Investigation of proinflammatory genes expression in STZ-induced diabetic rats treated with extract of Hibiscus rosa-sinensis flower

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Abstract

Background

Diabetes mellitus, a metabolic disorder of carbohydrates and fat, which results from the deficiency of insulin secretion or varying degree of insulin resistance, is a major public health problem and has become a global menace.

Aim

This study is aimed to investigate the expression of proinflammatory genes in STZ-induced diabetic rat model, treated with *Hibiscus rosa-sinensis* flower extract.

Methods

Diabetes was induced by intraperitoneal injection of streptozotocin (65 mg/kg). Thirty-six diabetic rats were divided into 6 groups which includes the diabetic control, another group treated with 100 mg/kg of sildenafil citrate and the 4 other groups treated with varying concentrations of aqueous extract of *Hibiscus rosa-sinensis* flower (50 mg/kg, 100 mg/kg, 200 mg/kg and 1000 mg/kg). Six animals were given only distilled water throughout the experiment as the normal control. The treatment was administered for 21 days. The mRNA expression of the pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) in the pancreas, kidney, liver and brain of the experimental animals were investigated using reverse-transcriptase polymerase chain reaction (RT-PCR). The fasting blood sugar and body weight of the experimental animals was also monitored throughout the experiment.

Results

Oral administration of *Hibiscus rosa-sinensis* flower extract (100, 200 and 1000 mg/kg) to STZ-induced diabetic rats significantly ($p < 0.05$) down-regulated the pancreatic mRNA expression of IL-6, IL-1β and TNF-α when compared with the diabetic control group. 200 and 1000 mg/kg of the extract down-regulated ($p < 0.05$) the mRNA expression of TNF-α and IL-6 in the kidney when compared with the diabetic and normal control group. 100, 200 and 1000 mg/kg of the extract significantly up-regulated ($p < 0.05$) the mRNA expression of IL-6 in the liver when compared with the diabetic and normal control group. 200 and 1000 mg/kg of extract significantly down-regulated the mRNA expression of TNF-α in the brain of diabetic rats. In addition, the oral administration of *Hibiscus rosa-sinensis* flower extract (50, 100, 200 and 1000 mg/kg) significantly ($P < 0.05$) reduces the fasting blood sugar and increases the body weight of experimental animals.

Conclusion

Observation drawn from this study suggests that *Hibiscus rosa-sinensis* flower extract possess antidiabetic potential and can suppress the JAK/STAT pathway thereby regulating the mRNA expression of the proinflammatory cytokines.

1.0 Introduction

Diabetes Mellitus (DM) is proving to be a global burden with the estimation of about 500 million people suffering from the metabolic disorder and about 12% cases in Nigeria [1, 2]. DM is characterized by hyperglycemia due to deficiency of insulin secretion and/or resistance which is associated with numerous disorders including cardiovascular diseases, organ damage and erectile dysfunction. It has been estimated that 90% of male diabetic patients suffer from erectile dysfunction, reduced libido, ejaculatory problems, infertility, among others, due to testicular dysfunction associated with protracted hyperglycemia [3, 4]. Almost 90% of diabetes cases are caused by insulin resistance, which is the main cause of type II diabetes mellitus. Resistance to insulin secretion damages the metabolic processes that produce proteins, lipids, and carbohydrates, increasing the risk of hyperglycemia in diabetic individuals. Hence, hyperglycemia has an osmotic effect on cells and sets off a series of events that lead to the generation of reactive oxygen species [5]. Overproduction of reactive oxygen species (ROS) in hyperglycemia causes oxidative stress conditions that mediate oxidative damage to the body's essential organs, including the kidney, liver, and pancreas [6].

Increases production of ROS also leads to the activation of Proinflammatory cytokines. Proinflammatory cytokines are group of protein that regulates the host responses to immune responses, infection and trauma, they also play a major role in inflammatory process and are usually upregulated in tissue injuries (e.g. tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6)) [7]. These proteins have been linked in classical immunology to gene expression alterations, cell proliferation, differentiation, and the creation of matrix proteins crucial for tissue repair and cell development. They stimulate the Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT) pathway, which plays a crucial role in the regulation of the liver, pancreas, and immune system function both in normal homeostasis and in diabetes, enabling an organism to respond quickly to an immune challenge [8, 9]. Hence, investigating the proinflammatory genes in a diabetic condition could be a good therapeutic approach in the management of the diseased condition.

Herbal remedies for diabetes have been shown to provide symptomatic relief and aid in the avoidance of subsequent problems. Certain plants have also been shown to aid in the regeneration of b-cells and the reduction of insulin resistance, while others have been reported to have antioxidant and anti-inflammatory properties in addition to their anti-hyperglycemic effect [10, 11]. *Hibiscus rosa-sinensis* is one of such medicinal plant that has been reported to show profound antidiabetic effects using the plant flower extracts [12, 13]. *Hibiscus rosa-sinensis Linn* (Malvaceae*)* is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colors of flowers ranging from white, pink, yellow and red, in medicine however, the red flowered variety is preferred [14]. The red flower is known as “Àdòdó” in Yoruba tribe of Nigeria and it is commonly used as an aphrodisiac. The

phytochemical investigation of its extract revealed the presence of tannins, phenols, flavanoides, alkaloids, terpenoids, saponins, cardiac glycosides, and steroids which validates its anti-diabetic, antioxidant, anti-inflammatory, and hypoglycemia activity of the plant [13, 15–16]. Despite the prevalence of pharmacological treatments for diabetes, finding a lasting cure remains a challenge. This study explores the potential of *Hibiscus rosa-sinensis* flower extract as a cost-effective and less toxic alternative for treating diabetes, investigating its anti-diabetic effects and impact on proinflammatory gene expression.

2.0 Materials and Methods

2.1 Chemicals

Steptozotocin (STZ), Trizol, Ethanol, Nuclease free water, Agarose gel, EZ vision, gene specific primers, Agarose gel, TBE buffer, Rutin, 100bp DNA ladder, Gel loading dye. All chemicals were purchased from sigma-aldrich (Germany) and are of analytical grade and the distilled water used was glass distilled.

2.2 Collection of plant materials

Fresh *Hibiscus rosa-sinensis* flowers was collected from lebbi street, okitipupa, ondo state and authenticated at the Department of Biological Sciences Olusegun Agagu University of Science and Technology, Okitipupa, Ondo state, Nigeria with the voucher number OAUSTECH/H/0657

2.3 Preparation of Plant extracts

The petals of the flower were separated and air dried for 1 week and was blended using electric blender and stored at room temperature in air tight container prior to use. 424.25g of powdered *Hibiscus rosa-sinesis* flower was measured and macerated in 6.4liters of distilled water for 24hrs and was filtered using muslin cloth to obtain an aqueous extracts. The filtrate was freeze dried and stored.

2.4 Animal purchase, experimental design, grouping and treatment

Forty-two (42) adult male wistar albino rats with an average weight of 150-250g were obtained from breeding colony of Zoology Department, in university of Ibadan, on the approval of animal ethnic committee. The rats were divided into groups of seven groups were housed in clean cages and maintained under standard laboratory condition (temperature 252 with dark/light cycle 12/12h). They were fed on rat chow and water was given as desired. The principles of laboratory animal care as stated by the animal ethic committee was strictly followed throughout the duration of the experiment. DM was induced by intraperitoneal (i.p) injection of freshly bufered (0.1 M citrate, pH 4.5) solution of STZ (65 mg/kg) to overnight fasted rats. The fasting blood sugar (FBS) and body weight was monitored at day 3, 7, 14 and 21 after 72 h induction of STZ, and rats with FBS ≥ 200 mg/dl were considered diabetic. There was no mortality after the STZ induction. The animal groups are as follows comprising six rats each:

Group I (Control): Normal control given distilled water;

Group II (Negative Control): Diabetic rats given distilled water;

Group III (Positive Control): Diabetic rats treated with sildenafil (100 mg/kg body weight);

Group IV (HB 50mg/kg): Diabetic rats treated with *Hibiscus rosa-sinensis* flower (50 mg/kg bw);

Group V (HB 100mg/kg): Diabetic rats treated with *Hibiscus rosa-sinensis* flower (100 mg/kg bw);

Group VI (HB 200mg/kg): Diabetic rats treated with *Hibiscus rosa-sinensis* flower (200 mg/kg bw);

Group VI (HB 1000mg/kg): Diabetic rats treated with *Hibiscus rosa-sinensis* flower (1000 mg/kg bw).

The doses was administered orally using oral gavage daily till the end of the experiment. All doses were started 72 h after STZ injection and the treatment lasted for 21 days. On the 22nd day, the animals were decapitated after an overnight fast by cervical dislocation and the organs liver, pancreas, kidney, and brain was collected and preserved in Trizol reagent.

2.5 Gene Expression Study

Total RNA was isolated from the Trizol-preserved tissues with Quick-RNA MiniPrep™ Kit (Zymo Research). The homogenate underwent treatment with a gradient separation media (chloroform) and was centrifuged at 1500 rpm for 30 minutes. The supernatant was combined with 100 mL of precipitating medium (isoamy alcohol) for 30 minutes at 1500 rpm, and the RNA pellet was collected before the supernatant was decanted. The precipitated RNA was suspended in nuclease-free water. To eliminate any DNA or phenol contamination, samples were digested with DNase and re-precipitated in ethanol. Total RNA concentration was measured using UV absorbance spectrophotometry (JENWAY 6305). Subsequently, the Proscript II second strand synthesis kit was utilized to convert the RNA to cDNA. PCR amplification was done using OneTaq® 2X Master Mix (NEB) using specific primer sequences for **(** IL-6, TNF- α, IL-1β**)** (Table 1).

2.6 Polymerase chain reaction (PCR) and Gel electrophoresis

The amplification of Polymerase Chain Reaction (PCR) was performed using One-Taq 2x Master Mix (NEB) with the gene-specific primer sets listed below. The primer design was executed using Primer Express v2.0 (Applied Biosystem, Foster City, Calif). Subsequently, the polymerase chain reaction amplicons were subjected to 1% agarose gel electrophoresis. Snapshots capturing the relative density of DNA bands were taken under UV-gel documentation.

Statistical Analysis

The intensities of the bands were quantified densitometrically using Image J software (http://imagej.en.softonic.com). Results were expressed as mean ± SD. Statistical analysis was carried

out using PRISM 8.01 (Graphpad software, Inc.) with $p < 0.05$ being considered as statistically significant

Table 1
Proinflammatory genes primers

Genes	Forward Primer Sequence	Reverse Primer Sequence
IL-6	5'-TCTCTCCGCAAGAGACTTCCA-3'	5'-ATACTGGTCTGTTGTGGGTGG-3'
TNF-α	5'-ACCACGCTCTTCTGTCTACTG-3'	5'-CTTGGTGGTTTGCTACGAC-3'
IL-1β	5'-CCGGATGGGTAGGATAAAGTT-3'	5'-ACCCACTGAGGTAGGAAAGA-3'

3.0 RESULTS

3.1 Hibiscus rosa-sinensis flower extract restored the fasting blood sugar level in diabetic rats and increased their body weights.

As showed in Table 2, the glucose levels of all the experimental rats are between a ranges below 200mg/dL before diabetes was induced. After 72 h of streptozotocin administration (day 0), the blood glucose level were significantly ($p<0.05$) elevated above 200mg/dL in the diabetic rats in relative to non-diabetic rats. The result also revealed that the blood glucose level of the treated groups (100mg/kg sildenafil, 50, 100, 200 and 1000 mg/kg *Hibiscus rosa-sinensis* flower extract) were significantly reduced in relative to the control group.

The effect of *Hibiscus rosa-sinensis* flower extract (50, 100, 200 and 1000 mg/kg) and sildenafil (100mg/kg) on the body weight of the experimental animals is as shown in Table 3. The body weight of the diabetic animals was significantly reduced in relative to the control however, the administration of sildenafil as well as the varying concentration of *Hibiscus rosa-sinensis* flower extract significantly ($p<0.05$) restored the body weight of the diabetic rats in relative to the control group.

3.2 The administration of *Hibiscus rosa-sinensis* flower extract lowers the mRNA expression of the pancreatic pro-inflammatory genes

As shown in figure 1, there is significant difference in the mRNA expression of the pancreatic pro-inflammatory genes (IL-6, IL-1β and TNF- α) in the STZ-induced diabetic group compared with the control. The pancreatic mRNA expression of pro-inflammatory genes (IL-6, IL-1β and TNF- α) were significantly ($p < 0.05$) up-regulated in diabetic control group compared to the control group. However, there was significant down-regulation of pancreatic mRNA expression of IL-6, IL-1β and TNF- α upon oral administration of *Hibiscus rosa-sinensis* flower extract (100, 200 and 1000 mg/kg), for 21days in relative to the diabetic group.

3.3 Hibiscus rosa-sinensis flower extract regulate the pro-inflammatory genes of the kidney

The effect of *Hibiscus rosa-sinensis* flower extract on the mRNA expression of the pro-inflammatory genes (TNF- α and IL-6) in the kidney of STZ-induced diabetic rats are shown in figure 2. A significant up-regulation of mRNA expression of TNF- α and IL-6 was observed in the kidney of diabetic rats compared to the control. The oral administration of *Hibiscus rosa-sinensis* flower extract at 200 and 1000 mg/kg for 21 days significantly ($p<0.05$) down-regulate the pro-inflammatory genes (TNF- α and IL-6) of the kidney compared to the diabetic group.

3.4 The effect of *Hibiscus rosa-sinensis* flower extract on IL-6 mRNA expression in the liver

There is significant difference in the IL-6 mRNA expression in the liver of STZ-induced diabetic group compared with the control as shown in figure 3. Group treated with *Hibiscus rosa-sinensis* flower extract (100 and 200 mg/kg) showno obvious difference ($p<0.05$) compared with the diabetic control.

3.5 Hibiscus rosa-sinensis flower extract reduces inflammation in the brain of

STZ-induced rats

The results in Figure 4 showed the effect of the plant extract on mRNA expression of TNF- α in the brain of diabetic rats. The result revealed that there is significant difference in the mRNA expression of TNF- α in the brain of STZ-induced diabetic rats compared to the control. The oral administration of *Hibiscus rosa-sinensis* flower extract at 200 and 1000 mg/kg as well as sildenafil (100 mg/kg) for 21 days significantly down-regulate the mRNA expression of TNF- α in relative to the diabetic group.

Table 2: Effect of oral administration of *Hibiscus rosa-sinensis* flower extracts on the fasting blood sugar (mg/dL) of STZ-induced diabetic albino rats. Treatment of diabetic rats with 50, 100, 200 and 1000mg/kg as well as 100mg/kg of sildenafil reduced the blood glucose**.** HB – *Hibiscus rosa-sinensis* extract. Results are expressed in Mean ± Standard deviation. *Statistical difference to (normal) control ($p<0.05$). $^{\#}$Statistical difference to negative (diabetic) control ($p<0.05$)

Treatments	Before STZ Induction (mg/dL)	72 h After STZ Induction (mg/dL)	Day 3 of Treatment (mg/dL)	Day 7 of Treatment (mg/dL)	Day 14 of Treatment (mg/dL)	Day 21 of Treatment (mg/dL)
Control	86 ± 1.32	89 ± 10.00$^{\#}$	89 ± 2.89$^{\#}$	106 ± 2.40$^{\#}$	90 ± 1.79$^{\#}$	62 ± 1.72$^{\#}$
Negative Control	106 ± 2.56	211 ± 6.33*	375 ± 25.59*	380 ± 13.13*	370 ± 12.61$^{*\#}$	432 ± 13.70*
Positive Control	105 ± 8.42	372 ± 4.30$^{*\#}$	117 ± 26.30$^{\#}$	173 ± 73.62$^{\#}$	115 ± 18.18$^{*\#}$	106 ± 9.73$^{*\#}$
HB 50mg/kg	95 ± 1.56	283 ± 6.30$^{*\#}$	385 ± 19.10*	88 ± 3.25$^{\#}$	94 ± 7.65$^{\#}$	78 ± 13.06$^{\#}$
HB 100mg/kg	96 ± 3.44	383 ± 6.24$^{*\#}$	416 ± 14.59*	250 ± 10.17$^{*\#}$	110 ± 10.92$^{*\#}$	100 ± 3.88$^{*\#}$
HB 200mg/kg	105 ± 6.25	421 ± 8.92$^{*\#}$	347 ± 29.75*	86 ± 10.13$^{\#}$	110± 4.07$^{*\#}$	88± 8.07$^{\#}$
HB 1000mg/kg	110 ± 2.89	463 ± 18.00$^{*\#}$	387 ± 63.08*	236 ± 84.13$^{*\#}$	121 ± 6.71$^{*\#}$	99± 10.17$^{*\#}$

Table 3: Effect of oral administration of *Hibiscus rosa-sinensis* flower extracts on the body weight (g) of STZ-induced diabetic albino rats. The positive control was treated with 100mg/kg body weight Sildenafil. HB – *Hibiscus rosa-sinensis* extract. Results are expressed in Mean ± Standard deviation. *Statistical difference to (normal) control ($p<0.05$). $^{\#}$Statistical difference to negative (diabetic) control ($p<0.05$)

Treatments	Before STZ Induction	72 h After STZ Induction	Day 3 of Treatment	Day 7 of Treatment	Day 14 of Treatment	Day 21 of Treatment
Control	110 ± 12.83	141 ± 11.94#	148 ± 11.20#	155 ± 14.09#	167 ± 14.13#	184 ± 16.09#
Negative Control	95 ± 3.86	106 ± 7.05*	103 ± 5.11*	98 ± 7.18*	94 ± 6.34*	98 ± 7.97*
Positive Control	121 ± 12.80	128 ± 1.59#	129 ± 5.18*#	123 ± 6.84*#	120 ± 10.44*#	120 ± 9.94*#
HB 50mg/kg	105 ± 8.63	104 ± 2.03*	107 ± 5.27*	114 ± 0.33*	117 ± 7.05*	118 ± 7.91*
HB 100mg/kg	128 ± 18.79	104 ± 11.48*	108 ± 9.74*	111 ± 9.39*	121 ± 10.97*#	121 ± 0.24*#
HB 200mg/kg	108 ± 9.36	112 ± 7.67*	113 ± 5.11*	123 ± 4.07*#	124 ± 4.58*#	135 ± 3.93*#
HB 1000mg/kg	118 ± 7.55	105 ± 15.02*	106 ± 12.66*	107 ± 15.55*	114 ± 15.83*#	117 ± 7.58*#

4.0 Discussion and Conclusion

Streptozotocin, a toxin produced by Streptomycetes achromogenes and known as (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranosyl), enters pancreatic cells through the glucose transporter 2, causing DNA alkylation and resulting in pancreatic cell death. Widely used to induce insulin insufficiency related to diabetes [17], streptozotocin administration at 65 mg/kg induces hyperglycemia, weight loss, and metabolic abnormalities in experimental rats. In this study, diabetic rats exhibited significantly ($p < 0.05$) elevated blood sugar and weight loss, both of which were effectively restored after 21 days of administering *Hibiscus rosa-sinensis* flower extracts (50, 100, 200, and 1000 mg/kg) and sildenafil (100 mg/kg). Previous studies have established the glucose-lowering and weight gain properties of *Hibiscus rosa-sinensis* flower extracts [13, 15, 18], validating the results of this study.

In response to oxidative stress that is brought on by the generation of reactive oxygen species (ROS), the JAK/STAT system, which plays an essential role in the mediation of cell proliferation, the transformation of healthy cells into malignant cells, and inflammatory signaling, is activated [19]. Oxidative stress is one of the clinical hallmarks of diabetes. This stress presented itself as a result of an imbalance between the body's natural antioxidant system and the generation of ROS. The formation of ROS caused tissue damage, which in turn triggered the release of pro-inflammatory cytokines such as tumor necrosis factor alpha, interleukin 1 beta, and interleukin 6. Because of the binding of TNF-α to its receptor, the receptor is able to undergo conformational modifications. These changes, in turn, cause JAKs to become activated. Phosphorylation of STATs by activated JAKs leads to their translocation in the nucleus, where they bind particular DNA sequences in order to initiate gene transcription.

The pancreas plays a crucial role in glucose homeostasis. The exocrine function of the pancreas is to facilitate digestion, while the endocrine function of the pancreas is to produce hormones that regulate blood glucose levels [20]. Hyperglycemia, which is a feature of diabetes, causes an increase in the production of reactive oxygen species and oxidative stress, both of which contribute to the activation of multiple signaling pathways, which ultimately results in the death of pancreatic beta cells and a reduction in beta-cell mass [21]. The pathogenesis of type 2 diabetes is characterized by an impairment, either absolute or relative, of insulin production by beta cells in response to insulin resistance. The present research found that the mRNA expression of pro-inflammatory cytokines (IL-1beta, IL-6, and TNF-α) was significantly higher in diabetes control compared to normal control, implying that the up-regulation of these cytokines promotes the activation of the JAK/STAT pathway in STZ-induced diabetic rats, causing pancreatic tissue inflammation. However, treatment of the rats with *Hibiscus rosa-sinensis* flower extract (100, 200, and 1000mg/kg) alleviated inflammation by down-regulating ($p < 0.05$) pro-inflammatory cytokines (IL-1beta, IL-6, and TNF-α), thereby suppressing the activation of the JAK/STAT pathway, preventing further inflammation in the pancreatic tissue. Interestingly, the findings in this present study is supported by Pillai and Mini reports, when they investigated the Attenuation of high glucose induced apoptotic and inflammatory signaling pathways in RIN-m5F pancreatic β cell lines by *Hibiscus rosa sinensis* L. petals and its phytoconstituents [22].

Diabetic nephropathy, also known as diabetic kidney disease, is characterized by a gradual decline in glomerular filtration rate as well as an increase in the amount of albumin that is excreted in urine, and these symptoms are almost always accompanied by an elevation in blood pressure [23]. The hypertrophy of the proximal tubules in a diabetic kidney can eventually lead to thickening of the glomerular basement membrane and growth of the mesangial tissue, both of which can lead to fibrosis as well as the reabsorption of urine filtrate. Diabetic nephropathy, which affects between one-third and one-fifth of all diabetes patients, can lead to end-stage renal failure if the condition is left untreated [24]. Several proinflammatory cytokines are thought to be the root cause of renal illness or a contributing factor in its progression. In addition, patients who have diabetes and diabetic nephropathy have higher amounts of TNF-α and IL-6 in their bodies compared to patients who do not have the nephropathy [25, 26]. Comparatively, diabetic individuals with kidney disease had higher levels of IL-6 mRNA expression in their glomerular, epithelial, and mesangial renal cells, as well as their kidney infiltrating cells, as compared to diabetic patients who do not have kidney disease [25]. In the present study, the mRNA gene expression of pro-inflammatory cytokines (TNF-α and IL-6) were elevated in the STZ-induced diabetic group however, the groups treated with *Hibiscus rosa-sinensis* flower extract (200 and 1000mg/kg) shows statistical difference ($p < 0.05$) when compared with the control group and diabetic control group. This suggests a potential protective role in alleviating diabetic nephropathy, possibly by mitigating inflammation and fibrosis in the kidneys.

The human liver is the largest internal organ in the body, and it is responsible for maintaining the normal level of glucose in the blood and, as a result, the body's energy metabolism [27]. In addition to fulfilling this function, this organ is critical to the process of lipid metabolism. It does so by absorbing, digesting, and encapsulating lipids in a variety of forms, which are then transported to other extra-hepatic tissues

[28]. Despite the fact that insulin is responsible for the exact regulation of these processes, the development of non-alcoholic fatty liver disease in type 2 diabetics has been linked to an insensitivity to or resistance to the hormone signals [29]. Inflammation also makes a significant contribution to host defense by producing pro-inflammatory cytokines such as IL-6, which help protect the host from being injured. In spite of this, chronic inflammatory illnesses lead to fibrosis, which in turn causes tissue damage and a loss of cellular function. In the present study, there is significant difference between the STZ-induced diabetic group compared to the normal control. Groups treated with *Hibiscus rosa-sinensis* flower extract (100, 200 and 1000 mg/kg) of the extract significantly up-regulated ($p < 0.05$) the mRNA expression of IL-6 in the liver when compared with the diabetic and normal control group. This could indicate a regulatory effect on inflammatory processes in the liver, potentially contributing to maintaining normal glucose levels and lipid metabolism.

Inflammation caused by diabetes can endure for a long time. This inflammation manifests itself in a number of different ways, including the formation of reactive oxygen species, the expression of proinflammatory cytokines, and the activation or expression of other inflammatory mediators. It suggests that after cerebral ischemia, diabetes-related proinflammatory pathways are further accelerated, resulting in more ischemic damage [30]. Diabetes has been demonstrated to aggravate damage after cerebrovascular illness [31, 32], and this is likely due to the fact that diabetes is involved in a wide variety of damaging pathways, including oxidative stress and inflammatory responses. Elevated levels of proinflammatory cytokines, including tumor necrosis factor alpha, are one of the primary complications associated with neuropathy. These cytokines are linked to significant comorbidities, including diabetes. As stated in Fig. 4, there is a statistical difference ($p < 0.05$) in the mRNA expression of TNF-α in the brain of STZ-induced diabetic rats compared to the control. The oral administration of *Hibiscus rosa-sinensis* flower extract at 200 and 1000 mg/kg as well as sildenafil (100 mg/kg) for 21 days significantly down-regulate the mRNA expression of TNF- α in relative to the diabetic group. The observed neuroprotective effects may contribute to mitigating ischemic damage and complications associated with neuropathy.

It is possible that the aqueous extraction of the *Hibiscus rosa-sinensis* flower and its constituent phytochemicals are responsible for the down-regulation of the mRNA expression of the pro-inflammatory cytokines that were observed in this study. Studies have shown that the blossoms of the *Hibiscus rosa-sinensis* plant contain high levels of a variety of bioactive chemicals, including myricetin, apigenidin, citric acid, pelargonidin, quercetin, and ascorbic acid [33, 34]. These chemicals may reduce inflammation by reducing the synthesis of pro-inflammatory cytokines such as IL-1beta, IL-6, and TNF-α in the organs. This, in turn can reduces the activation of the JAK/STAT pathway, which eventually results in less inflammation.

Declarations

Ethical approval

The experiments were approved by the institutional committee for the Ethical use of Research Animals of the Olusegun Agagun University of Science and Technology (Ethical approval No: OAUSTECH/ETH/2022/013).

Declaration of Competing Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

OC and OA: conceptualization, analysis, manuscript editing, and review; BO, and OP: original drafting of the manuscript

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Figures

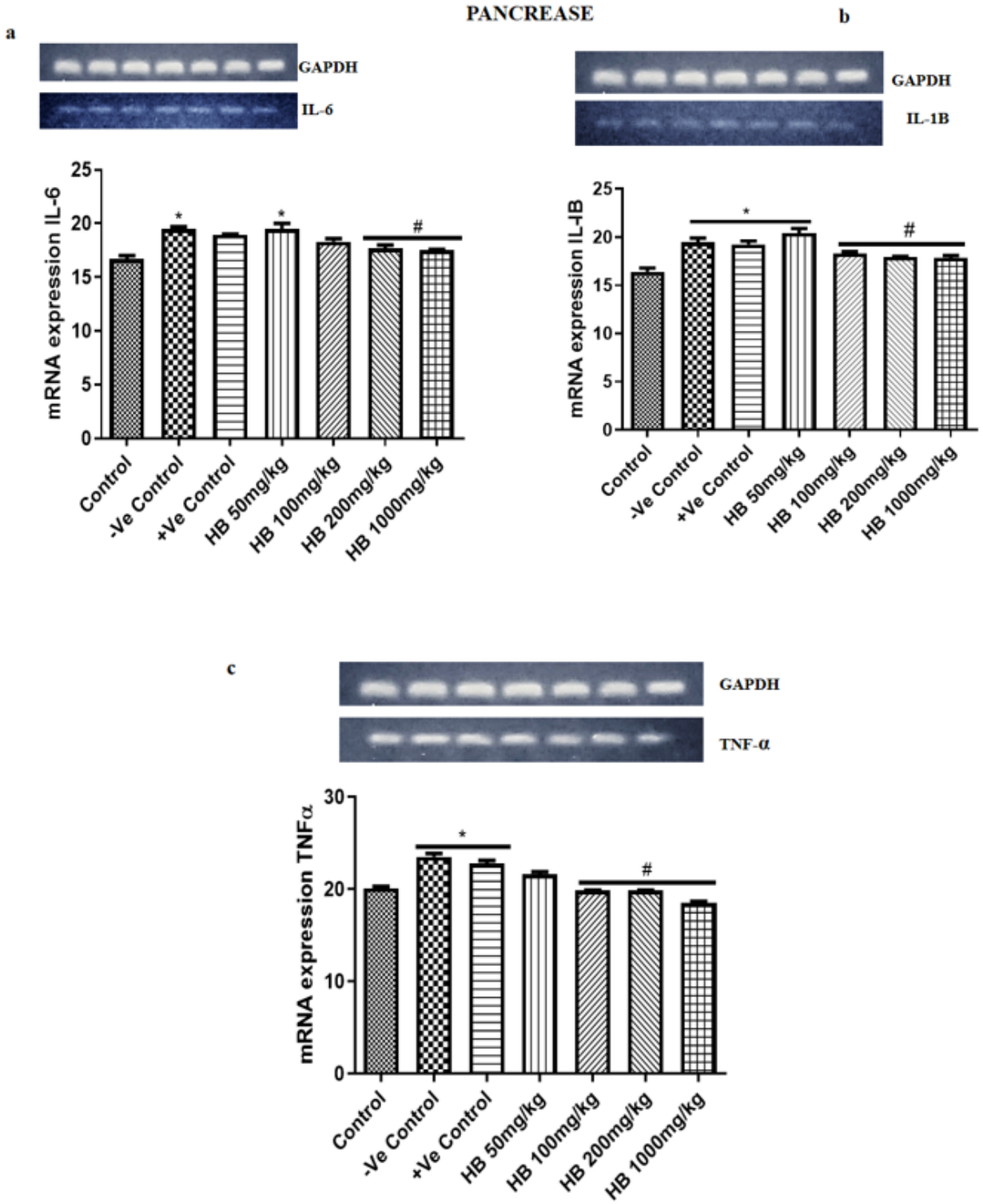

Figure 1

(a) Dose dependent expression of IL-6 measured by PCR from the pancreas tissues of control and STZ model diabetic rats treated with *Hibiscus rosa-sinensis* flower extract and Sildenafil using IL-6 primer. * Indicate statistical difference (P 0.05) to the control while # indicate statistical difference (P 0.05) to diabetic control **(b)** Dose dependent expression of IL-1β measured by PCR from the pancreas tissues of control and STZ model diabetic rats treated with *Hibiscus rosa-sinensis*flower extract and Sildenafil using

IL-1β primer. * Indicate statistical difference (P 0.05) to the control while # indicate statistical difference (P 0.05) to diabetic control **(c)** Dose dependent expression of TNF-α measured by PCR from the pancreas tissues of control and STZ model diabetic rats treated with *Hibiscus rosa-sinensis* flower extract and Sildenafil using TNF-α primer. * Indicate statistical difference (P 0.05) to the control while # indicate statistical difference (P 0.05) to diabetic control

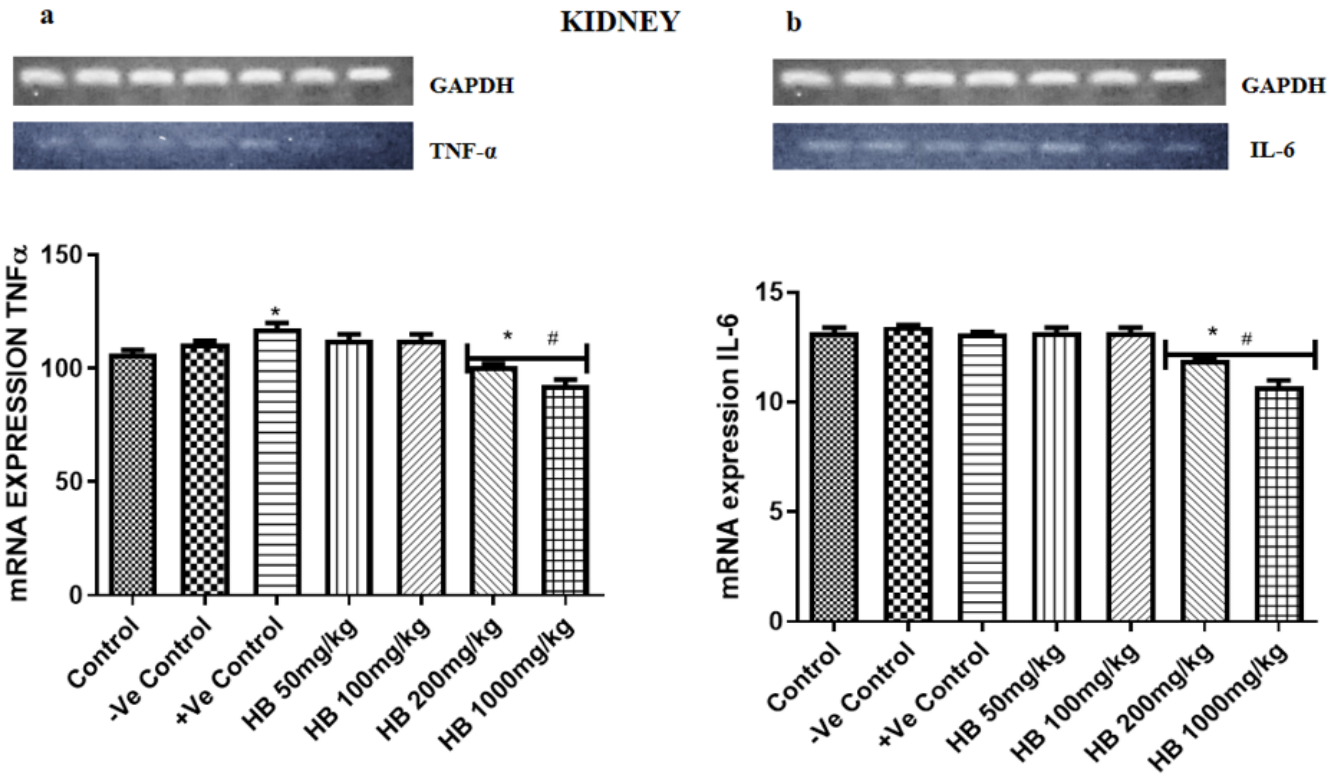

Figure 2

(a) Dose dependent expression of TNF-α measured by PCR from the kidney tissues of control and STZ model diabetic rats treated with *Hibiscus rosa-sinensis* flower extract and Sildenafil using TNF-α primer. * Indicate statistical difference (P 0.05) to the control while # indicate statistical difference (P 0.05) to diabetic control **(b)** Dose dependent expression of IL-6 measured by PCR from the kidney tissues of control and STZ model diabetic rats treated with *Hibiscus rosa-sinensis*flower extract and Sildenafil using IL-6 primer. * Indicate statistical difference (P 0.05) to the control while # indicate statistical difference (P 0.05) to diabetic control

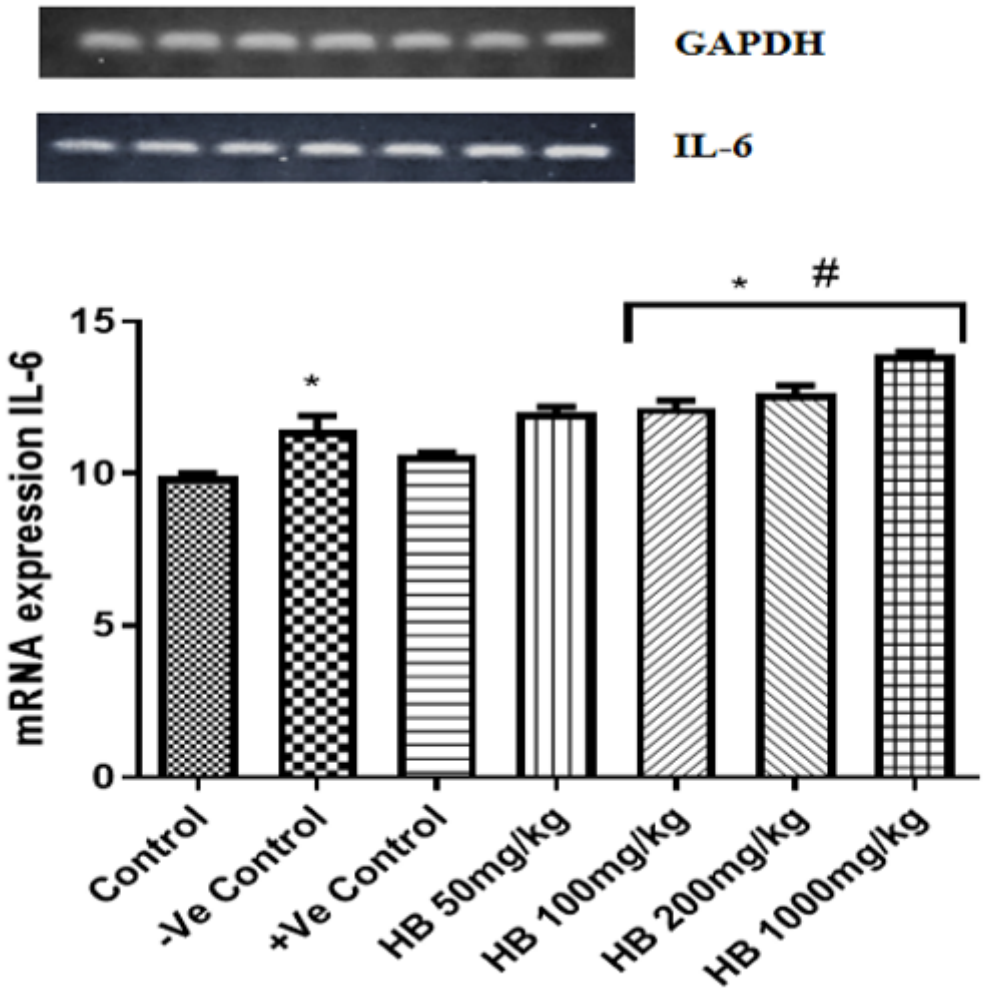

Figure 3

Dose dependent expression of IL-6 measured by PCR from the liver tissues of control and STZ model diabetic rats treated with *Hibiscus rosa-sinensis* flower extract and Sildenafil using IL-6 primer. * Indicate statistical difference (P 0.05) to the control while # indicate statistical difference (P 0.05) to diabetic control

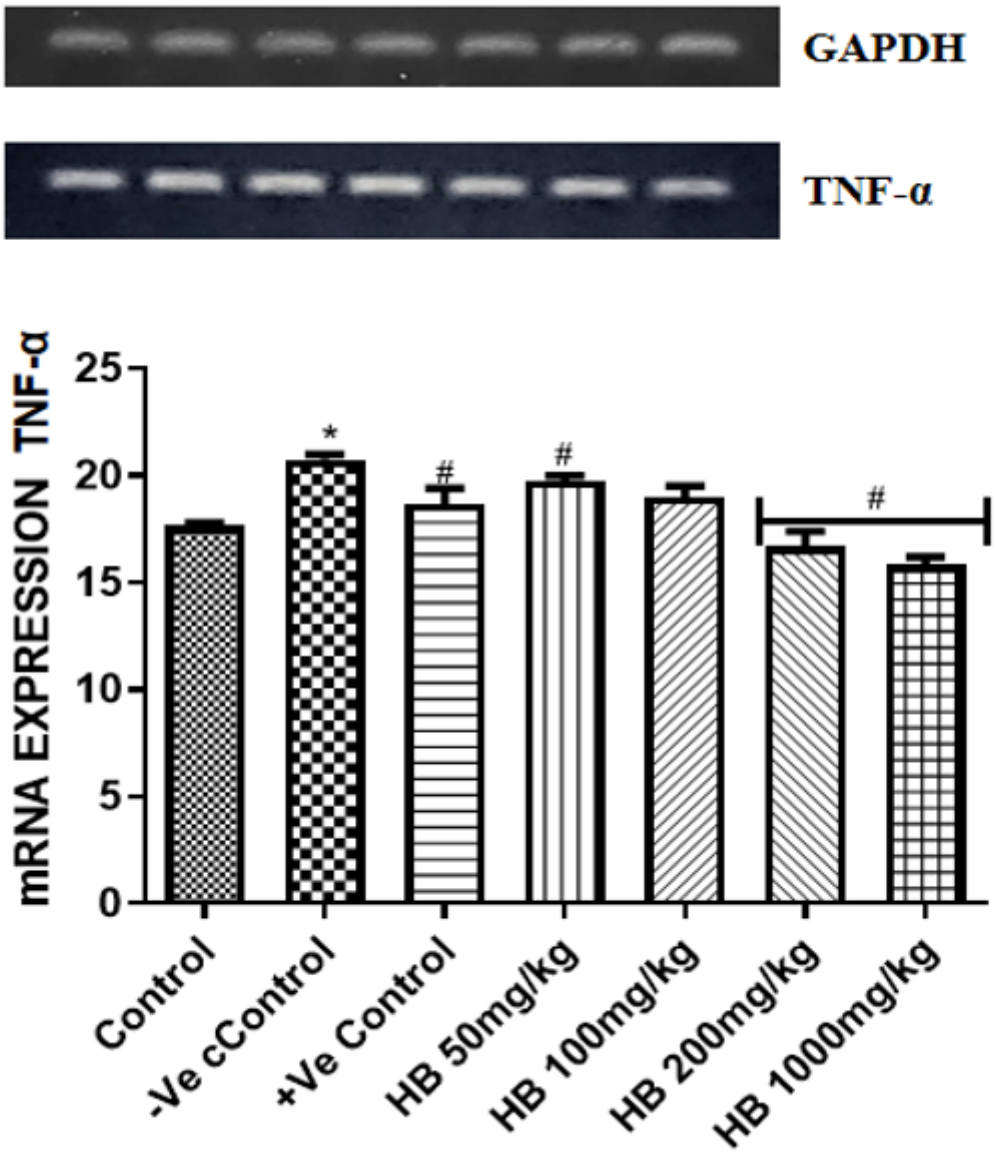

Figure 4

Dose dependent expression of TNF-α measured by PCR from the brain tissues of control and STZ model diabetic rats treated with *Hibiscus rosa-sinensis* flower extract and Sildenafil using TNF-α primer. * Indicate statistical difference (P 0.05) to the control while # indicate statistical difference (P 0.05) to diabetic control