membranes, and plasma lipids. This damage leads to the activation of inflammatory mediators, including nuclear factor-kappa B (NF-κB) [4]. Once activated, NF-κB stimulates the production of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumour necrosis factoralpha (TNF-α). Elevated levels of these cytokines are commonly observed in pre-diabetic and diabetic individuals [5]. Given the detrimental effects of oxidative stress and inflammation in diabetes, developing antioxidant and anti-inflammatory therapeutic strategies has become a critical area of research.

Hibiscus sabdariffa (HS), commonly known as Roselle, is a plant rich in bioactive compounds. It has been reported to exhibit multiple therapeutic properties, including antihypertensive, antimicrobial, hepatoprotective, antioxidant, antihyperlipidaemic, anticancer, anti-inflammatory, and antidiabetic activities. The antioxidant and anti-inflammatory properties of bioactive compounds in medicinal plants have been extensively studied in the context of diabetes management [6-8]. Research by A. Hamadjida *et al*. [9] identified triterpenes, flavonoids, phenolic compounds, polysaccharides, organic acids, vitamins, and tannins as the major bioactive compounds present in the calyces of HS, contributing to its antidiabetic properties in alloxan-induced diabetic rats. Similarly, N. Herdiani & E.A. Wikurendra [10] demonstrated that Roselle petal extract effectively reduces malondialdehyde (MDA) levels – a biomarker of lipid peroxidation – in diabetic rats, suggesting its potential to mitigate oxidative stress.

Despite the documented benefits of HS, its specific effects on insulin signalling pathways and the interrelationship between oxidative stress and inflammation remain underexplored. Therefore, this study aimed to investigate the impact of *Hibiscus sabdariffa* on insulin signalling, oxidative stress, and inflammation in alloxan-induced diabetic rats.

MATERIALS AND METHODS

The study was conducted in May 2024 at the University of Ilorin, Nigeria. Thirty albino Wistar rats, weighing between 150 and 212 g, were used in this experiment. They were obtained from Temmy Concept, Gaa Akanbi, Ilorin, Kwara State. The rats were housed in a controlled environment (ambient room temperature and a 12-hour light/dark cycle) and divided into six groups. They were fed a standard rat diet and provided with ad libitum access to water. Cages were cleaned daily, and food and water were replenished, while the rats were acclimatised for two weeks before the experiment commenced.

Ethical approval for this experiment was obtained from the University of Ilorin Ethical Review Committee through the Faculty of Basic Medical Sciences (protocol identification code UERC/BMS/238 and approval number UERC/ASN/2024/2935). The study complied with the Institutional Animal Care and Use Committee (IACUC) guidelines, ensuring the humane treatment and welfare of all animals used in the research. These guidelines align with internationally recognised ethical standards for the use of animals in scientific research, including the principles of Replacement, Reduction, and Refinement (3Rs) [11].

The calyces of HS were purchased from Ipata Market, Ilorin, Kwara State, Nigeria, and identified at the Herbarium of the Department of Plant Biology, University of Ilorin. The calyces were air-dried in a dust-free environment at the Pharmacy Department of the University of Ilorin. The dried calyces were ground into a fine powder using a mechanical grinder. The powdered material was soaked in ethanol for 48 hours with occasional agitation. The mixture was then filtered using filter paper, and the resulting extract was concentrated to a final concentration of 100 mg/mL. The extract was stored at 4°C in tightly sealed, amber-coloured glass bottles until use. Extract administration was performed orally. Male Wistar rats were randomly assigned to six groups of five animals each (n = 5) (Table 1).

Table 1. Distribution of rats (n = 30)

Groups	Description	No. of animal	Treatment
Group I	Non-diabetic control	5	Normal saline
Group II	Diabetic control	5	Normal saline
Group III	Non-diabetic	5	HS (200 mg/kg)
Group IV	Non-diabetic	5	HS (300 mg/kg)

Table 1. Continued

Groups	Description	No. of animal	Treatment
Group V	Diabetic	5	HS (200 mg/kg)
Group VI	Diabetic	5	HS (300 mg/kg)

Source: compiled by the authors

Diabetes mellitus was induced with an intraperitoneal injection of alloxan at a dose of 150 mg/kg of body weight, prepared at a concentration of 20 mg/mL in normal saline. The rats underwent a 36-hour fasting period prior to injection, and the injection was administered in the morning at pH 4.5, according to the method described by O.L. Sheriff *et al*. [12]. The diabetic state was assessed by measuring blood glucose levels 72 hours post-induction using an On-Call Plus glucometer. Rats with blood glucose levels above 200 mg/dL were classified as diabetic.

Fasting blood glucose (FBG) was measured before and after the experiment using an On-Call Plus glucometer. At the end of the experiment, ketamine was used as the anaesthetic agent, and blood samples were collected. The serum was separated by centrifuging the blood at 3,000 rpm for 20 minutes at 4°C. Serum insulin levels were measured using the Insulin Mouse ELISA Kit (Thermo Fisher Scientific, MA, USA), following the manufacturer's instructions. Lipid peroxidation refers to the oxidative degradation of lipids caused by reactive oxygen species. MDA is a key by-product of this process and serves as a reliable marker of lipid oxidative damage.

To assess lipid peroxidation, MDA levels were quantified using the Thiobarbituric Acid Reactive Substances (TBARS) method. Specifically, MDA levels were determined in skeletal muscle, pancreas, kidney, and liver homogenates and normalised to protein content [13]. Superoxide dismutase (SOD) and catalase are key endogenous antioxidant enzymes that neutralise free radicals. SOD activity was assessed according to the method described by H.P. Misra & I. Fridovich [14], based on the inhibition of epinephrine auto-oxidation at pH 10.2. Catalase activity was determined using the method of A.K. Sinha [15], based on dichromate reduction.

Plasma levels of the pro-inflammatory cytokines IL-6 and TNF-α were measured using a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit (EDM Millipore, MA, USA). The assay involved incubating samples with a biotin-conjugated solution, followed by streptavidin-HRP, and measuring absorbance spectrophotometrically at 450 nm. Plasma samples were collected using the retro-orbital technique under anaesthesia, whereby a needle was inserted into the retro-orbital sinus to obtain 2-3 mL of blood. Plasma was carefully separated by centrifugation of the blood at 2,000 rpm for 15 minutes. Statistical analysis was conducted using SPSS version 17 (SPSS Inc., Chicago, USA) and GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, USA). Data are presented as mean ± SEM. One-way ANOVA with Tukey's multiple comparison tests was employed for statistical comparisons, and a p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The FBG levels of diabetic and non-diabetic rats were measured on days 0 and 21 to assess the effect of HS administered at doses of 200 and 300 mg/kg. On day 0, the non-diabetic control group had an average FBG of 90 ± 3.17 mg/dL, which decreased significantly to 78 ± 2.26 mg/dL by day 21 (p = 0.005). Similarly, the non-diabetic group treated with 300 mg/kg HS showed a significant reduction in FBG from 86 ± 1.12 to 69.5 ± 1.20 mg/dL (p = 0.002), indicating the glucose-lowering potential of HS. In contrast, the non-diabetic group treated with 200 mg/kg HS showed a slight increase in FBG levels from 85.5 ± 0.18 to 90.5 ± 0.07 mg/dL, which was not statistically significant (p = 0.12).

For the diabetic groups, the control rats exhibited persistently elevated FBG levels, with no significant change between day 0 (239.5 ± 3.10 mg/dL) and day 21 (235 ± 0.20 mg/dL, p = 0.45). Diabetic rats treated with 200 mg/kg HS showed increased FBG levels from 368 ± 0.9 to 426 ± 2.09 mg/dL, but this change was not statistically significant (p = 0.15). However, 300 mg/kg HS treatment led to a statistically significant reduction in FBG levels, from 367.6 ± 2.14 mg/dL on day 0 to 88.5 ± 3.018 mg/dL on day 21 (p = 0.001). The results are shown in Figure 1.

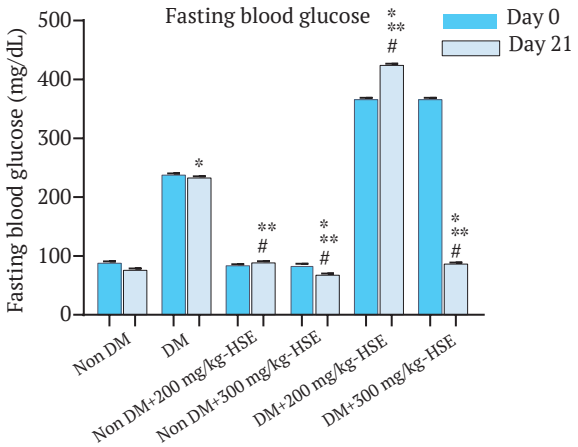

Figure 1. Hypoglycaemic effect of HS on FBG in alloxan-induced diabetic Wistar rats

Notes: * – p < 0.05 vs control group; ** – p < 0.05 vs diabetic control; # – p < 0.05 vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)

Source: compiled by the authors

The serum insulin level in the control group was 2.59 ± 0.05 µIU/mL, which was significantly higher (p < 0.05) than in the diabetic control group (0.79 ± 0.06 µIU/mL). Treatment with HS resulted in increased serum insulin levels in diabetic rats in a dose-dependent manner. Diabetic rats treated with 200 mg/kg HS had 1.96 ± 0.06 µIU/mL (p < 0.01), while those treated with 300 mg/kg HS had 2.68 ± 0.06 µIU/mL (p < 0.001). This is shown in Figure 2. In non-diabetic rats, serum insulin levels were also significantly elevated (p < 0.05) following HS treatment. The

control group had 2.59 ± 0.05 µIU/mL, while the 200 mg/kg HS group had 3.41 ± 0.06 µIU/mL ($p < 0.001$), and the 300 mg/kg HS group had 3.91 ± 0.05 µIU/mL ($p < 0.001$).

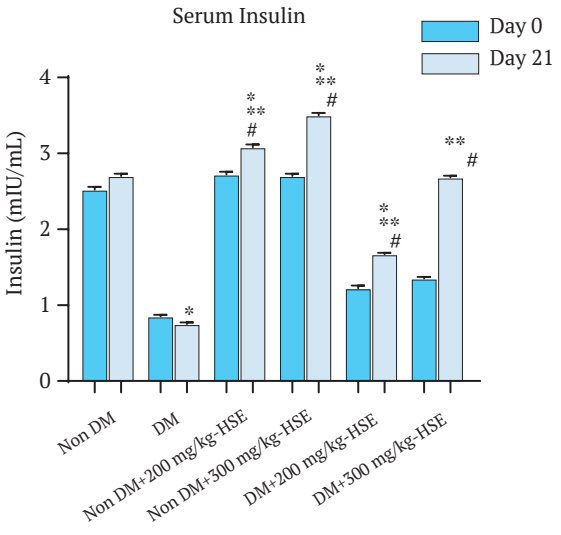

Figure 2. Hypoglycaemic effect of HS on insulin levels in alloxan-induced diabetic Wistar rats
Notes: * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

The antioxidant parameters, including catalase activity, SOD activity, SOD inhibition, and MDA concentrations, were assessed in skeletal muscle, pancreas, liver, and kidney tissues of alloxan-induced diabetic Wistar rats treated with *Hibiscus sabdariffa* at doses of 200 and 300 mg/kg. Comparisons were made between the non-diabetic control, non-diabetic HS-treated groups, and diabetic groups with and without HS treatment. Catalase activity, a key antioxidant marker, was significantly higher in the control group (71.9 ± 0.33 U/mL) compared to the diabetic control (1.6 ± 0.02 U/mL, $p < 0.001$). Treatment with HS at 200 and 300 mg/kg restored catalase activity in diabetic rats (34.9 ± 0.29 and 10.4 ± 0.30 U/mL, respectively, $p < 0.01$ for both). Similarly, MDA levels, an indicator of oxidative stress, were elevated in the diabetic control group (0.358 ± 0.03 mmol/mL) and significantly reduced following HS treatment at 300 mg/kg (0.213 ± 0.31 mmol/mL, $p < 0.05$) (Fig. 3).

In the pancreas (Fig. 4), the diabetic control group exhibited significantly reduced catalase activity (1.0 ± 0.12 U/mL) compared to the control group (0.7 ± 0.08 U/mL, $p < 0.001$). Treatment with 200 mg/kg HS significantly increased catalase activity in diabetic rats (21.9 ± 0.81 U/mL, $p < 0.01$). Additionally, MDA levels were highest in the non-diabetic 300 mg/kg group (0.468 ± 0.22 mmol/mL), with a marked reduction in the diabetic group treated with 300 mg/kg HS (0.096 ± 0.04 mmol/mL, $p < 0.001$), demonstrating the dose-dependent antioxidative effect of HS.

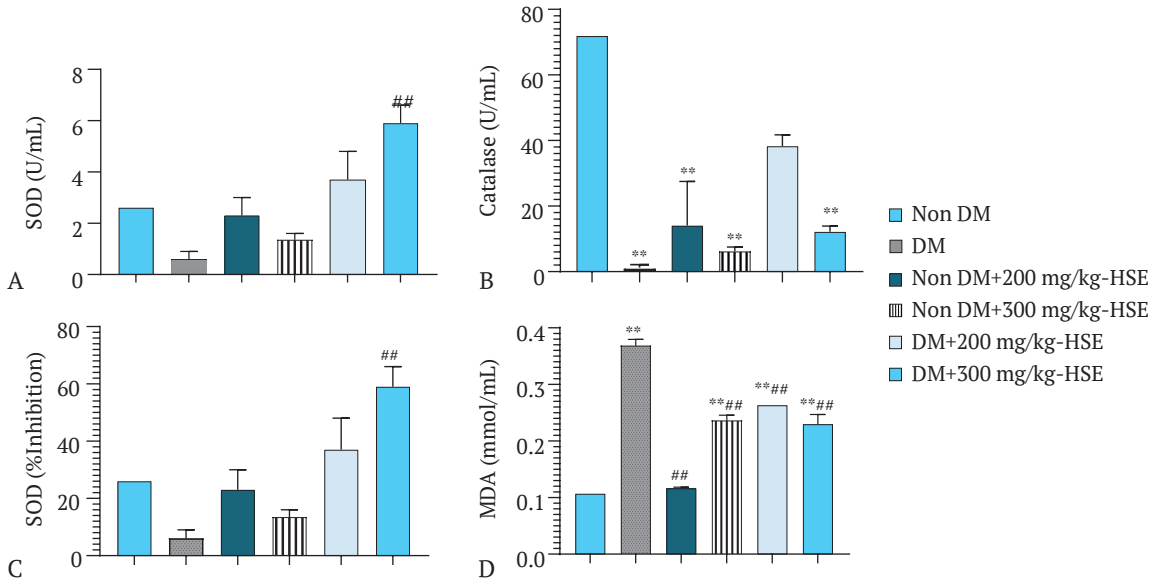

Figure 3. Antioxidant effect of HS on antioxidant enzyme parameters in skeletal muscle
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

The diabetic control group showed the lowest catalase activity in the liver (1.0 ± 0.45 U/mL), which was significantly lower than that of the control group (55.9 ± 0.23 U/mL, $p < 0.001$). Administration of HS to diabetic rats improved catalase activity to 32.4 ± 0.67 U/mL (200 mg/kg, $p < 0.05$) and 18.5 ± 0.42 U/mL (300 mg/kg, $p < 0.01$). A similar trend was observed for SOD activity, which improved with HS treatment but remained significantly lower than in the control group. This is shown in Figure 5.

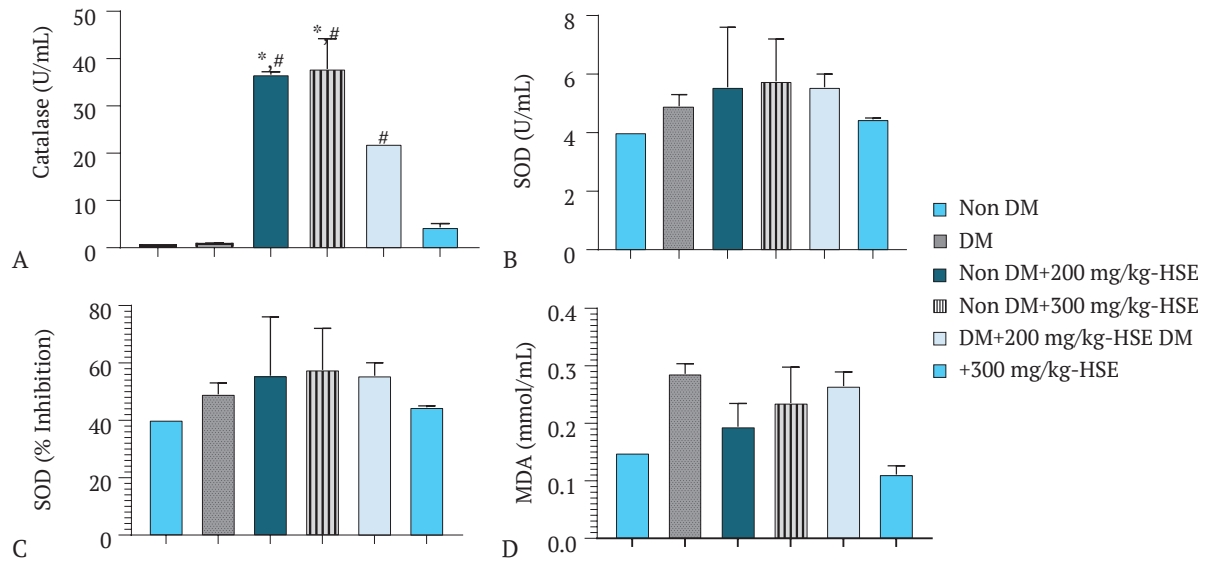

Figure 4. Antioxidant effect of HS on antioxidant enzyme parameters in the pancreas
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

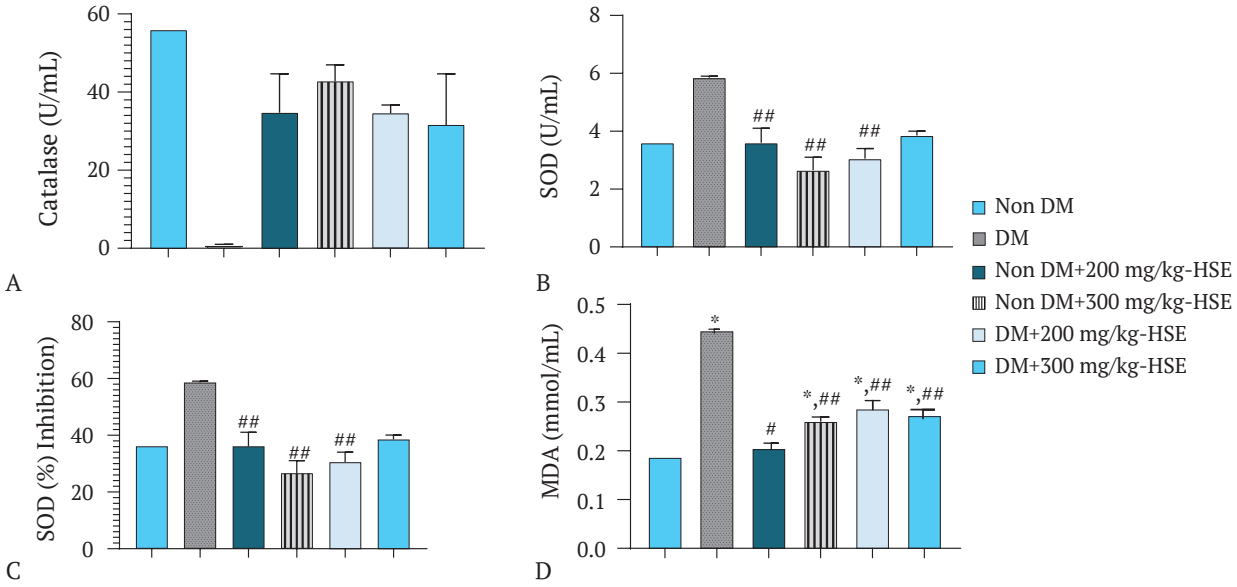

Figure 5. Antioxidant effect of HS on antioxidant enzyme parameters in the liver
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

In the kidney (Fig. 6), HS treatment resulted in significant improvements in catalase activity in diabetic rats (17.6 ± 0.30 U/mL for 200 mg/kg and 3.6 ± 0.32 U/mL for 300 mg/kg) compared to the control group (1.4 ± 0.02 U/mL, $p < 0.001$). MDA levels, which were elevated in the diabetic control group (0.400 ± 0.41 mmol/mL), were significantly reduced following 200 mg/kg HS treatment (0.234 ± 0.26 mmol/mL, $p < 0.01$).

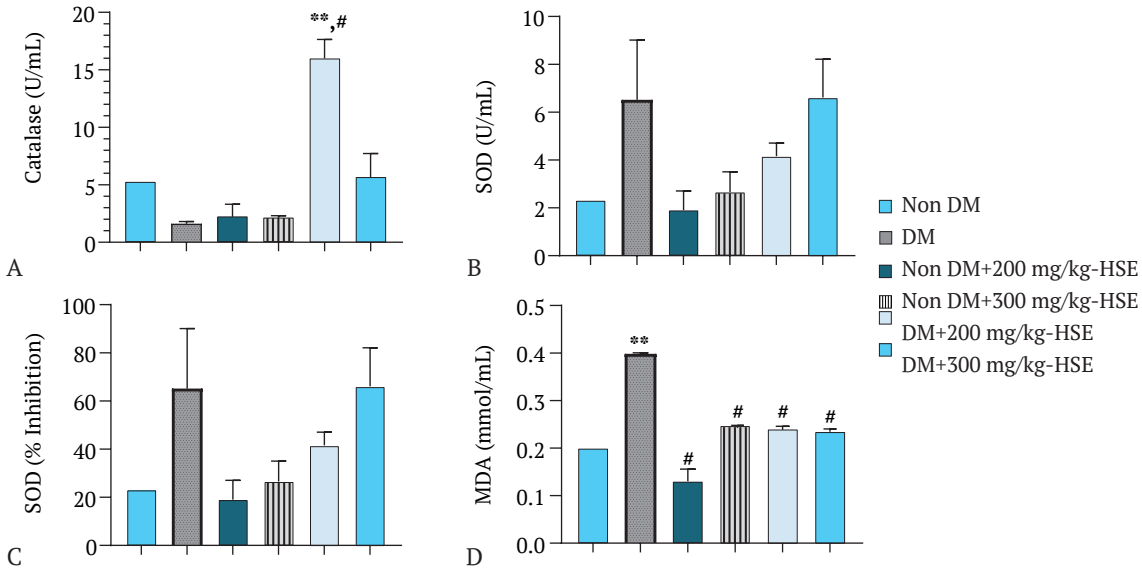

Figure 6. Antioxidant effect of HS on antioxidant enzyme parameters in the kidney
Notes: A – Catalase; B – SOD; C – SOD inhibition; D – MDA; * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

The effect of HS treatment on TNF-α and IL-6 levels was evaluated in control, diabetic, and HStreated groups. The treatment demonstrated a capacity to modulate inflammatory cytokine levels in both diabetic and non-diabetic rats. Figure 7 illustrates that the TNF-α level in the control group was 42.22 ± 3.53 pg/mL, while it increased significantly in the diabetic control group to 110.19 ± 9.46 pg/mL ($p = 0.007$). In the non-diabetic groups treated with HS, TNF-α levels decreased to 20.14 ± 2.61 pg/mL in the 200 mg/kg group ($p = 0.010$) and 25.67 ± 3.44 pg/mL in the 300 mg/kg group ($p = 0.035$). In diabetic rats, HS treatment also reduced TNF-α levels. The group treated with 200 mg/kg HS had a TNFα level of 81.03 ± 3.85 pg/mL, while the 300 mg/kg HS-treated group showed a level of 75.86 ± 3.71 pg/mL. Although these reductions, compared to the diabetic control group, did not reach statistical significance ($p = 0.081$ and $p = 0.074$, respectively), the downward trend suggests an antiinflammatory effect.

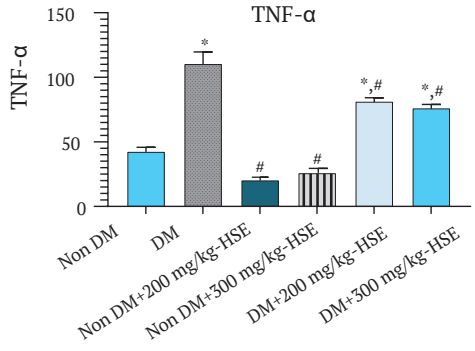

Figure 7. Anti-inflammatory effect of HS on inflammatory parameters (TNF-α)
Notes: * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

Figure 8 shows that the IL-6 level in the control group was 0.113 ± 0.003 pg/mL. In the diabetic control group, IL-6 levels increased significantly to 0.150 ± 0.001 pg/mL ($p = 0.001$). Non-diabetic rats treated with HS exhibited reductions in IL-6 levels, with the 200 mg/kg group recording 0.103 ± 0.0001 pg/mL ($p = 0.047$) and the 300 mg/kg group recording 0.105 ± 0.001 pg/mL ($p = 0.107$). In diabetic rats, HS treatment at 200 mg/kg reduced IL-6 levels to 0.118 ± 0.001 pg/mL ($p < 0.001$), while the 300 mg/kg group recorded a further reduction to 0.106 ± 0.001 pg/mL ($p < 0.001$).

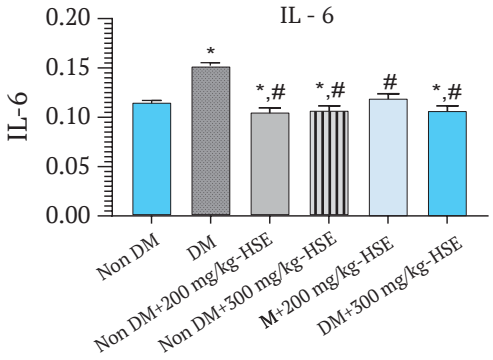

Figure 8. Anti-inflammatory effect of HS on inflammatory parameters (IL-6)
Notes: * – $p < 0.05$ vs control group; ** – $p < 0.05$ vs diabetic control; # – $p < 0.05$ vs blood glucose on day 0. Data are presented as mean ± SEM (n = 5)
Source: compiled by the authors

Elevated fasting blood glucose serves as a hallmark of impaired glucose metabolism and is a critical diagnostic indicator of diabetes mellitus. In this study, alloxan-induced diabetes resulted in a significant elevation of FBG levels in the diabetic control group compared to the normal control

group, consistent with its mechanism of β-cell destruction. However, administering *Hibiscus sabdariffa* extract at a dose of 300 mg/kg significantly reduced FBG levels, bringing them closer to the values observed in the nondiabetic control group. This effect aligns with findings reported by G.I. Kasimu *et al*. [16], who demonstrated the hypoglycaemic efficacy of HS in untreated diabetic rats. The observed reduction in FBG may be attributed to improved insulin secretion, enhanced glucose transport, or the regeneration of pancreatic β-cells, supported by the bioactive phytochemicals (polyphenols – especially anthocyanins – polysaccharides, and organic acids) in HS extract [17]. These findings emphasise the therapeutic potential of HS in managing hyperglycaemia and improving insulin sensitivity in diabetic conditions. A study by T. Suárez-Diéguez *et al*. [18] evaluated HS extracts in diabetic rats over an 80-day period. The authors found that doses of 200, 400, and 600 mg/kg significantly reduced fasting blood glucose levels by 35.2, 41.63, and 50.1%, respectively. Additionally, the highest dose improved lipid metabolism by lowering total cholesterol, triglycerides, VLDL, and LDL while increasing HDL levels. This suggests that HS extract can modulate glucose and lipid metabolism while offering potential as a functional ingredient or nutraceutical for managing diabetes. In another study, B.O. Ajiboye *et al*. [19] investigated the effects of flavonoid extracts from HS on nephropathy in streptozotocin-induced rats. The extracts mitigated kidney damage, indicating potential benefits for diabetic complications.

The present studies demonstrate that HS extracts can effectively reduce blood glucose levels and improve lipid profiles in diabetic models. However, the results of this study specifically highlight potential mechanisms, such as β-cell regeneration and improved insulin sensitivity, which are not explored in detail in other studies. This distinction adds a unique dimension to understanding how HS exerts its antidiabetic effects. Additionally, while the other studies focus on specific aspects such as nephropathy or lipid metabolism, the present research provides a more comprehensive view by addressing the hypoglycaemic, anti-inflammatory, and antioxidant effects of the extract. This holistic approach offers a more thorough insight into the potential therapeutic benefits of HS in diabetes management. Insulin is a key hormone involved in glucose storage in the liver, muscle, and adipose tissue. Following diabetes induction with alloxan, insulin levels decreased in the DM groups compared to the non-DM groups. The subsequent increase in insulin levels, particularly at the higher HS dose, aligns with reports from D. Jamrozik *et al*. [20] and M. Bule *et al*. [21], who proposed that gallic acid, a component of HS (Roselle), is responsible for stimulating insulin secretion through β-cell regeneration in the islets of Langerhans., This process enhances insulin sensitivity and reduces insulin resistance. D.B. Koval *et al*. [22] conducted a study similar to the present one, using the same species of rats but obtaining slightly different statistical results. The authors also emphasised the benefits of the chemical compound microcrystalline cellulose rather than those of plant-based treatments.

Diabetes-induced oxidative stress was evident in this study, as reflected by increased levels of MDA, a marker of lipid peroxidation, and reduced activity of antioxidant enzymes such as SOD and CAT in the diabetic control group. These alterations were observed across key organs involved in glucose regulation, including skeletal muscle, the pancreas, liver, and kidney. Elevated MDA levels in diabetic rats suggest heightened peroxidative injury, contributing to the development of DM. Oxidative stress activates IL-6 and TNF-α, worsening insulin sensitivity and exacerbating hyperglycaemia. D. Jamrozik *et al*. [20] reported that patients with high FBG had increased MDA levels and decreased SOD and CAT activity, alongside elevated IL-6 and TNF-α, forming a cycle that perpetuates the diabetic state. In this study, oxidative stress – characterised by elevated MDA levels and reduced SOD and CAT activities – was significantly mitigated in the HS-treated groups. The improvement in antioxidant activity across multiple organs indicates the protective role of HS against oxidative damage. Comparative studies have further elucidated the antioxidant properties of HS in diabetic models. Ajiboye *et al.* [19] demonstrated that an aqueous HS extract ameliorated diabetic nephropathy in streptozotocin-induced type 1 diabetic rats by reducing lipid peroxidation and increasing catalase and glutathione activity in the kidney, suggesting that HS exerts protective effects via modulation of oxidative stress pathways. Additionally, a systematic review by D. Jamrozik *et al*. [20] and M. Bule *et al*. [21] highlighted that HS exhibits hypoglycaemic, antioxidant, hypotensive, and anti-lipidaemic activities, indicating its potential as a complementary therapy in diabetes management. These findings align with the results of the present experiment, reinforcing the notion that HS's antioxidant properties contribute significantly to its therapeutic effects in diabetes.

Inflammation, evidenced by elevated IL-6 and TNF-α levels in diabetic rats, was significantly reduced following HS treatment, further demonstrating its anti-inflammatory properties. This is consistent with previous studies that have reported the anti-inflammatory effects of HS [23, 24]. The observed anti-inflammatory response is likely due to the presence of phytochemical compounds in HS extract, which modulate inflammatory pathways [25]. This study underscores the multifaceted role of HS in mitigating hyperglycaemia, enhancing insulin secretion, reducing oxidative stress, and suppressing inflammation in diabetic rats. The interplay between fasting blood glucose, insulin levels, oxidative stress, and inflammation is well-documented in the pathogenesis of DM. This study also identifies a correlation between antioxidant enzymes and pro-inflammatory cytokines such as IL-6 and TNF-α in diabetes. The administration of *Hibiscus sabdariffa* over a 21-day period significantly affected FBG, insulin levels, antioxidant enzymes, and pro-inflammatory cytokines in alloxan-induced diabetic rats.

CONCLUSIONS

This study demonstrates the potential of *Hibiscus sabdariffa* as a therapeutic agent for managing diabetes and its complications. Alloxan administration induced hyperglycaemia, oxidative stress, and inflammation in diabetic rats, replicating key pathological features of diabetes mellitus. However, treatment with HS extract at 300 mg/kg significantly reduced fasting blood glucose levels, likely through enhanced insulin secretion and the regeneration of pancreatic β-cells. This suggests that HS may contribute to improved glucose homeostasis, making it a promising

candidate for diabetes management. Beyond its hypoglycaemic effect, HS exhibited strong antioxidant and anti-inflammatory properties. The extract reduced malondialdehyde levels, a marker of lipid peroxidation while increasing the activities of key antioxidant enzymes such as superoxide dismutase and catalase. These antioxidative effects suggest that HS helps counteract diabetes-induced oxidative damage, protecting vital metabolic organs such as the liver, pancreas, kidneys, and skeletal muscles. Additionally, the extract significantly reduced the proinflammatory cytokines IL-6 and TNF-α, indicating its potential to alleviate inflammation, a major contributor to insulin resistance and diabetes progression.

Comparative studies further reinforce these findings, with prior research demonstrating similar hypoglycaemic, antioxidative, and anti-inflammatory effects of HS in various diabetic models. The presence of bioactive compounds, such as anthocyanins and polyphenols, in HS, likely underpins these effects by modulating multiple pathological pathways, including insulin sensitivity, glucose metabolism, and oxidative stress responses. These findings have significant implications, particularly in regions with limited access to conventional antidiabetic medications. As a natural, cost-effective alternative, HS holds promise for improving diabetes management while mitigating its complications. However, further research is necessary to optimise its dosage, evaluate its long-term effects, and validate its efficacy in human clinical trials. Additionally, investigating its interactions with standard antidiabetic drugs may provide insights into its potential role as a complementary treatment. Overall, *Hibiscus sabdariffa* offers a promising natural intervention for diabetes care, warranting further investigation in translational and clinical settings.

ACKNOWLEDGEMENTS

The authors thank the College of Health Sciences, University of Ilorin, Ilorin, Nigeria, for providing the necessary support and services for the successful completion of this study.

FUNDING

None.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Laakso M. Biomarkers for type 2 diabetes. Mol Metab. 2019;27:139–46. DOI: 10.1016/j.molmet.2019.06.016
[2] International Diabetes Federation (IDF). Diabetes and kidney disease (Atlas report) [Internet]. 2023 [cited 2024 August 13]. Available from: https://diabetesatlas.org/atlas/diabetes-and-kidney-disease/
[3] Liss DT, Cherupally M, O'Brien MJ, Kang RH, Aikman C, Wallia A, et al. Treatment modification after initiating second-line medication for type 2 diabetes. Am J Manag Care. 2023;29(12):661–8. DOI: 10.37765/ajmc.2023.89466
[4] Yulianti E, Sunarti, Wahyuningsih MSH. The effect of *Kappaphycus alvarezii* active fraction on oxidative stress and inflammation in streptozotocin and nicotinamide-induced diabetic rats. BMC Complement Med Ther. 2022;22:15. DOI: 10.1186/s12906-021-03496-8
[5] Araujo LS, da Silva MV, da Silva CA, Borges MDF, da Cunha Palhares HM, Rocha LP, et al. Analysis of serum inflammatory mediators in type 2 diabetic patients and their influence on renal function. PloS One. 2020;15(3):e0229765. DOI: 10.1371/journal.pone.0229765
[6] Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, et al. Organic acids from Roselle (*Hibiscus sabdariffa* L.) – a brief review of its pharmacological effects. Biomedicines. 2020;8(5):100. DOI: 10.3390/biomedicines8050100
[7] Islam MM. Food and medicinal values of Roselle (*Hibiscus sabdariffa* L. Linne Malvaceae) plant parts: A review. Open J Nutr Food Sci. 2019;1(1):1003.
[8] Montalvo-González E, Villagrán Z, González-Torres S, Iñiguez-Muñoz LE, Isiordia-Espinoza MA, Ruvalcaba-Gómez JM, et al. Physiological effects and human health benefits of *Hibiscus sabdariffa*: A review of clinical trials. Pharmaceuticals. 2022;15(4):464. DOI: 10.3390/ph15040464
[9] Hamadjida A, Metechie LC, Tchiengang FDT, Otto GLN, Eteme ON, Njintang NY, et al. Antidiabetic potential of *Hibiscus sadariffa* extract in alloxan-induced diabetic rats. GSC Biol Pharm Sci. 2023;23(1):193–203. DOI: 10.30574/gscbps.2023.23.1.0158
[10] Herdiani N, Wikurendra EA. Effect of Roselle petal extract on decreased levels of MDA in rats with type 2 diabetes. J Health Sci. 2021;14(1):48–52. DOI: 10.33086/jhs.v14i1.1688
[11] Institutional Animal Care and Use Committee Guidebook [Internet]. 2002 [cited 2024 August 13]. Available from: https://grants.nih.gov/grants/olaw/guidebook.pdf
[12] Sheriff OL, Olayemi O, Taofeeq AO, Riskat KE, Ojochebo DE, Ibukunoluwa AO. A new model alloxan-induced diabetes mellitus in rats. J Bangladesh Soc Physiol. 2020;14(2):56–62. DOI: 10.3329/jbsp.v14i2.44785
[13] Kil HN, Eom SY, Park JD, Kawamoto T, Kim YD, Kim H. A rapid method for estimating the levels of urinary thiobarbituric acid reactive substances for environmental epidemiologic survey. Toxicol Res. 2014;30(1):7–11. DOI: 10.5487/TR.2014.30.1.007
[14] Misra HP, Fridovich I. The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170–5.
[15] Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389–94. DOI: 10.1016/0003-2697(72)90132-7
[16] Kasimu GI, Ababubakar MB, Audu BB, Mainasara AS. Effect of co-administration of glibenclamide and aqueous calyx extract of hibiscus sabdariffa on oxidative stress markers in streptozotocin-induced diabetic rats. Niger J Physio Sci. 2021;36(1):77–84.

[17] Adeyemi DO, Adewole OS. *Hibiscus sabdariffa* renews pancreatic β-cells in experimental type 1 diabetic model rats. Morphologie. 2019;103(341(2)):80–93. DOI: 10.1016/j.morpho.2019.04.003
[18] Suárez-Diéguez T, Palma-Morales M, Camacho Bernal GI, Valdez López EN, Rodríguez-Pérez C, del Socorro Cruz-Cansino N, et al. Modulation of the hyperglycemia condition in diabetic lab rats with extracts of the creole Jamaica Flower (*Hibiscus sabdariffa* L.) from the Morelia region (Mexico). Antioxidants. 2024;13(8):1010. DOI: 10.3390/antiox13081010
[19] Ajiboye BO, Famusiwa CD, Nifemi DM, Ayodele BM, Akinlolu OS, Fatoki TH, et al. Nephroprotective effect of *Hibiscus sabdariffa* leaf flavonoid extracts via KIM-1 and TGF-1β signaling pathways in streptozotocin-induced rats. ACS Omega. 2024;9(17):19334–44. DOI: 10.1021/acsomega.4c00254
[20] Jamrozik D, Borymska W, Kaczmarczk-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
[21] Bule M, Albelbeisi AH, Nikfar S, Amini M, Abdollahi M. The Antidiabetic and antilipidemic effects of *Hibiscus sabdariffa*: A systematic review and meta-analysis of randomized clinical trials. Food Res Int. 2020;130:108980. DOI: 10.1016/j.foodres.2020.108980
[22] Koval DB, Malyarchuk HR, Levenets OO. The effect of microcrystalline cellulose on the microflora of the colon. Int J Med Med Res (IJMMR). 2021;7(2):91–6. DOI: 10.11603/ijmmr.2413-6077.2021.2.12516
[23] Janson B, Pramsomthong 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 Pharmacother. 2021;138:111438. DOI: 10.1016/j.biopha.2021.111438
[24] Prasomthong J, Limpeanchob N, Daodee S, Chonpathompikunlert P, Tunsophon S. *Hibiscus sabdariffa* extract improves hepatic steatosis, partially through IRS-1/Akt and Nrf2 signaling pathways in rats fed a high fat diet. Sci Rep. 2022;12:7022. DOI: 10.1038/s41598-022-11027-9
[25] Hamadjida A, Ayissi Mbomo RE, Minko SE, Ntchapa F, Mingoas JPK, Nnanga N. Antioxidant and anti-inflammatory effects of *Boswellia dalzielii* and *Hibiscus sabdariffa* extracts in alloxan-induced diabetes rats. Metab Open. 2024;21:100278. DOI: 10.1016/j.metop.2024.100278

Оцінка гіпоглікемічної, протизапальної та антиоксидантної дії *Hibiscus sabdariffa* у щурів з алоксановим діабетом

Лекан Шериф Оджуларі
Кандидат фізіологічних наук, доцент
Університет Ілорину
Ілорин, Нігерія
https://orcid.org/0000-0002-3405-0116

Нгайтад Станіслаус Нджінга
Кандидат фармацевтичних наук, доцент
Університет Ілорину
Ілорин, Нігерія
https://orcid.org/0000-0001-5566-5905

Рідван Абіодун Ганію
Бакалавр фізіологічних наук
Університет Ілорину
Ілорин, Нігерія
https://orcid.org/0009-0009-8563-7652

Таофік Олареваджу Айінде
Кандидат фізіологічних наук, доцент
Університет Ілорину
Ілорин, Нігерія
https://orcid.org/0000-0003-2092-0026

Еніола Ріскат Кадір
Кандидат анатомічних наук, доцент
Університет Ілорину
Ілорин, Нігерія
https://orcid.org/0000-0002-0097-4783

Анотація. *Hibiscus sabdariffa* корисний при лікуванні цукрового діабету. Дослідження було присвячено вивченю гіпоглікемічної, протизапальної та антиоксидантної дій *Hibiscus sabdariffa* у щурів з алоксановим діабетом. 30 щурів лінії Вістар були розділені на шість груп по п'ять щурів і акліматизовані протягом двох тижнів перед початком експерименту. Група I: контроль без діабету; група II: контроль з діабетом; група III: без діабету з 200 мг/кг *Hibiscus sabdariffa*; група IV: без діабету з 300 мг/кг *Hibiscus sabdariffa*; група V: з діабетом з 200 мг/кг *Hibiscus sabdariffa*; група VI: з діабетом з 300 мг/кг *Hibiscus sabdariffa*. Щури отримували одноразову внутрішньоочеревинну ін'єкцію алоксану (150 мг/кг маси тіла), а щури з діабетом отримували *Hibiscus sabdariffa* протягом 21 дня. У дослідженні вимірювались рівень глюкози в крові натще, рівень інсуліну, супероксиддисмутази, каталази, малонового діальдегіду, інтерлейкіну-6 та фактора некрозу пухлин-альфа, а також відбиралися зразки органів та крові. Результати були проаналізовані за допомогою дисперсійного аналізу з рівнем значущості <0,05, а дані були візуалізовані за допомогою GraphPad. Це дослідження продемонструвало, що *Hibiscus sabdariffa* має значний вплив на діабетичні параметри, прозапальні цитокіни та антиоксидантні ферменти. Щоденний пероральний прийом протягом 21-го дня знижував рівень глюкози в крові натще, інтерлейкіну-6, фактора некрозу пухлин-альфа та малонового діальдегіду. Він також посилював вироблення інсуліну, активність супероксиддисмутази та каталази в скелетних м'язах, печінці, підшлунковій залозі та нирках. Можна зробити висновок, що *Hibiscus sabdariffa* має потенціал для боротьби з гіперглікемією та запаленням, одночасно покращуючи активність антиоксидантних ферментів. Крім того, він може служити природним джерелом або засобом для лікування або профілактики діабету

Ключові слова: біомаркери оксидативного стресу; прозапальні цитокіни; біологічно активні сполуки; β-клітини підшлункової залози