Pharmacological Study of the Effect of Cold and Hot Aqueous Extracts of *Hibiscus sabdariffa* on Vascular Activity of Diabetic Albino Rats

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**Authors’ contributions**

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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**Original Research Article**

**ABSTRACT**

The oxidative stress has been indicated to play an important role in the development and progression of diabetic complications such as decreased vascular reactivity, hypertension…etc. Previous studies show that *Hibiscus sabdariffa* possesses a hypoglycemic effect and useful in some diseases including hypertension. Our study aimed to evaluate the possible protective effect of *Hibiscus sabdariffa* aqueous extract on diabetic vascular complications and blood pressure and compare between cold and hot aqueous extracts by studying their effects on the reactivity of the isolated rat aorta to norepinephrine and acetylcholine in the diabetic albino rats. The results show that the rats systolic blood pressure and, the contractile response of the isolated rat aortae to norepinephrine are increased significantly and the relaxant response of the rat aortae to acetylcholine is decreased significantly as compared with those obtained from the nondiabetic rats. Treatment with both cold and hot *hibiscus* as well as glibenclamide decreased significantly the systolic blood pressure and the contractile response of the isolated aortae to norepinephrine as well as increased significantly the relaxant response of the isolated aortae to acetylcholine. In conclusion, *Hibiscus sabdariffa* aqueous extracts, either cold or hot can protect against the diabetes.
mellitus-induced vascular complications by decreasing the elevated blood pressure and the increased norepinephrine-induced contractile effect as well as by improving the decreased relaxant responses to acetylcholine in the Streptozotocin-induced diabetic male albino rats.

Keywords: *Hibiscus sabdariffa*; vascular activity; streptozotocin; diabetic rats; blood pressure.

1. **INTRODUCTION**

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion and/or insulin action. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels [1]. DM is associated with an increased risk of micro- and macro vascular complications, causing considerable morbidity and mortality [2]. Blood glucose control can reduce the risk of these vascular complications but does not prevent them altogether. Endothelial dysfunction, a non-traditional cardiovascular risk marker, has been strongly associated with the reduced vascular reactivity occurring in patients with type 2 DM, thereby playing a major role in the development of complications of the micro- and macro-circulation (Vinik et al. 2003). The protective effect of a polyphone extract of *Hibiscus sabdariffa* was studied in a type II diabetic rat model; the extract demonstrated anti-insulin resistance properties as it reduced hyperglycemia and hyperinsulinemia. It decreased serum triacylglycerol, cholesterol and the ratio of low density lipoprotein/high-density protein (LDL/HDL), as well as reduced the plasma advanced glycation end products (AGE) formation and lipid per oxidation [3]. Extract of the calyces of *Hibiscus sabdariffa* indeed reduce both the systolic and diastolic pressures, lowering heart rate and working as a vasodilator [4].

1.1 **Aim of the Work**

The present work aimed for evaluation of the possible protective effect of *Hibiscus sabdariffa* aqueous extract on the diabetes mellitus-induced vascular complications and to show whether heating can affect its effect by studying its effect on the blood pressure and the contractile and relaxant responses of the isolated rat aorta to norepinephrine and acetylcholine respectively as compared with the standard antidiabetic drug glibenclamide in the Streptozotocin-induced diabetic male albino rats.

2. **MATERIALS AND METHODS**

2.1 **Experimental Animals**

Adult male albino rats were chosen as an animal model for this study. Rats were brought from animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, and were maintained on a balanced diet with water supply freely in clean containers. They were kept for two weeks to adapt to the laboratory conditions before the start of the experiment. Fifty age-matched male albino rats with initial body weights ranging from 150 to 200g were used. The rats were divided into five groups (10 rats each).

- **Group I**: Normal untreated non diabetic rats (control).
- **Group II**: Diabetic untreated rats.
- **Group III**: Diabetic rats treated with glibenclamide (0.6 mg/kg body wt daily) [5].
- **Group IV**: Diabetic rats treated with hot aqueous extract of *Hibiscus sabdariffa* (150 mg/kg/day) [6].
- **Group V**: Diabetic rats treated with cold aqueous extract of *Hibiscus sabdariffa* (150 mg/kg/day) [6].

2.2 **Drugs and Chemicals**

- Acetylcholine (ACH) (Fluka, Switzerland).
- Glibenclamide (Sigma Chemical Company – Aldrich, USA).
- Norepinephrine (NE) (Sigma Chemical Company – Aldrich, USA).
- Streptozotocin (STZ) (MP biomedicals, LLC- France).
- *Hibiscus sabdariffa* calyces (Faculty of Agriculture– Al-Azhar University (Assiut).

2.3 **Procedures**

2.3.1 **Induction of diabetes mellitus**

The animals were injected by a single intraperitoneal injection of streptozotocin, 55 mg/kg body weight [7]. Diabetes was confirmed through detecting blood glucose concentrations
by glucose oxidase method using glucometer with glucose test strip (One Touch Basic). Animals having blood glucose concentrations over 250 mg/dl were considered diabetic and selected for use [8].

2.3.2 Preparations of hibiscus extracts

Cold aqueous extraction: 250 g of air dried powder of calyces of Hibiscus sabdariffa was weighed and soaked separately in 500 mL cold water in a conical flask stopper with rubber cork and left undisturbed for 24 hours and then filtered off using sterile filter paper (What Man No: 1) into a sterile conical flask and subjected to water bath evaporation, where the aqueous solvent was evaporated at its boiling temperature 100°C. The extract was filtered with the help of muslin cloth and was subjected to centrifugation at 5000Xg for 5 minutes and the supernatant was obtained and stored at 4°C for further use [9].

Hot aqueous extract: 250 g of air dried powder of calyces of Hibiscus sabdariffa was weighed and soaked separately in 500 mL of hot water which was then boiled for 30 minutes and kept in a conical flask for 24 hours undisturbed and then filtered off using sterile filter paper (What Man No: 1) into a sterile conical flask and subjected to water bath evaporation, where the aqueous solvent was evaporated at its boiling temperature 100°C. The extract was filtered with the help of muslin cloth and was subjected to centrifugation at 5000Xg for 5 minutes and the supernatant was obtained and stored at 4°C for further use (Vimaiin, 2010).

2.3.3 Drug administration

- Glibenclamide was given orally in a single dose of 0.6 mg/kg body wt /day orally for 8 weeks. Glibenclamide was dissolved in distilled water and administered via gastric tube [5].
- Hot aqueous extract of Hibiscus sabdariffa was given orally in a dose of 150 mg/kg/day [6] for 8 weeks. Cold aqueous extract of Hibiscus sabdariffa was given orally in a dose of 150 mg/kg/day [6] for 8 weeks.

2.3.4 Blood pressure measurement procedure

Rats were trained daily for the measurement of blood pressure (BP) by indirect rat tail cuff method (Harvard apparatus 52-0338). The system is an electronic version of the traditional sphygmomanometer cuff method used to determine blood pressure by non-invasive technique. Rats were placed (9 AM) in their maintenance cages for 2 hours. Afterward, systolic BP was measured in untrained animals. Once the rats were considered to be trained and not susceptible to stress from the tail-cuff procedure, systolic BP measurements were performed and at eighth week, systolic BP was measured on 2 consecutive days at the same time of the day (11 AM).

2.3.5 Collection of blood samples

The animals were anaesthetized with ether by placing the rats in an anesthetic box filled with ether vapor which is maintained by periodically applying liquid ether to a cotton wool on the base of the box. When surgical stage of anesthesia is reached (judged by loss of withdrawal reflexes), the animal was removed and placed on a table and blood was collected from the retro-orbital plexus using capillary tube (0.75-1.0 mm internal diameter) inserted in the medial canthus medial to the eye globe.

After eight weeks, blood was collected from carotid artery after sacrificing the animals. The blood was collected into a dry clean graduated glass centrifuge tube. It was rapidly set to centrifuge at 5000 r.p.m for 10 minutes. About half of the supernatant serum was sucked out into a clean dry glass serology tube using Pasteur pipette.

2.3.6 Preparation of the Isolated rat aortic rings

On the day of experiment, animals were killed by a blow on the head and cutting the throat. Abdominal and thoracic walls were opened and the thoracic aortae dissected and cut and then placed in a dish containing Kreb,s-Henseleit solution composed of the following reagents in g/L: (NaCl: 118.4, KCl: 4.69, kH2PO4: 1.17, MgSO4: 1.18, CaCl: 2.52, D-glucose: 11.10 and NaHCO3: 25) aerated with carbogen (95% oxygen and 5% carbon dioxide). The isolated aortae put under the dissecting microscope, cleaned from the surrounding attached tissues and cut into small rings (about 4 mm length). The aortic rings were suspended in an isolated organ bath (30 ml capacity) containing Kreb,s-Henseleit solution maintained at 37°C and aerated with carbogen. Aortic rings were subjected to an initial tension 1g, and were kept in the organ bath (for equilibration) for approximately 90 minutes, the physiological solution is renewed every 15 minutes. Response of the aortic rings to drugs were measured isometrically with a Grass FT O3
force-displacement transducer, and recorded on a polygraph. The viability and stability of the tissue was documented by a two equal contractile responses to the same concentration of norepinephrine ($10^{-7}$). Norepinephrine contained 1% HCL to prevent auto-oxidation. Tissues were then washed several times and allowed to relax to the base line level.

- Cumulative dose–response curves to norepinephrine were performed on each ring. Molar solutions of norepinephrine ($1\times10^{-8}$ to $3\times10^{-3}$) were used. During performing the dose-response curves of norepinephrine, each dose was added after reaching the plateau of the response of the previous dose. Each ring was serially washed after obtaining the maximum response to norepinephrine and allowed to relax to the base line level.

- For relaxation study; aortic rings were precontracted by norepinephrine ($10^{-6}$). This concentration produced a submaximal response. When the contractile responses of norepinephrine reached the plateau level, cumulative concentration response curves of acetylcholine molar solutions ($1\times10^{-8}$ to $3\times10^{-3}$) were performed and each ring was serially washed after obtaining the maximum response to norepinephrine, and allowed to relax to the base line level.

2.3.7 Blood glucose measurements

The blood glucose level was determined by enzymatic colorimetric method [10], by using diamond diagnostic kits.

2.4 Statistical Analysis

Statistical analysis was done using the computer program (SPSS). The quantitative data were presented as mean ± Standard Error (S.E). Statistical analysis of the difference between groups was performed, using one-way analysis of variance (ANOVA) followed by Tukey-Kramer test for differences between means. A value of $P<0.05$ was used as the limit for statistical significance.

3. RESULTS

3.1 Effects of Hot and Cold Aqueous Extract of *Hibiscus sabdariffa* and Glibenclamide on the Contractile Response of the Diabetic Rat Isolated Aortae to Norepinephrine

The results show that the contractile response of the aorta is increased significantly ($P<0.01$) in the diabetic untreated rats in comparison with the normal rats. Treatment with hot and cold hibiscus aqueous extracts decreased significantly ($p<0.01$) the contractile response of the isolated aorta of the diabetic rats in comparison with the diabetic untreated rats, but still there is significant increase in comparison with the normal rats. The results show that there was no significant difference between hot and cold aqueous extracts of *Hibiscus sabdariffa* in their effects on the contractile response of the diabetic rats isolated aorta. The contractile response of the isolated aorta of the diabetic rats was also decreased significantly ($P<0.01$) in the diabetic rats treated with glibenclamide, in comparison with the diabetic untreated rats. As shown in Fig. 1.

3.2 Effects of Hot and Cold Aqueous Extract of *Hibiscus sabdariffa* and Glibenclamide on the Relaxant Response of the Diabetic Rat Isolated Aortae to Acetyl Choline

Cumulative concentration-response curves elicited by acetylcholine on norepinephrine precontracted aortic ring preparations obtained from the normal rats, the diabetic untreated rats and the diabetic rats treated with glibenclamide (0.6 mg/kg) for 8 weeks. The results show that the relaxant response of the aorta was decreased significantly ($P<0.01$) in the diabetic untreated rats in comparison with the normal rats. The results show that the relaxant responses of the isolated aorta to acetylcholine are increased significantly ($P<0.01$) in the diabetic rats treated with glibenclamide and both hot and cold aqueous extracts of *Hibiscus sabdariffa* as compared with those of the untreated diabetic rats with no significant difference between effects of the hot and cold aqueous extract of *Hibiscus sabdariffa* on the relaxant response of the isolated diabetic rats aortae as shown in Fig. 2.
Fig. 1. Effects of hot and cold aqueous extract of *Hibiscus sabdariffa* and glibenclamide on the contractile response of the diabetic rat’s isolated aorta to norepinephrine.

Fig. 2. Effects of hot and cold aqueous extract of *Hibiscus Sabdariffa* and glibenclamide on the relaxant response of the diabetic rat’s isolated aorta to acetyl choline.
3.3 Effects of Hot & Cold Aqueous Extract of *Hibiscus sabdariffa* as well as Glibenclamide on Systolic Blood Pressure of the Diabetic Rats

The systolic blood pressure of the diabetic rats is increased significantly (p<0.01) as compared with the normal rats. Treatment with cold and hot aqueous extracts of hibiscus as well as glibenclamide decreased significantly (p<0.01) the systolic blood pressure of the diabetic rats with no significant difference between the effect of hot and cold aqueous extracts of *Hibiscus sabdariffa* or glibenclamide on the systolic blood pressures of the diabetic rats as shown in Fig. 3.

4. DISCUSSION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion and/or insulin action. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels [1].

The protective effect of a polyphone extract of *Hibiscus sabdariffa* was studied in a type II diabetic rat model; the extract demonstrated anti-insulin resistance properties as it reduced hyperglycemia and hyperinsulinemia [3] and reduce both the systolic and diastolic blood pressures, lowering heart rate and working as a vasodilator [4]. Since the *Hibiscus sabdariffa* is one of the known medicinal herbs and results of the previous studies on *Hibiscus sabdariffa* are very interesting, encouraging and shows many benefits, the present study aims to evaluate the effect of *Hibiscus sabdariffa* aqueous extracts on DM-induced changes in vascular activity and blood pressure in streptozotocin induced diabetes mellitus in male albino rats.

The results of the present study showed that the contractile response of the isolated rat aorta to noradrenaline is increased significantly in the diabetic untreated rats in comparison with the normal rats, in disagreement with results of Dhein et al. [11], who showed that smooth muscle contractile function not altered by diabetes mellitus, as evident from the nearly unchanged KCl-induced contractions.

![Fig. 3. Effects of hot & aqueous extract of *Hibiscus sabdariffa* and glibenclamide on the systolic blood pressure of the diabetic rats](image)
The results of the present study showed that the contractile responses of the isolated aortae of the diabetic rats treated with hot and cold hibiscus extracts were decreased significantly in comparison with the diabetic untreated rats. The results of this study are in agreement with the results obtained by Yoswaris et al. [12] who reported that daily treatment with hibiscus extract for 6 weeks could restore the vascular responses to vasoactive agents, both vasodilators and vasoconstrictors, in STZ-induced chronic diabetic rats.

The results of this study showed that the relaxant responses of the isolated rat aorta to acetylcholine were decreased significantly in the diabetic untreated rats in comparison with the normal rats and the relaxant responses of the isolated aortae of the diabetic rats treated with hot and cold aqueous extract of Hibiscus sabdariffa were increased significantly in comparison with the diabetic untreated rats. The results of the present study are in agreement with the results obtained by Mamadou et al., [13] who showed that in adrenaline-precontracted isolated aortic rings, the crude hydro-alcoholic extract of Hibiscus sabdariffa induced a vasorelaxation. This relaxation is dose-dependent. Also, Ajay et al. [14], reported that a concentration of 1 mg/ml of hibiscus extract, produce a maximal relaxation on adrenaline-precontracted isolated aortic rings. Anunkaorn et al., [15], reported that the daily supplementation with Hibiscus sabdariffa extract and gallic acid decreased the blood glucose, normalized blood pressure, and restored the vascular response to vasoactive agents both the vasodilator and vasoconstrictor in STZ-induced chronic diabetic rats.

The result of this study showed that systolic blood pressure (SBP) was increased significantly in the diabetic untreated rats in comparison with the normal rats and the systolic blood pressure of the diabetic rats treated with hot and cold hibiscus extracts was decreased significantly in comparison with the diabetic untreated rats. The results of the present study are in agreement with the results obtained by Mozaffari-Khosravi et al., 2009, who reported that the SBP in Hs consumers is decreased. Ojeda et al. [16] reported that the anti-hypertensive activity might be through inhibition of angiotensin-converting enzymes (ACE). Mojiminiyi et al., [17] found that the anti-hypertensive activity might be through diuretic effect. Inuwa et al. [4] showed that the anti-hypertensive activity might be through reduction in the diffusion distance between capillaries and myocytes, as well as new vessel formation. Extract of the calyces of Hibiscus sabdariffa indeed reduce both the systolic and diastolic pressures, lowering heart rate and working as a vasodilator [4]. Several studies both in vitro [18] and in vivo [19] have shown that extracts of Hs have a potent antioxidant effect. The antioxidant activity of the extract is due to its strong scavenging effect on reactive oxygen and free radicals [18].

The results of the present study showed that treatment of the diabetic rats with the antidiabetic drug glibenclamide decreased significantly the contractile response of the rat aorta to norepinephrine and increased significantly the relaxant effect of the acetylcholine in the diabetic rats and also decreased significantly the systolic blood pressure. Elif and Mukkader 2005 showed that exposure of endothelium-intact rat aortic rings to glibenclamide raised the tissue content of cyclic guinesene monophosphate (cGMP) by approximately two fold and this raise was abolished by presence of the nitric oxide synthase inhibitor. The present results disagree Chaim et al. 2004 who showed that glibenclamide worsened blood pressure control possibly by elevation of insulin levels and activation of the sympathetic system.

5. CONCLUSION

In conclusion, treatment with both of the hot and the cold aqueous extracts of the Hibiscus sabdariffa aqueous extracts, decreased the contractile responses of the isolated aorta to norepinephrine and increased the relaxant responses of the isolated aorta to acetylcholine as well as decrease the systolic blood pressure in the streptozotocin induced diabetic male albino rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
REFERENCES


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