RESEARCH ARTICLE

Hibiscus sabdariffa L. extract ameliorates the diabetic late complications: Cardioprotective and nephroprotective effect in streptozotocin-induced diabetic rats

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Abstract

Introduction: *Hibiscus sabdariffa* L. belongs to family Malvaceae and is used worldwide as a food, local medicine, and as a home remedy for various illnesses. Conventionally, the dried calyx is used as a diuretic, antidiabetic, antihypertensive, galactagogue, and for cardiovascular complications. Information concerning its protective effect against late diabetic complications is scarce. Hence, the present investigation is aimed to evaluate cardioprotective and nephroprotective activity of ethanolic extract (HSE) and aqueous extract (HSA) of H. sabdariffa L. on late complications associated with streptozotocin (STZ)-induced diabetes in rats. **Materials and Methods:** Diabetes was induced in male Wistar rats by single intravenous injection of STZ 45 mg/kg. Rats showing serum glucose (SG) level >200 mg/dl were included in the study and treated with HSE and HSA (200 and 400 mg/kg) and glibenclamide (10 mg/kg) for 8 weeks. At the end of the study, intraperitoneal glucose tolerance test, insulin tolerance test, blood glucose, lipid profiles, liver antioxidant levels, and cardiac and renal parameters were estimated. **Results:** Treatment with HSE and HSA resulted in a significant reduction in blood glucose, cholesterol, triglyceride, low-density lipoprotein-cholesterol (LDL-c), very-LDL-c, urea, and creatinine accompanied by an increase in total protein, albumin, and high-density lipoprotein-cholesterol in diabetic rats. Moreover, the cardiac markers such as creatinine kinase-MB and lactate dehydrogenase were also increased. HSE and HSA improved glucose tolerance, insulin tolerance, and decreased oxidative stress by improving endogenous antioxidant levels. **Conclusion:** The present investigation concludes that HSE and HSA ameliorate the cardiotoxicity and nephrotoxicity associated with late complications of diabetes mellitus in STZ-induced diabetic rats.

Key words: Cardiotoxicity, diabetes mellitus, diabetic late complications, *Hibiscus sabdariffa*, nephrotoxicity

INTRODUCTION

Patients with long-term diabetes mellitus (DM) pose a greater risk of other serious health issues such as hyperlipidemia, nephropathy, hepatopathy, cardiopathy, and retinopathy.[1] According to the WHO, diabetes is one of the leading causes of death, about 3.7 million deaths in 2012 were due to high blood sugar level, of which about 2.2 million deaths were associated with cardiovascular disease and chronic kidney disease. The kingdom of Saudi Arabia stands second among Middle East countries for prevalence of diabetes and overall seventh globally.[2]

In diabetic patients, cardiovascular disease is 40 times more common than non-diabetic patients. Clinical symptoms associated with diabetic nephropathy start from asymptomatic proteinuria and nephrotic syndrome to progressive renal failure.[3]

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In early 1970s, the term cardiomyopathy was used to define the cardiac dysfunction in diabetic patients with no coronary artery disease and hypertension.[4] The Framingham Heart Study demonstrated that there is 2.5–5 fold increase in the incidence of heart failure in diabetic patients, independent of hypertension, and coronary artery disease.[5] In diabetic patients, chronic insulin resistance or insulin deficiency leads to increased metabolism of lipids that lead to accumulation of fatty acid (FA) oxidation products and free radicals.[6] As on date, there are no drugs available that can cure as well as can inhibit diabetes and related complications. The WHO has recommended the evaluation of plants for treatment of diabetes. This has led to an increase in demand of research on antidiabetic natural products.

The plant *Hibiscus sabdariffa* L., red sorrel in English and Karkade in Arabic, is used worldwide as a food and local medicine for various illnesses and used as diuretic, antipyretic, antidiabetic, antihypertensive, galactagogue, and for cardiovascular complications.[7] Previous studies have shown that polyphenol extract of *H. sabdariffa* L. has nephroprotective effect in diabetic rats.[8] However, scientific data concerning its protective effect in diabetic late complications, particularly nephrotoxicity and cardiotoxicity, are scarce. Hence, we thought it worthwhile to investigate the cardioprotective and nephroprotective effect of ethanolic and aqueous extract in 8 weeks model of streptozotocin (STZ)-induced diabetes in rats.

MATERIAL AND METHODS

Animals

Seventy young male Wistar rats, 3–4 weeks old with 150–180 g body weight, were purchased from the Animal House of College of Pharmacy, Najran University, Najran, Saudi Arabia. Animals were housed in groups of five rats in standard clear polycarbonate cages with food and water available *ad libitum*. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%), and 12-h light-dark cycles. All the animals were acclimatized for 7 days before the study. All experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Preparation of Extracts

H. sabdariffa L. dried flowers were purchased from a local market, in Najran, KSA, and air-dried for 1 week at room temperature. The shade dried, coarse powdered flowers (2 kg) were extracted exhaustively with ethanol (95%) in a Soxhlet apparatus and subsequently extracted with water on a hot water bath. The extractives were concentrated to a small volume and evaporated to dryness in a vacuum desiccator to yield ethanol extract (HSE) and aqueous extract (HSA). Weighed quantity of HSE and HSA was suspended in water using 0.5% tragacanth and administered orally to experimental animals. Suspension of the extract was prepared freshly. The extracts were administered at a constant volume of 10 ml/kg for each animal.

Induction of DM

Diabetic condition (Type-I) was induced in male Wistar rats by single intravenous injection of STZ (45 mg/kg) after overnight fasting for 12 h. Rats showing serum glucose (SG) level >200 mg/dl 7 days after STZ administration were considered diabetic and included in the study.[9]

Experimental Protocol

The experimental rats were divided into seven groups of five rats each and treated for 8 weeks as follows:

1. Group 1: Normal control (NC) received 0.5% tragacanth 10 ml/kg, p.o.
2. Group 2: Diabetic control (DC) received 0.5% tragacanth 10 ml/kg, p.o.
3. Group 3: Diabetic rats treated with HSE 200 mg/kg, p.o.
4. Group 4: Diabetic rats treated with HSE 400 mg/kg, p.o.
5. Group 5: Diabetic rats treated with HSA 200 mg/kg, p.o.
6. Group 6: Diabetic rats treated with HSA 400 mg/kg, p.o.
7. Group 7: Diabetic rats treated with glibenclamide (GLB) 10 mg/kg, p.o.

Intra Peritoneal Glucose Tolerance Test (IPGTT)

Rats from all groups were subjected to the IPGTT on 52nd day. Briefly, food was withheld from the rats overnight (~12 h) and glucose challenge was initiated with an intraperitoneal injection of glucose (2 g/kg).[10] Glucose concentrations were measured in blood drops obtained by clipping the tail of the rats immediately before glucose challenge, as well as at 30, 60, and 120-min intervals. Blood glucose concentrations were measured using ACCU-CHEK Aviva Plus test strips (Roche Diagnostics).

Insulin Tolerance Test (ITT)

ITT was carried out on 54th day of dosing, and insulin (2 U/kg, i.v) was administered to 6 h-fasted rats. Blood samples were collected before insulin load, i.e., at 0 min and at 10, 20, and 30 min afterward.[11] Blood glucose concentrations were measured using ACCU-CHEK Aviva Plus test strips (Roche Diagnostics).

The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$), which was calculated by trapezoid rule.

Estimation of Lipid Profile

At the end of the study, blood samples were collected from retro-orbital plexus. Serum was collected and analyzed for triglyceride (STG), total cholesterol (STC), and high-density lipoprotein-cholesterol (HDL-c), using diagnostic reagent kits.[12] Very low-density lipoprotein-cholesterol (VLDL-c) and LDL-c in serum were calculated as per Friedewald's equation.

$$\text{VLDL-c} = \frac{\text{Triglyceride}}{5}$$

$$\text{LDL-c} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL-c}$$

The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were calculated.

Estimation of Cardiovascular Parameters

At the end of the study, blood samples were collected from retro-orbital plexus. Serum was collected and analyzed for creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) using diagnostic reagent kits.[9]

Estimation of Renal Parameters

At the end of the study, blood samples were collected from retro-orbital plexus. Serum was collected and analyzed for serum urea nitrogen, serum albumin, serum total protein, and serum creatinine using diagnostic reagent kits.[13,14]

Endogenous Enzymatic and Non-enzymatic Antioxidant Levels

Glutathione (GSH)

The GSH content of the supernatant of liver homogenate was assayed using Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid [DTNB solution]).[15]

Total thiols

The assay is based on the formation of a relatively stable yellow product when sulfydryl groups react with DTNB. Briefly, 0.2 ml of liver homogenate was mixed with phosphate buffer (pH = 8), 40 µl of 10 mM DTNB, and 3.16 ml of methanol. This mixture was incubated for 10 min, and the absorbance was measured at 412 nm against appropriate blanks.[16]

Lipid peroxidation (thiobarbituric acid reactive substances [TBARS])

Lipid peroxidation was estimated by the measurement of malondialdehyde levels. It is an end product of lipid peroxidation and its level will be determined spectrophotometrically by the use of TBARS method.[17]

Catalase

The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate, and 0.4 ml of 2M H2O2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then, the absorbance was read at 620 nm.

Histopathological studies

The whole heart and kidney from animals were removed after sacrificing the animal, collected and preserved in 10% formalin solution. The samples were submitted for histological examination.

Statistical evaluation

The data are expressed as a mean ± standard error of the mean (SEM). Statistical comparisons are performed by one-way ANOVA followed by Tukey's post-test using GraphPad Prism version 5.0, USA.

RESULTS

IPGTT

The NC rats did not show a significant increase in SG level after intraperitoneal administration of glucose (2 g/kg) at 120 min interval. Whereas, the diabetic rats exhibited a significant elevation in fasting SG (at time zero) and showed significant impairment in glucose tolerance to exogenously administered glucose compared to normal rats [Figure 1a]. Treatment of diabetic rats with HSE (400 mg/kg), HSA (400 mg/kg), and GLB showed significantly ($P < 0.001$) improved glucose tolerance and marked fall in SG level over 120 min compared to DC [Figure 1a].

The DC group showed significantly higher ($P < 0.001$) integrated area under the glucose curve over 120 min ($AUC_{glucose}$) compared to NC. Treatment with HSE (400 mg/kg), HSA (400 mg/kg), and GLB showed significantly ($P < 0.001$) decreased $AUC_{glucose}$ compared to DC [Figure 1b]. Collectively, both the tested extracts (200 mg/kg and 400 mg/kg) showed marked improvement in glucose tolerance.

ITT in Diabetic Rats

SG levels were measured following insulin challenge (2 U/kg, i.v) and diabetic rats subjected to insulin challenge exhibited a marked fall in SG levels, suggesting that these diabetic rats were able to utilize the exogenously administered insulin and reduce the SG levels. As shown in Figure 2a, HSE (400 mg/kg) exhibited a significant fall in SG level after insulin administration. This reduction in SG level was comparable to GLB which showed significant reduction

compared to DC group. Figure 2b depicts $AUC_{glucose}$ over 30 min time period of post-insulin administration. Integrated $AUC_{glucose}$ for HSE (400 mg/kg) and HSA (400 mg/kg)-treated diabetic rats was found to be significantly ($P < 0.001$) lower compared to DC group. These data suggest that the sensitivity to exogenously administered insulin was increased by both higher and lower doses of HSE and HSA which is comparable with GLB (10 mg/kg).

Estimation of Cardiac Serum Enzyme Markers

A significant increase in CK-MB and LDH levels was observed in DC rats [Table 1]. Diabetic rats treated with higher dose of HSE, HSA (400 mg/kg), and GLB showed a significant decrease ($P < 0.001$) in the level of LDH as compared to diabetic rats. Whereas, HSE (400 mg/kg), HSA (400 mg/kg), and GLB exhibited significant decrease ($P < 0.001$) in CK-MB level [Table 1].

Heart Weight

There was a significant increase in heart weight of DC rats compared to normal rats. Rats treated with HSE, HSA (400 mg/kg), and GLB (10 mg/kg) exhibited a decrease in the heart weight which was near to normal [Table 1].

Estimation of Renal Serum Enzyme Markers

A significant increase in the levels of serum urea and creatinine was observed in DC rats compared to NC, whereas a decrease in the level of albumin and total protein was observed [Table 2]. Rats treated with higher doses of HSE, HSA (400 mg/kg), and GLB showed a significant decrease in the level of serum urea and creatinine and showed significantly ($P < 0.001$) increased total protein and serum albumin as compared to diabetic rats [Table 2].

Estimation of Lipid Parameters

DC rats showed a significant increase ($P < 0.001$) in STG, STC, VLDL-c, and LDL-c levels compared to normal rats increased, whereas HDL-c was decreased. Dyslipidemia markers such as TC/HDL-c and LDL-c/HDL-c ratios were significantly elevated in the DC rats [Table 3].

In Vivo Antioxidant Parameters

Reduced GSH

DC rats showed marked fall in levels of GSH (25.3 ± 2.04 mg/100 g of protein) compared to normal rats (54.9 ± 2.5 mg/100 g of protein). Treatment of diabetic rats with HSE (400 mg/kg) and HSA (400 mg/kg) showed significant ($P < 0.001$; $P < 0.01$) improvement in GSH levels, respectively, whereas lower dose of extract exhibited significant ($P < 0.05$) improvement in GSH levels [Table 4].

Total thiols

DC rats exhibited a reduction in levels of total thiols (82.19 ± 1.5 nmoles/100 mg of protein) in comparison to normal rats (184.4 ± 3.4 nmoles/100 mg of protein). Treatment with HSE, HSA, and GLB showed a significant ($P < 0.001$) increased levels of total thiols [Table 4].

Lipid peroxidation

TBARS levels of NC rats were 0.77 ± 0.03 mM/100 g of liver tissue, and diabetic rats showed an increase in TBARS levels (1.91 ± 0.08 mM/100 g of tissue). Treatment of diabetic rats with HSE, HSA, and GLB significantly ($P < 0.001$) reduced the increase in TBARS levels induced by STZ [Table 4].

Catalase

Diabetic rats showed marked fall in levels of catalase (43.79 ± 2.33 U/mg of protein) in comparison to normal rats (86.6 ± 2.59 U/mg of protein). Diabetic rats treated with HSE, HSA, and GLB exhibited significantly ($P < 0.001$) increased levels of catalase [Table 4].

Histopathological studies

Histopathological study of cardiac tissue of DC rats showed various degrees of localized lesions along with hemorrhage and degeneration of myofibers with confluent retrogressive

Table 1: Effect of *H. sabdariffa* L. on cardiac biomarkers in diabetic rats

Treatment	CK-MB	LDH	Heart weight (g)
NC	421.5±11.17	259.9±23.48	0.58±0.086
DC	556.4±10.31	984.2±32.26	0.70±0.0194
GLB (10 mg/Kg)	454.3±9.97***	551.8±25.08***	0.61±0.021
HSE (200 mg/Kg)	501.3±17.42*	827.9±25.87*	0.65±0.010
HSE (400 mg/Kg)	452.3±3.972***	775.1±15.50***	0.62±0.007
HSA (200 mg/Kg)	513.1±10.58	965.5±57.76	0.64±0.016
HSA (400 mg/Kg)	465.5±12.63***	789.8±24.69**	0.60±0.008

Each value represents mean±SEM n=5 *P<0.05,**P<0.01,***P<0.001 compared to DC. NC: Normal control, DC: Diabetic control, GLB: Glibenclamide, HSE: *Hibiscus sabdariffa* ethanolic, HSA: *Hibiscus sabdariffa* aqueous, CK-MB: Creatinine Kinase-MB, LDH: Lactate dehydrogenase, H. sabdariffa: *Hibiscus sabdariffa*, SEM: Standard error of the mean

lesions in heart, congestion of vascular bundles, interstitial edema, inflammation of myocardial cells, and intravascular hemolyse. Whereas, rats treated with HSE (200 and 400 mg kg), GLB (10 mg/kg), and HSA (400 mg/kg) showed mild interstitial edema, moderate loss of myofibrils, mild intravascular hemolysis, and decreased degree of localized lesions in the heart tissue [Figure 3a].

Histopathological study of kidney tissue of DC rats showed various degrees of localized lesions in many regions, tubular congestion, tubular inflammation, cytoplasmic vacuoles, proximal convoluted tubule hemolysis, loss of brush border, glomerular congestion, glomerular necrosis, and interstitial swelling. Whereas, rats treated with HSE, HSA (200 and 400 mg/kg), and GLB (10 mg/kg) exhibited significant protection against pathological changes such as tubular inflammation, loss of tubular congestion, and interstitial swelling [Figure 3b].

DISCUSSION

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs,

Table 2: Effect of HSE and HSA extracts on renal profile in STZ-induced diabetic rats

Treatment	Serum urea (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Kidney weight
NC	64.6±2.9	0.38±0.04	9.2±0.39	7.05±0.23	1.25±0.009
DC	162.6±4.8	0.66±0.02	5.3±0.32	2.85±0.25	1.35±0.009
GLB (10 mg/Kg)	85.4±2.3***	0.39±0.03***	8.8±0.16***	6.08±0.34***	1.24±0.011
HSE (200 mg/Kg)	141.4±3.6**	0.52±0.02**	7.8±0.41***	4.38±0.3*	1.28±0.013
HSE (400 mg/Kg)	108.0±3.8***	0.41±0.03***	8.2±0.23***	5.25±0.25***	1.26±0.008
HSA (200 mg/Kg)	145.9±3.2*	0.53±0.02*	7.3±0.31**	4.25±0.26*	1.30±0.018
HSA (400 mg/Kg)	142.5±4.1**	0.49±0.01**	7.6±0.37***	4.68±0.46**	1.28±0.020

Each value represents mean±SEM n=5, *P<0.05, **P<0.01, ***P<0.001 compared to DC. NC: Normal control, DC: Diabetic control, GLB: Glibenclamide, HSE: *Hibiscus sabdariffa* ethanolic, HSA: *Hibiscus sabdariffa* aqueous, SEM: Standard error of the mean, STZ: Streptozotocin

Table 3: Effect of HSE and HSA on lipid profile in STZ-induced diabetic rats

Serum parameters	NC	DC	HSE 200 mg/kg	HSE 400 mg/kg	HSA 200 mg/kg	HSA 400 mg/kg	GLB 10 mg/kg
STG (mg/dl)	60.6±4.4	198.7±4.5	112.1±4.8***	169.3±4.5**	121.3±2.8***	173.5±5.1**	117.8±4.1***
STC (mg/dl)	63.9±3.8	128.4±4.3	69.9±3.1***	83.9±2.9***	78±3.2***	111.5±2.5*	81.8±3.2***
HDL-c (mg/dl)	33.9±1.8	17.02±1.1	31.1±0.5***	24.5±1.5**	32.1±1.6***	21.8±1.5	29.4±0.7***
VLDL-c (mg/dl)	12.1±0.8	39.7±0.9	22.4±0.9***	33.8±0.9**	24.2±0.5***	34.6±1.01**	23.5±0.8***
LDL-c (mg/dl)	17.8±4.8	71.6±4.2	16.5±3.4***	25.5±4.4***	21.5±2.6***	55.7±3.4	28.9±3.3***
TC/HDL-c ratio	1.9±0.1	7.5±0.2	2.2±0.1***	3.4±0.3***	2.4±0.1***	5.1±0.34***	2.7±0.1***
LDL-c/HDL-c ratio	0.54±0.1	4.2±0.1	0.52±0.1***	1.1±0.2***	0.66±0.07***	2.6±0.26***	0.98±0.13***

Each value represents mean±SEM n=5 *P<0.05, **P<0.01, ***P<0.001 compared to DC. NC: Normal control, DC: Diabetic control, HSE: *Hibiscus sabdariffa* ethanolic, HSA: *Hibiscus sabdariffa* aqueous, SEM: Standard error of the mean, STZ: Streptozotocin, STG: Serum triglyceride, STC: Serum total cholesterol, HDL-c: High-density lipoprotein-cholesterol, VLDL-c: Very low-density lipoprotein-cholesterol, LDL-c: low-density lipoprotein-cholesterol, GLB: Glibenclamide

Table 4: Effect of HSE and HSA on antioxidant enzymes in STZ-induced diabetic rats

Antioxidant Parameters	NC	DC	GLB (10 mg/kg)	HSE (200 mg/kg)	HSE (400 mg/kg)	HSA (200 mg/kg)	HSA (400 mg/kg)
Catalase	86.6±2.59	43.79±2.33	69.05±1.93***	66.61±2.01***	73.30±1.9***	64.06±1.68***	66.47±1.67***
TBARS	0.77±0.03	1.91±0.08	1.19±0.06***	1.30±0.05***	1.44±0.02***	1.47±0.04***	1.50±0.04***
GSH	54.9±2.5	25.3±2.04	42.4±1.9***	33.01±1.6*	37.3±1.1***	32.6±1.1*	34.15±1.5**
Total thiol	184.4±3.4	82.19±1.5	171.0±3.5***	128.7±3.7***	158.7±4.17***	102.4±4.1	108.1±5.5**

Each value represents mean±SEM n=5 *P<0.05, **P<0.01, ***P<0.001 compared to DC. TBARS: Thiobarbituric acid reactive substances, NC: Normal control, DC: Diabetic control, HSE: *Hibiscus sabdariffa* ethanolic, HSA: *Hibiscus sabdariffa* aqueous, SEM: Standard error of the mean, GLB: Glibenclamide, GSH: Glutathione

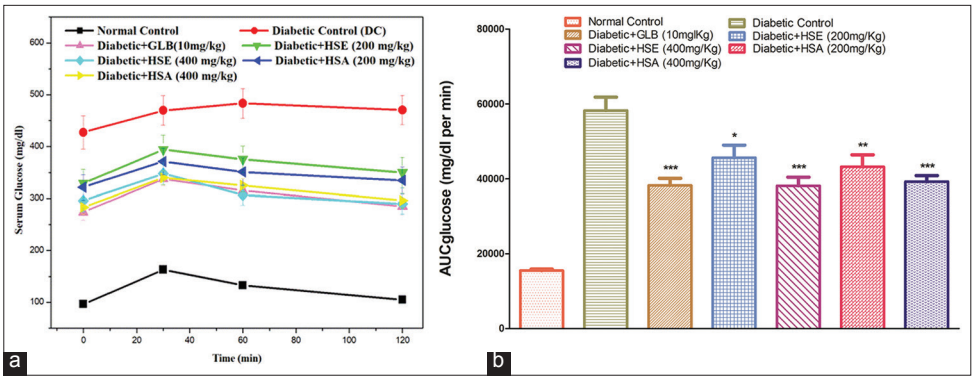

Figure 1a: Effect of *Hibiscus sabdariffa* ethanolic (HSE) and *Hibiscus sabdariffa* aqueous (HSA) on SG levels after i.p administration of glucose alone (2 g/kg body weight) or in combination with HSE, HSA, or glibenclamide, **1b:** Area under curve for glucose ($AUC_{glucose}$) values for 0–120 min post glucose load. Data represent the mean ± standard error of the mean, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with diabetic control rats

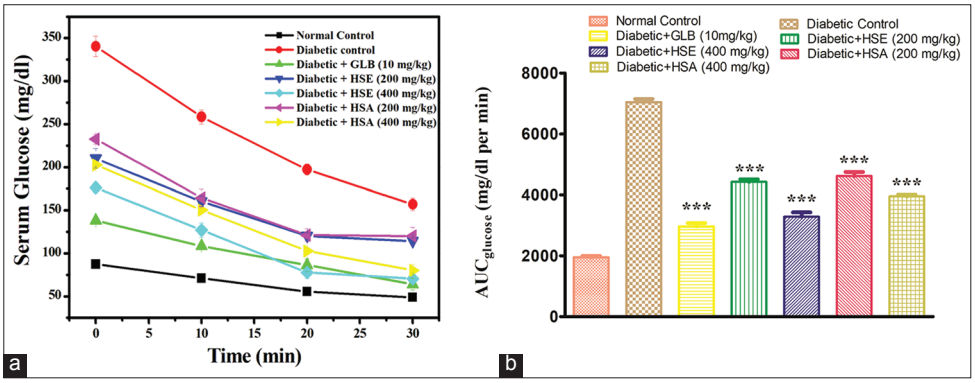

Figure 2a: Serum glucose levels were measured before and after i.p. administration of insulin alone (2 U/kg body weight) or in combination with *Hibiscus sabdariffa* ethanolic, *Hibiscus sabdariffa* aqueous, or glibenclamide, **2b:** Area under curve for glucose ($AUC_{glucose}$) values for 0–30 min post-insulin injection. Data represent the mean ± standard error of the mean, $n = 5$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with diabetic rats

especially the eyes, kidneys, nerves, heart, and blood vessels. Long-term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, and cardiovascular complications with an increased risk of coronary heart disease and cardiomyopathy.[18]

In the present study, STZ-induced diabetic rats showed marked hyperglycemia and impaired glucose tolerance compared to normal rats. Impaired glucose tolerance in diabetic rats was attenuated by both ethanolic and aqueous extracts. HSE (400 mg/kg) and HSA (400 mg/kg) significantly reduced serum glucose level in STZ-induced diabetic rats.

In Type-1 diabetes, cardiac lipid accumulation commonly occurs and has been suggested to play a direct causal role in the development of cardiomyopathy and heart failure in a process termed as cardiac lipotoxicity.[5]

Autoimmune β-cell destruction leads to insulin insufficiency and reduced myocardial glucose uptake, and this results in decreased glucose oxidation. As a result, the heart becomes heavily reliant on FAs as a source of energy.[19] These metabolic changes promote reactive oxygen species production, mitochondrial dysfunction, and altered calcium handling, contributing to the development of cardiomyopathy in Type-1 diabetes.[20,21] High levels of STC and more importantly LDL-c are a major coronary risk factor, whereas increase in HDL-c is associated with a decrease in coronary risk.[22]

In this study, DC rats exhibited significantly elevated STC and STG, LDL-c, VLDL-c levels, and reduction in HDL-c levels as compared to NC rats. Chronic administration of both doses of HSE and HSA significantly reduced STC, STG, VLDL-c, and LDL-c levels, whereas HDL-c level was significantly raised. Therefore, normalization of lipids in diabetic rats treated with *H. sabdariffa* L. extract may be attributed to its stimulatory effect on insulin secretion from pancreatic β-cells as well as activation of lipoprotein lipase, insulin-mediated lipolytic activity or inhibition of lipogenic enzymes, and hormone-sensitive lipase as previously suggested for antidiabetic plants exhibiting antihyperlipidemic activity.[23] It has been reported that lipid lowering is an important factor in preventing diabetic cardiotoxicity.[1] It is a well known fact that STZ-induced diabetes leads to cardiotoxicity which is manifested by an increase in serum CK-MB and LDH levels as was observed in untreated diabetic rats. Diabetic rats

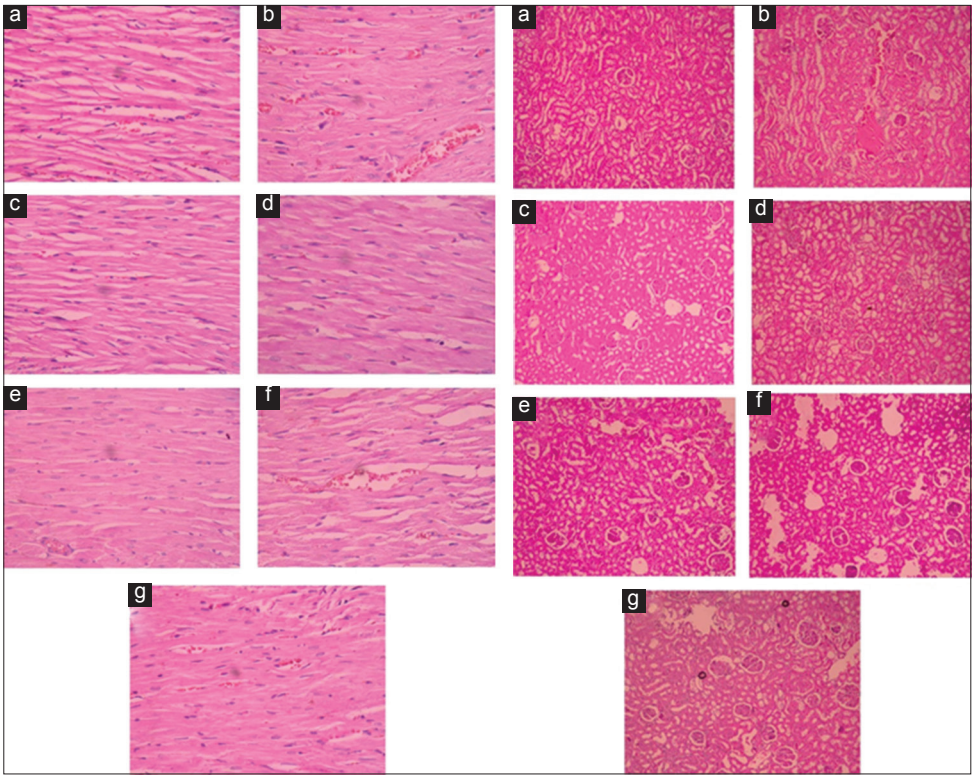

Figure 3a: Histopathology of heart: (a) Normal control, (b) diabetic control, (c) diabetic+glibenclamide (10 mg/Kg), (d) diabetic+*Hibiscus sabdariffa* ethanolic (HSE) (200 mg/Kg), (e) diabetic+HSE (400 mg/Kg), (f) Diabetic+*Hibiscus sabdariffa* aqueous (HSA) (200 mg/Kg), (g) diabetic+HSA (400 mg/Kg), **3b:** Histopathology of kidney: (a) Normal control, (b) diabetic control, (c) diabetic+glibenclamide (10 mg/kg), (d) diabetic+*Hibiscus sabdariffa* ethanolic (HSE) (200 mg/Kg), (e) diabetic+HSE (400 mg/Kg), (f) diabetic+*Hibiscus sabdariffa* aqueous (HSA) (200 mg/Kg), (g) diabetic+HSA (400 mg/Kg)

treated with *H. sabdariffa* L. extract showed a significant decrease in the levels of LDH and CK-MB which was comparable with GLB.

Moreover, the present study revealed a significant decrease in serum total protein and albumin levels in the untreated diabetic rats, whereas administration of *H. sabdariffa* L. extracts significantly increased the levels of serum total protein and albumin. The serum total protein and albumin levels serve as an indicator of liver function. In uncontrolled diabetic individuals, blood urea and creatinine concentrations are increased and this increase could be a result of impaired renal function due to increased blood glucose level. Through this study, it has been revealed that blood urea and creatinine levels in the blood increased in untreated diabetic rats while significantly decreased after the administration of the extracts. Thus, the extract has the potential to improve the renal function. Previously, it has been reported that diabetic individuals had lower serum albumin concentrations as well as higher serum uric acid and urea levels than non-diabetic individuals.[24] Thus, it can be said that reduction in blood glucose levels probably leads to the reduction in serum urea and creatinine levels as was evident in our study.

Patients with DM are at a higher risk of developing cardiovascular diseases, and the elevated serum uric acid levels further exacerbate these complications, especially when accompanied with high triglyceride and low HDL-c. Moreover, high levels of serum uric acid, urea, and creatinine act as an indicator of kidney problems. Thus, our data suggest that *H. sabdariffa* L. extract might play an important role in reducing the risk of kidney problems as well as cardiovascular diseases through lowering serum urea, uric acid, creatinine as well as improving lipid profile.

It is a well-established fact that free radicals are one of the main contributors to the development of DM as well as its complications.[25,26] Lipid peroxidation is one of the most upshots of free radical-mediated tissue injury and is an indicator for oxidative damage. Treatment of diabetic rats with different doses of extracts showed a reduction in lipid peroxidation and enhanced levels of antioxidant enzymes such as catalase, GSH, and total thiols. The potent antioxidant potential of the extract can be attributed to the presence of high levels of antioxidant compounds such as flavonoids, anthocyanins, quercetin, sabdaretin, β-sitosterol, as well as rutin found in the title plant.[27,28] Furthermore, collectively, these findings indicate that the lipid-lowering, antioxidant, and hypoglycemic effects of extracts may be an important contributor to its cardioprotective and nephroprotective potential.

CONCLUSION

We conclude that *H. sabdariffa* L. on oral administration showed potent antidiabetic, cardioprotective,

nephroprotective, hypolipidemic, and antioxidant activity. These findings clearly support the traditional use of this medicinal plant in the treatment of DM and late complications associated with it and shed more light on the efficacy of this plant. Thus, *H. sabdariffa* L. is ideally suited to be used in the treatment of DM and prevent or delay the onset of its complications in humans.

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