

Pharmacological study of the effect of cold and hot aqueous extract of *Hibiscus sabdariffa* on vascular activity of diabetic albino rats

Abstract

Oxygen derived free radicals are produced and 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 its effect on isolated rat aorta to norepinephrine and acetylcholine in the Streptozotocin- induced diabetic male albino rats. The results show that the rat's systolic blood pressure and, the contractile response of the isolated rat aorta to norepinephrine are increased significantly and the relaxant response of the rat aorta to acetylcholine is decreased significantly as compared with 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 aorta to norepinephrine as well as increased significantly the relaxant response of the isolated aorta to acetylcholine. **In conclusion**, hibiscus sabdariffa aqueous extracts, either cold or hot as well as glibenclamide, can protect against the diabetes mellitus- induced vascular complications by decreasing the elevated blood pressure and the increased norepinephrine- induced contractile effect and by improving the decreased relaxant responses to acetylcholine in the Streptozotocin- induced diabetic male albino rats.

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 (**Craig et al., 2009**). DM is associated with an increased risk of micro- and macro vascular complications, causing considerable morbidity and mortality (**Cade, 2008**). 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 (**Peng et al., 2011**). Extract of the calyces of hibiscus sabdariffa indeed reduce both the systolic and diastolic pressures, lowering heart rate and working as a vasodilator (**Inuwa et al., 2012**).

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

Materials and methods

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) (**Arulselvan and Subramanian, 2008**).

Group IV: Diabetic rats treated with hot aqueous extract of hibiscus sabdariffa (150 mg/kg/day) (**Saravanan et al.,2001**).

Group V: Diabetic rats treated with cold aqueous extract of hibiscus sabdariffa (150 mg/kg/day) (**Saravanan et al.,2001**).

Drugs and chemicals:

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

Procedures:**1-Induction of diabetes mellitus:**

The animals were injected by a single intraperitoneal injection of streptozotocin 55 mg / kg body weight (**Mifsud et al., 2002**). Diabetes was confirmed through detecting blood glucose concentration by glucose oxidase method using glucometer with glucose test strip (One Touch Basic). Animals having blood glucose concentration over 250 mg/dl were considered diabetic and selected for use (Olanlokun 2008).

2- Preparations of hibiscus extract:

A-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 (**Farombi ,2003**).

B-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 in 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 a muslin cloth and was subjected to centrifugation 5000Xg for 5 minutes and the supernatant was stored at 4°C for further use (**Vimaiin, 2010**).

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 (Arulselvan and Subramanian, 2008).

-Hot aqueous extract of hibiscus sabdariffa was given orally in a dose of (150 mg/kg/day) (Saravanan et al.,2011)for 8 weeks . Cold aqueous extract of hibiscus sabdariffa was given orally in a dose of (150 mg/kg/day) (Saravanan et al., 2011) for 8 weeks.

4- Blood Pressure Measurement Procedure:

Rats were trained daily for the measurement of blood pressure (B P) 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).

5- Collection of blood samples:

A-The animal is anaesthetized with ether by placing the rat 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.

B-After eight weeks, blood is collected from carotid artery after sacrificing of 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.

6- Preparation of the Isolated rat aortic rings:

On the day of experiment, animals are killed by a blow on the head and cutting the throat. Abdominal and thoracic walls were opened. The thoracic aorta was dissected and cut, placed in dish containing Krebs-Henseleit solution of the following composition (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% carbogen dioxide), put under the dissecting microscope, cleaned from the surrounding attached tissues and cut into small rings (about 4mm length). The aortic ring was suspended in an isolated organ bath (30 ml capacity) containing Krebs-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. Tissue were then washed several times and allowed to relax to base line level.

-Cumulative dose-response curves to norepinephrine are performed on each ring. Molar solutions of norepinephrine (1×10^{-8} to 3×10^{-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 baseline and equilibrated.

-For relaxation study; aortic ring is precontracted by norepinephrine (10^{-6}) this concentration produced a submaximal response. When the response reached its plateau, cumulative concentration response curves of acetylcholine molar solutions (1×10^{-8} to 3×10^{-3}) is done and each ring was serially washed after obtaining the maximum response to reach the baseline and equilibrated.. During performing the dose-response curves of acetylcholine each dose was added after reaching the plateau of the response of the previous dose, to evaluate endothelium-dependent versus endothelium-independent vasodilation.

7-Blood glucose measurements: The blood glucose level is determined by enzymatic colorimetric method (Trinder and Ann 1969). Using diamond diagnostics kits.

Statistical analysis: Statistical analysis was done using the computer program (SPSS). The quantitative data were presented in the form of mean $\pm$ standard error (S.E). Statistical analysis of the difference between groups was performed by 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.

Results

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

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 is 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 is also decreased significantly ($P < 0.01$) in the diabetic rats treated with the glibenclamide, in comparison with the diabetic untreated rats. figure (1).

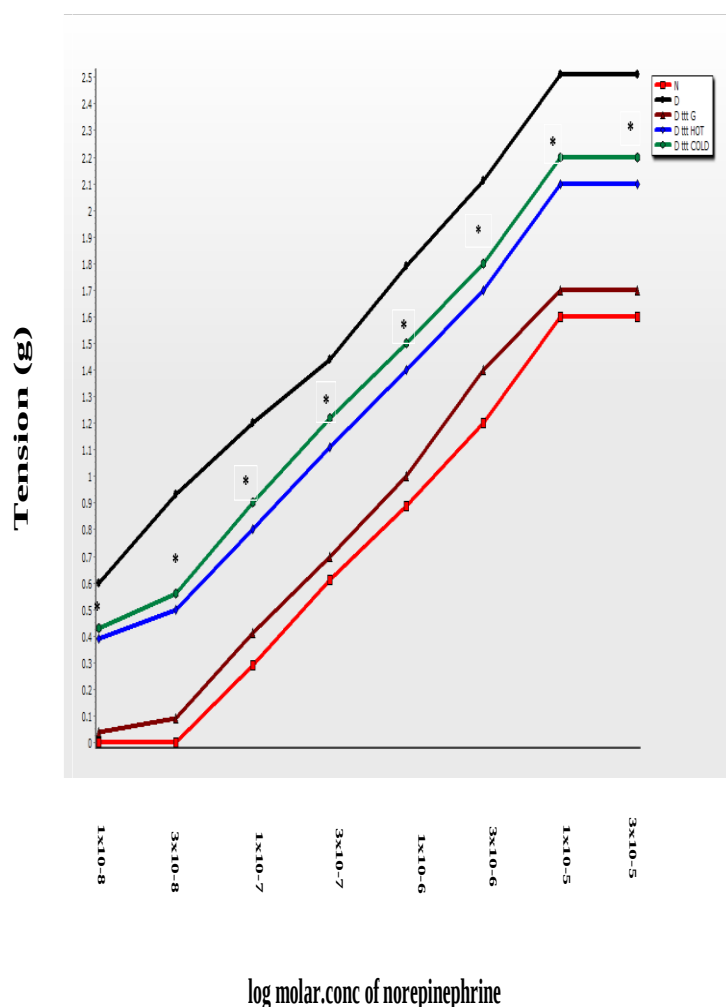


Figure-1:

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

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

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 (0.6mg/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 figure (2).

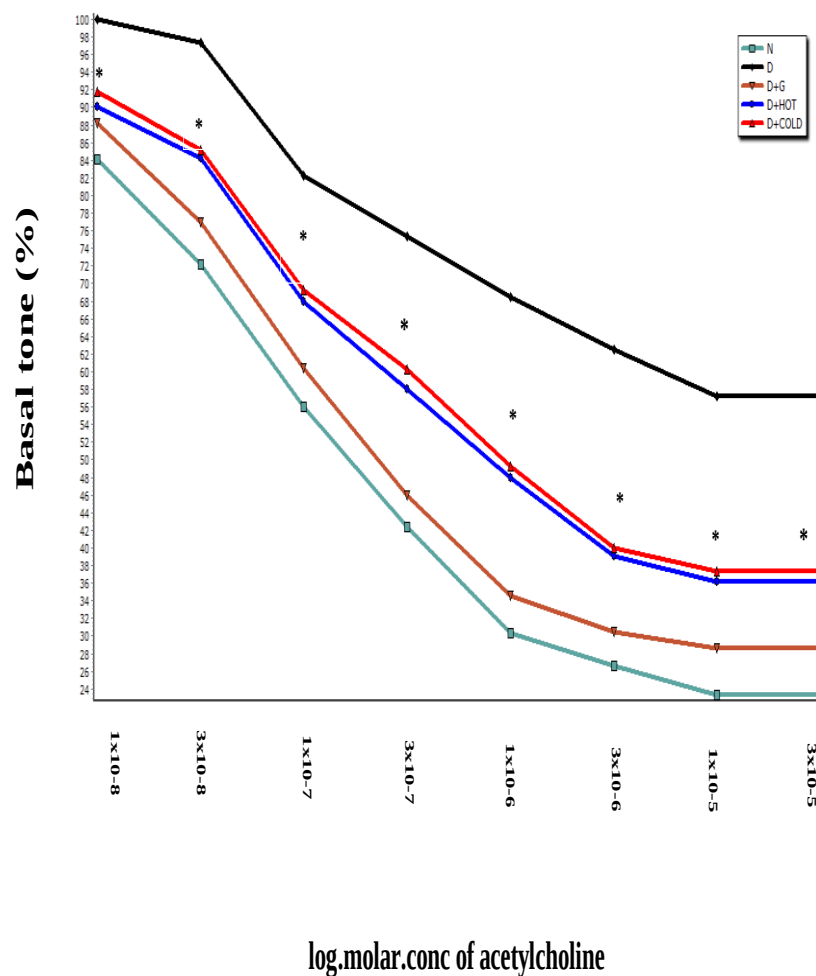


Figure-2:

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.

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 figure (3).

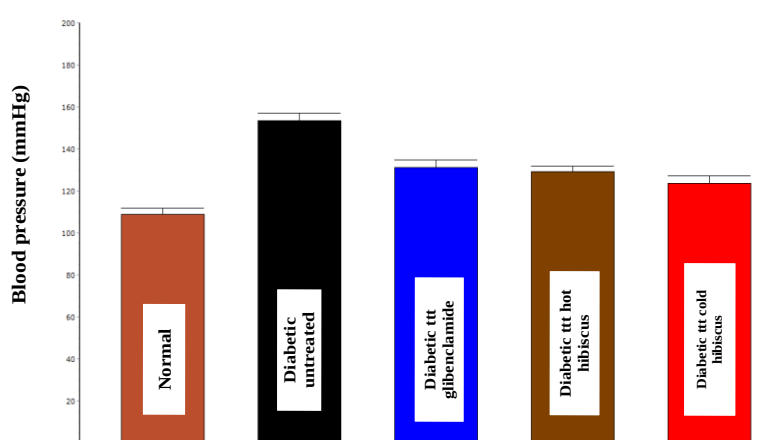


Figure -3: Effects of hot & aqueous extract of hibiscus sabdariffa and glibenclamide on the systolic blood pressure of the diabetic rats

Discussion

Diabetes mellitus is a disease with multiple manifestations, the most dangerous being its vascular complications. Atherosclerosis, involving cardiac, cerebral, and peripheral vasculature, develops earlier in diabetic than non diabetic subjects and accounts for the largest fraction of excess morbidity and mortality (Clark and Lee 1995). Diabetes mellitus is also associated with premature microvascular disease which contributes importantly to complications such as retinopathy and nephropathy (Merimee 1990).

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 evaluation of 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 agreement with the results obtained by **Chang et al., 1993** who found that streptozotocin (STZ) increased the maximal contractile response of the aorta to norepinephrine (NE), phenylephrine and high K⁺ and **Karasu et al., 1997** who showed that the maximal contractile response of aorta to noradrenaline was augmented in STZ-diabetic rats. However, the results of the present study are in disagreement with **results of Dhein et al., 2003** who showed that smooth muscle contractile function not altered by diabetes mellitus, as evident from the nearly unchanged KCl-induced contractions.

The results of the present study showed that the contractile response of the isolated aorta of the diabetic rats treated with hot and cold hibiscus extracts is decreased significantly in comparison with the diabetic untreated rats. The results of this study are in agreement with the results obtained by other workers as, **Yoswaris et al., 2016** 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. **Obiefuna et al., 1993** reported that reduced vascular resistance due to enhanced endothelial function and inhibition of Ca²⁺ influx has been proposed in rats.

The results of this study showed that the relaxant response of the isolated rat aorta to acetylcholine was decreased significantly in the diabetic untreated rats in comparison with the normal rats and the relaxant response of the aorta of the diabetic rats treated with hot and cold aqueous extract of hibiscus sabdariffa was 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., 2009** 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., 2007** reported that a concentration of 1 mg/ml of hibiscus extract, produce a maximal relaxation on adrenaline-precontracted isolated aortic rings. **Anunkaorn et al., 2010** 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) is 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 extract is 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 decreased and **Jonadet et al., 1990** who showed that the extract of the calyces reduce both the systolic and diastolic pressures, lowering heart rate and working as a vasodilator. However, the results of this work are in disagreement with the results obtained by **Adegunloye et al., 1996** who showed that pretreatment of rats with roselle calyx extract in anaesthetized rats did not have a significant effect on the increase in blood pressure induced by bilateral carotid occlusion. **Ojeda et al., 2010** reported that the anti-hypertensive activity might be through inhibition of angiotensin-converting enzymes (ACE). **Adegunloye et al., 1996** showed that the anti-hypertensive activity might be through acetylcholine-like and histamine-like mechanisms, while, **Mojiminiyi et al., 2000** found that the anti-hypertensive activity might be through diuretic effect. **Inuwa et al., 2012** 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 (**Inuwa et al., 2012**). Several studies both in vitro (**Mohd-Esa et al., 2010**) and in vivo (**Mossalam et al., 2011**) 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 (**Mohd-Esa et al., 2010**).

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 guanosine monophosphate (cGMP) by approximately two fold and this raise was abolished by presence of the nitric oxide synthase inhibitor. The present results disagree with **Purnima et al., 1997** who reported that systolic responsiveness to intravenous norepinephrine and angiotensin II infusions is increased during

chronic glibenclamide therapy compared with metformin therapy and **Chaim et al., 2004** who showed that glibenclamide worsened blood pressure control possibly by elevation of insulin levels and activation of the sympathetic system. Also, Nelson et al., 1998 found that glibenclamide inhibit release of vasodilator substances.

In conclusion, treatment with both of the hot and the cold aqueous extracts of the hibiscus sabdariffa aqueous extracts as well as glibenclamide 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.

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