Original Article

Study of the Possible Prophylactic Effect of Hibiscus Sabdariffa Extract on Experimental Hypertension in Rats

Nabila N. El Maraghy*, Mona F. Mahmoud,* Doaa A. Sourour****, Mohamad I. Abozaid****

*Faculty of Pharmacy, Zagazig University, Egypt
**Nuclear Materials Authority, Egypt

A B S T R A C T

Background: Hypertension is a common global health problem with significant mortality and morbidity. Hibiscus sabdariffa (HS) is a plant known in many countries and is consumed as hot and cold drinks. **Aim of the work**: The present study was designed to assess the possible prophylactic effect of aqueous extract of HS on experimental hypertension in rats. **Methods:** Renovascular hypertension was induced in rats by left renal artery ligation with 4-0 sterile surgical silk. Sham-operated rats served as control. The animals were divided into: control group, sham group, hypertensive group, hypertensive group pretreated with HS (250mg/kg/day) given orally 30 days before ligation of left renal artery and 30 days after ligation. **Results:** Pretreatment of hypertensive group with HS extract prevented the development of hypertension and significantly reduced heart rate with significant decrease in serum levels of cholesterol, triglyceride and low density lipoprotein cholesterol while it increased significantly serum high density lipoprotein cholesterol and nitric oxide levels as compared to non-treated hypertensive group. Hibiscus sabdariffa also prevented the decrease in aortic reduced glutathione content and superoxide dismutase enzyme activity while it did not cause any significant change in serum levels of sodium or potassium as compared to non-treated hypertensive group. **Conclusion:** The present study showed that HS prevented the development of hypertension in an experimental model of hypertension which may be attributed to its negative chronotropic, nitric oxide preserving and antioxidant effects and is not related to serum electrolytes.

Key Words: Hibiscus sabdariffa; Renovascular Hypertension; Lipid profile; Nitric oxide; Antioxidant.

Corresponding Author: Doaa Abdallah Sourour   **Email:** sourour209@yahoo.com

1. INTRODUCTION

Hypertension is a major risk factor for many cardiovascular diseases including stroke, coronary heart disease, cardiac failure, and end stage renal disease (Sugiyama et al., 2007). Therefore, prevention of hypertension becomes an important goal to control blood pressure and reduce the incidence of hypertension-related cardiovascular and renal complications and outcome (Slama et al., 2002).

Previous studies indicate that the consumption of polyphenol-rich foods may induce beneficial changes in pathways related to cardiovascular health (Erlund et al., 2008). Meanwhile, recommendations for the consumption of plant-based beverages (except for fruit juices) such as black tea and herbal teas are absent despite their being particularly rich sources of

phytochemicals, especially polyphenols (McKay et al., 2010).

Hibiscus sabdariffa (HS), an ingredient found in many herbal tea blends and other beverages, is a genus of the Malvaceae family and contains anthocyanins, flavonoids and polyphenols (Lin et al., 2007). It is known in many countries and is consumed as hot and cold drinks. In folk medicine, HS has been used to treat hypertension, inflammatory disease and cancer (Kuriyan et al., 2010). It is also used as an antibacterial, antifungal, hypocholesterolemic, diuretic, uricosuric and mild laxative (Farnworth and Bunyapraphatsara, 1992). Concentrated HS beverages lower blood pressure in patients with hypertension (Haji Faraji and Haji Tarkhani, 1999) and type 2 diabetes (Mozaffari-Khosravi et al., 2009) compared

with black tea and have an effect similar to common hypotensive drugs (Herrera-Arellano et al., 2007). However compared to literature, little is known as to whether HS has a prophylactic effect against the development of hypertension.

A decrease in nitric oxide (NO) bioavailability and an increase in oxidative stress markers such as increased levels of biomarkers of lipid peroxidation, decreased antioxidant enzyme activity (superoxide dismutase and catalase) and reduced levels of reactive oxygen species scavengers [vitamins E and C and reduced glutathione (GSH)] are present in human hypertension (Redon et al., 2003; Touyz, 2004). Oxidative stress can potentially contribute to generation and maintenance of hypertension via inactivation of NO (Vaziri et al., 1999). Lipid abnormalities that cause endothelial dysfunction are also common in essential hypertension (Oparil et al., 2003).

Therefore, the present study was designed to evaluate the possible prophylactic effect of aqueous extract of HS against the development of hypertension induced in rats by ligation of left renal artery. The effect of the extract on heart rate, serum lipid profile and NO levels as well as aortic reduced GSH & antioxidant enzyme activity will be also studied.

2. MATERIALS AND METHODS

2.1 Animals

The present study was conducted on 40 adult male Wistar albino rats weighing 200-250 g. They were housed in conventional cages with free access to water and rodent chow at 20-22 °C, constant humidity 60±5% with a 12-h light/dark cycle. All animal procedures were performed after approval from the ethics committee of the National Research Centre Cairo, Egypt and in accordance with the international regulations for the use and care of experimental animals (Canadian Council on Animal Care Guidelines, 1993).

2.2 Preparation of HS

Extract of HS was prepared as previously described by Obiefuna et al. (1993). Dry calyces of HS were authenticated by a source staff of the Department of Pharmacognosy, College of Pharmacy, Zagazig University. These calyces were ground to powder, dissolved in hot water (100°C), and allowed to stand for about 1 h at room temperature. The mixture was stirred vigorously and intermittently. At the end of this period, the residue was sieved off using a piece of gauze. The solution was filtered at least twice and the residue was discarded. The obtained filtrate was left 1-2 days to evaporate to a pasty residue at room temperature. The pasty residue was stored in a deep freezer at -20°C until required. The

residual extract of HS was given via gastric tube as a single daily oral dose of 250 mg/kg/ day (Obiefuna et al., 1993) dissolved in 4 ml distilled water so that each rat received 1ml contained 62.5 mg of hibiscus extract.

2.3 Induction of renovascular hypertension

Renovascular hypertension [two-kidneys, one-clip, (2K1C)] was induced in rats according to the method described by Cangiano et al. (1979). Rats were anaesthetized by single intraperitoneal injection of thiopental sodium (40 mg/kg) (Bearnes and Eltherington, 1964). After shaving the hair and sterilization of the skin with topical antiseptic and alcohol, 2 cm-long incision was made in the left side just below the ribs and 0.5 cm away from the vertebral column. The left renal artery was identified then stretched by means of a retractor placed between the kidney and the muscle layer then the artery was separated from the vein with a hook and dissected from the surrounding connective tissue (Douglas et al., 1976). The exposed left renal artery was completely ligated with 4-0 sterile surgical silk as close as possible to the aorta. The incision was closed by careful continuous suturing of the muscle layer of 4-0 silk with a non- cutting needle then the skin is approximated and closed with interrupted sterile surgical O-silk sutures. Postoperatively, the rats were given penicillin G (100,000 units I.M per rat) for three successive days (Douglas et al., 1976). Sham operated rats served as controls. They received identical surgical procedures except that the ligation was placed loosely around the renal artery to evaluate the effect of surgical intervention on the arterial blood pressure (Cangiano et al., 1979).

2.4 Experimental Design

The animals were divided into four groups, 10 rats each, as follows:

Group I: Normal control group (n=10)

Group II: Sham operated group (n= 10): Rats were surgically manipulated without ligation of left renal artery and this group served as controls.

Group III: Hypertensive group (n=10): Rats in this group underwent ligation of left renal artery as previously described.

Group IV: Hypertensive + hibiscus group (n=10): Rats in this group were administered a single daily oral dose of hibiscus extract (250mg/kg/day) 30 days before ligation of left renal artery and for another 30 days after ligation of left renal artery.

At the end of the experiment, blood samples were collected from rat tail vein in overnight (12-h) fasting animals, after measuring the blood pressure and heart rate in the various groups. Serum was

separated by centrifugation at 3000 rpm for 20 min. and then clear serum was obtained and divided into three aliquots, which were then stored at -20 °C for determination of various biochemical parameters. All rats were sacrificed by decapitation and their aortas were removed, washed with saline and were kept in foil paper and frozen at -70 °C until assayed.

2.5 Measurement of blood pressure and heart rate

The mean arterial blood pressure (MAP) and heart rate were measured at the end of the experiment in all groups according to the method of Burden et al. (1979). The rats were anaesthetized with urethane (ethylcarbamate) in a dose of 1.75-2.0 gm/kg bodyweightinjected intraperitoneally as 25% freshly prepared aqueous solution (Iwamoto et al., 1987). After stabilization of anesthesia, the animal was placed on a board in the supine position. The four limbs were extended and fixed to the sides of the board. A midline longitudinal skin incision started just below the neck and extended to the sternum was done, the skin was removed and pretracheal muscles and fascia were separated away. The trachea was then exposed and dissected for a suitable distance. Lateral to trachea in the left side, the pulsation of the common carotid artery was located. The artery was separated from accompanying nerves (vagus and cervical sympathetic nerves) and internal jugular vein and was carefully freed from connective tissue for a distance as long as possible. A tight ligature of the artery was applied at its distal end (cephalic end) while a bullodog clamp was applied around the artery as near as possible to the sternum. A loose ligature was applied around the artery at its proximal end (thoracic end). A small snip across the artery was opened by a small sharp scissors and the polyethylene arterial cannula filled with heparinized saline solution was inserted gently and pushed towards the heart with a ligature tied around the cannula. The bullodog clamp was then removed, and valve of the cannula was turned to connect the cannula to the blood pressure transducer. A 4 channel oscillograph (MD4) was used. One FC123 (ECG) facility coupler was fixed to one channel, and one FC137 strain gauge coupler was fixed to another channel of the oscillograph. ECG limb cable was attached to the FC123 ECG facility coupler, and a PT400 blood pressure transducer was connected to the FC137 strain gauge coupler (All from Bioscience, USA). The ECG limb cable was attached through hypodermic needles inserted and fixed subcutaneously, in shoulders (for each forelimb), just above the ankle (for each hind limb) and to the chest above the apex of the heart followed by switching on the chart paper of the oscillograph. The heart rate was obtained from ECG recording by counting the number of the heart cycles per fixed distance of chart paper (e.g. 5 cm), with speed of 50 mm/sec and this number was multiplied by division of 300/5 to give the heart rate as beats/minute (Chan et al., 1987).

2.6 Biochemical analysis

2.6.1 Determination of serum lipid profiles

Serum cholesterol, triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) levels were determined by enzymatic colorimetric method using commercially available kits (Biodiagnostic kit, Egypt) according to Allain et al. (1974), Fassati and Prencipe (1982); and Lopez-Virella et al. (1977), respectively. Serum low density lipoprotein cholesterol (LDL-C) level was calculated from the formula [LDL-C= Total cholesterol - (HDL-C+TGs/5)] as described by Friedewald et al. (1972).

2.6.2 Determination of serum NO

NO was determined in serum according to the method of Montgomery and Dymock (1961), by colorimetric determination of nitrite as an indicator of NO production using Biodiagnostic kit (Egypt). In acid medium and in the presence of nitrite, the formed nitrous acid diazotises sulphanilamide and the product are coupled with N-(1-napthyl) ethylenediamine. The resulting azo dye has a bright reddish-purple color, which can be measured against standard and blank at 540 nm

2.6.3 Determination of serum electrolytes

For sodium (Na^+) and potassium (K^+) ions, a flame photometer was used and adjusted to give a direct reading of the concentration of the ion in solution in mEq/L using prepared solutions of different concentrations of a standard solution of the particular electrolyte (sodium chloride and potassium chloride salts).

2.6.4 Determination of aortic antioxidant parameters

The content of GSH in the aortic tissue homogenate was determined by colorimetric method (Biodiagnostic kit, Egypt) as described by Beutler et al. (1963). This method is based on the reduction of 2-nitrobenzoic acid with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405nm.

The activity of superoxide dismutase (SOD) enzyme in the aortic tissue homogenate was determined by colorimetric method (Biodiagnostic kit, Egypt) as described by Nishikimi et al. (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

2.6.5 Methods used for tissue homogenization

2.6.5.1 Tissue homogenization for determination of aortic GSH content

Aorta was perfused with a phosphate buffered saline solution, PH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. The tissues were homogenized in 5-10 ml cold buffer (50 mM potassium phosphate, PH 7.5 1Mm EDTA) per gram tissue. Tissues were centrifuged at 4000 rpm for 15 minutes at 4^0C. The supernatants were removed for assay and stored on ice.

2.6.5.2 Tissue homogenization for determination of aortic SOD enzyme

Aorta was perfused with 0.9%NaCL containing 0.16 mg/ml heparin. Tissues were washed in ice-cold 0.25M sucrose. Tissues were blot on paper and weighed. Tissues were minced in ice-cold 0.25M sucrose. Minced tissues were homogenized using Teflon pestle. Tissue homogenates were diluted in ice-cold 0.25M sucrose to approximately 10%w/v. Diluted homogenates was centrifuged at 4000g and 4^0C for 20 minutes. Supernatants were collected then stored at -70^0C. 0.5 ml of ice-cold extraction reagent was added to 1.0 ml of supernatant in glass test tube. The mixture was vigoursly shaked for 30 seconds using vortex. Then it was centrifuged at 3000g and 4^0C for 10 minutes. The aqueous upper layer was collected and kept at 0-4^0C for the assay.

2.7 Statistical analysis

The data were expressed as means ± standard error of the means (SEM). Analysis of variance (ANOVA) was performed on the means to determine whether there were significant (P < 0.05) differences among the groups. When ANOVA indicated statistical significance, Tukey-Kramer's multiple comparison test follows up, for intergroup comparisons. SPSS version 12 (Chicago, Illinois, USA) was used for all statistical analyses. The results were considered significant when P value ≤0.05.

3. RESULTS

There were no statistical significant difference (P>0.05) in MAP, heart rate, serum levels of lipid, NO and electrolytes as well as aortic antioxidant parameters (GSH and SOD enzyme) in sham operated group as compared to control group (Tables, 1 and 2).

3.1 Effect of hibiscus extract on MAP and heart rate

In the present study, the MAP of the 2K1C hypertensive rats (group III) reached 128.33±2.55 mmHg 30 days after clipping of the left renal artery and these values were significantly higher (P ≤ 0.001)

than that for sham operated rats (91.11±6.07 mmHg; table, 1).

The 2K1C hypertensive rats pretreated with hibiscus extract (group IV) given daily orally for 30 days before ligation of left renal artery and for 30 days after ligation showed a significant decrease (P ≤ 0.001) in MAP (101.11±6.65 mmHg) compared to untreated hypertensive rats (128.33±2.55 mmHg; table, 1). Meanwhile, hibiscus extract given prophylactically in hypertensive rats (group IV) did not normalize MAP as there was significant difference (P ≤ 0.05) in group IV as compared to sham operated group (table, 1).

Regarding heart rate, the present study showed significant increase (P ≤ 0.001) in heart rate in 2K1C hypertensive rats (group III) as compared to sham operated group (370±13.78 beats/min vs 300±17.32 beats/min, respectively; table, 1). Pretreatment of 2KIC hypertensive rats with hibiscus extract (group IV) induced a significant decrease (P ≤ 0.001) in heart rate (182.5±12.5 beats/min) as compared to untreated 2KIC hypertensive group (370±13.78 beats/min) and sham operated group (300±17.32 beats/min; table, 1).

3.2 Effect of hibiscus extract on serum lipid profile

In this study, induction of hypertension in the 2K1C hypertensive rats (group III) led to significant increase (P ≤ 0.001) in serum levels of cholesterol, TG and LDL-C to 105.09±6.26 mg/dl; 40.22±2.13 mg/dl and 75.71±6.06 mg/dl, respectively when compared with sham operated group (64.99±5.96 mg/dl, 21.76±0.72 mg/dl and 28.22±1.75 mg/dl, respectively). There was also significant decrease (P ≤ 0.001) in serum HDL-C level in 2K1C hypertensive group as compared to sham operated group (21.34±1.24 mg/dl vs 38.28±2.35 mg/dl, respectively; table, 1).

Pretreatment of hypertensive rats with hibiscus **extract** (group IV) significantly decreased (P ≤ 0.001) serum levels of cholesterol (69.51±5.48 mg/dl), TG (23.83±2.05 mg/dl) and LDL-C (34.23±3.22 mg/dl) as compared to untreated hypertensive rats (table, 1) together with significant increase (P ≤ 0.001) in HDL-C (32.63±2.91 mg/dl) as compared to untreated hypertensive group (21.34±1.24). Hibiscus extract pretreatment in hypertensive rats (group IV) normalized serum lipid levels as there was non-significant difference (P > 0.05) in serum lipid levels in group IV as compared to sham operated group (table, 1)

3.3 Effect of hibiscus extract on serum NO

The 2K1C hypertensive rats (group III) in this study showed significant decrease (P ≤ 0.05) in serum NO levels compared with sham operated group

(19.19±0.57 μmol/L vs 22.74±0.74 μmol/L, respectively; table, 2).

Hypertensive rats pretreated with hibiscus **extract** (group IV) showed significant increase (P ≤ 0.001) in serum NO levels compared to untreated hypertensive group (26.89±0.63 μmol/L vs 19.19±0.57 μmol/L, respectively) as well as significant increase (P ≤ 0.05) compared to sham operated group (26.89±0.63 μmol/L vs 22.74±0.74 μmol/L, respectively; table, 2).

3.4 Effect of hibiscus extract on serum electrolytes

In the present work, 2K1C hypertensive rats (group III) showed non-significant difference (P > 0.05) in serum Na^+ as compared to sham group (138.38±4.31 mEq/L vs 137.48±2.32 mEq/L, respectively) but with significant decrease (P ≤ 0.05) in serum K^+ compared to sham group (3.86±0.19 mEq/L vs 4.86±0.15 mEq/L, respectively; table, 2).

Hypertensive rats pretreated with hibiscus **extract** (group IV), showed non-significant difference (P > 0.05) in serum Na^+ (136.35±3.09 mEq/L) compared to untreated hypertensive group (138.38±4.31 mEq/L) and sham operated group (137.48±2.32 mEq/L; table, 2). Regarding serum K^+, the 2KIC hypertensive rats pretreated with HS showed non-significant difference (P > 0.05) as compared to non-treated hypertensive group (3.4±0.17

mEq/L vs 3.86±0.19 mEq/L, respectively) but with significant decrease (P ≤ 0.05) in serum K^+, as compared to sham operated group (3.4±0.17 mEq/L vs 4.86±0.15 mEq/L, respectively; table, 2).

3.5 Effect of hibiscus extract on aortic antioxidant parameters

In the present study, the mean aortic GSH content and aortic SOD enzyme activity in 2K1C hypertensive rats (group III) decreased significantly ((P ≤ 0.001) to 24.11±2.12 nmol/gm tissue and 36.14±2.37 U/gm tissue, respectively as compared to sham group ̃±4.05 nmol/gm tissue and 60.23±2.26 U/gm tissue, respectively; table, 2).

Pretreatment of hypertensive rats with hibiscus **extract** (group IV) produced significant increase (P ≤ 0.001) in aortic GSH content (49.72±2.87 nmol/gm tissue) and aortic SOD enzyme activity (45.51±2.17 U/gm tissue) compared to untreated hypertensive group (24.11±2.12 nmol/gm tissue and 36.14±2.37 U/gm tissue, respectively; table, 2). Hibiscus extract pretreatment in hypertensive group (group IV) normalized aortic GSH content as there was non-significant difference in aortic GSH (P > 0.05) in group IV as compared to sham group. Meanwhile, there was significant difference in aortic SOD enzyme activity (P ≤ 0.001) in group IV as compared to sham group (tabe, 2).

Table (1): Effect of pretreatment with Hibiscus extract on MAP, heart rate and serum lipid profile in 2K1C-hypertensive rats

Parameters	Control (Group I)	Sham operated (Group II)	Hypertensive (Group III)	Hypertensive+Hibiscus (Group IV)
MAP(mmHg)	96.66 ±3.22	91.11±6.07	128.33±2.55[*]	101.11±6.65[#***]
Heart rate(beats/min)	297.5± 21	300±17.32	370±13.78[*]	182.5±12.5[#*]
Serum Cholesterol (mg/dl)	71.91± 6.68	64.99±5.96	105.09±6.26[*]	69.51±5.48[#]
Serum TGs (mg/dl)	19.25± 1.33	21.76±0.72	40.22±2.13[*]	23.83±2.05[#]
Serum HDL-C (mg/dl)	37.80 ±2.69	38.28±2.35	21.34±1.24[*]	32.63±2.91[#]
Serum LDL-C (mg/dl)	27.57± 1.78	28.22±1.75	75.71±6.06[*]	34.23±3.22[#]

Values are presented as means ±SEM (n=10 / group). **MAP:** mean arterial blood pressure, **2K1C**: two kidneys-one clip, **TGs:** triglyceride, **HDL-C:** high density lipoprotein cholesterol, **LDL-C:** low density lipoprotein cholesterol. Analysis of variance (ANOVA) followed by Tukey-Kramer analysis was used for the comparison between the groups.

[*] P ≤ 0.001, [**] P ≤ 0.05: significantly different from sham operated group.

[#] P ≤ 0.001: significantly different from hypertensive group.

Table (2): Effect of pretreatment with Hibiscus extract on serum NO, serum electrolytes and aortic antioxidant parameters in 2K1C-hypertensive rats

Parameters	Control (Group I)	Sham operated (Group II)	Hypertensive (Group III)	Hypertensive+Hibiscus (Group IV)
Serum NO (µmol/L)	23.67±1.26	22.74±0.74	19.19±0.57[**]	26.89±0.63[#**]
Serum Na $^+$ (mEq/L)	140.59±3.62	137.48±2.32	138.38±4.31	136.35±3.09
Serum K $^+$ (mEq/L)	5.00±0.17	4.86±0.15	3.86±0.19[**]	3.4±0.17[**]
Aortic GSH (nmol/gm tissue)	55.98±2.15	55.23±4.05	24.11±2.12[*]	49.72±2.87[#]
Aortic SOD (U/gm tissue)	62.178±1.47	60.23±2.26	36.14±2.37[*]	45.51±2.17[#*]

Values are presented as means ± SEM (n=10 / group). **2K1C**: two kidneys-one clip, **NO:** nitric oxide, **GSH:** reduced glutathione, **SOD:** superoxide dismutase. Analysis of variance (ANOVA) followed by Tukey-Kramer analysis was used for the comparison between the groups.

[*] P ≤ 0.001, [**] P ≤ 0.05: significantly different from sham operated group.

[#] P ≤ 0.001: significantly different from hypertensive group.

4. DISCUSSION

The 2K1C model of hypertension was used in this study as it is similar to human renovascular hypertension in many aspects including endothelial dysfunction and increased oxidative stress (Basso and Terragno, 2001).

In the present work, induction of renovascular hypertension in rats led to significant increase in MAP, heart rate and serum levels of cholesterol, TG and LDL-C while it decreased significantly serum HDL-C level together with significant decrease in serum NO and aortic antioxidant parameters levels 30 days after clipping of left renal artery as compared to sham operated group.

The 2K1C hypertension model is an inducible model of hypertension where hypertension is usually established 3 to 4 weeks after clipping (Iversen et al., 1983). It is an angiotensin II–dependent model where the earliest phase of hypertension is characterized by a rapid rise in plasma renin in response to low renal arterial pressure and by the consequent increase in circulating angiotensin II which stimulates the production of aldosterone, a mineralocorticoid that exerts sodium and water retaining effects on the distal tubule. In the chronic phase, hypertension is maintained by a continuously activated renin-angiotensin system because pressure diuresis of the contralateral normal kidney prevents hypervolemia (Wiesel et al., 1997). Increased angiotensin II results in vasoconstriction, increased endothelin release, vascular remodeling, accelerated atherogenesis, glomerulosclerosisand increases the activity of the sympathetic nervous systems. Angiotensin II also stimulates nicotinamide adenine dinucleotide

phosphate (NADPH)/nicotinamide adenine dinucleotide (NADH) oxidase in endothelium, smooth muscle cells, and the adventitia of blood vessels to generate reactive oxygen species, leading to endothelial dysfunction, growth, and inflammation (Schiffrin, 2002).

The mechanisms involved in development and maintenance of 2K1C renal hypertension were discussed by Guo et al. (2000). They stated that deficiency of NO production and increase in renin-angiotensin system activity may contribute to vascular endothelial dysfunction of 2K1C rats and these factors may be involved in development and maintenance of 2K1C renal hypertension. Free radicals generated by angiotensin II have the ability to scavenge endothelial NO and peroxynitrite produced from the reaction between NO and O_2^- can oxidize tetrahydrobiopterin, the critical cofactor for nitric oxide synthase leading to uncoupling of the enzyme with a consequent reduction of NO production (Laursen et al., 2001).

In the current study the mechanism by which 2K1C rats showed an increase in heart rate may be due to interactions between renin angiotensin system and sympathetic nervous system since it has been well recognized that strong sympathetic stimulation can increase the heart rate (Schroeder et al., 2003).

In this work, induction of renovascular hypertension did not make any change in the levels of serum Na^+ but caused a significant decrease in K^+ levels compared to sham operated group. A previous study by Odigie et al. (2003) found that plasma electrolytes in 2K1C hypertensive rats were not different from sham operated controls. On the contrary, serum Na^+ concentration was significantly higher with non-significant change in serum K^+

concentration in the 2K1C hypertensive group as compared to control group (Othman and Mahmud, 2012). Seto et al. (1991) suggested that sodium retention was not a major factor in the acute and chronic phases of 2K1C hypertension in rabbits and that pressure natriuresis by the intact contralateral kidney increases sodium excretion and therefore, there is no sodium retention. Furthermore, they stated that derangement in the intracellular potassium metabolism may be associated with the maintenance rather than the development of hypertension.

The hypokalemia found in the present study in the 2K1C model of renovascular hypertension is due to increased production of aldosterone secondary to left renal artery ligation. Epidemiologic data suggest that K^+ intake and blood pressure are correlated inversely. Blood pressure elevation during K^+ depletion occur due to Na^+ retention, altered response to vasoactive hormones i.e. increasing cardiovascular reactivity to norepinephrine or angiotensin II, direct vasoconstrictive effects of hypokalemia, and calcium depletion (Krishna, 1994).

In our study, hypertensive rats pretreated with HS extract for 30 days before clipping of left renal artery and for 30 days after clipping of left renal artery, showed significant reduction in MAP as compared to non-treated hypertensive group. These results indicate that hibiscus extract pretreatment was effective in preventing the development of hypertension in the 2K1C rats. Compared to literature, daily consumption of hibiscus tea, in an amount readily incorporated into the diet, lowers blood pressure in pre- and mildly hypertensive adults and may be useful in preventing the progression to moderate or more severe hypertension (McKay et al., 2010). The antihypertensive activity of HS was previously reported in patients with essential hypertension (Haji-Faraji and Haji-Tarkhani, 1999), the spontaneously hypertensive rats (Onyenekwe et al., 1999), the 2K1C rat (Odigie et al., 2003) and in salt-induced and L-NAME (N^ω-L-arginine methyl ester)-induced hypertension (Mojiminiyi et al., 2007).

This study showed significant decrease in heart rate in hypertensive rats pretreated with hibiscus as compared to non-treated hypertensive rats as well as compared to sham control group. This suggests a negative chronotropic action of HS which is in agreement with the findings of Odigie et al. (2003) and Mojiminiyi et al. (2007).

The present study also showed that HS extract improved all lipid profile in pretreated hypertensive rats compared to non-treated hypertensive group. These findings confirm the hypolipidemic effects of HS extract detected previously in human and animal

studies by several investigators (Lin et al., 2007; Kumar et al., 2009 and Gosain et al., 2010).

The hypolipidemic effects of HS extract found in this study were discussed by Gaet (1999) who stated that the calyx of HS contains many phyto-constituents such as ß-sitosterol and pectin. These phyto-constituents have been reported to possess hypocholesterolemic effects according to previous investigators (Hexeberg et al. 1994; Wang and Ng, 1999 and Wu et al., 2009).

In this study, HS extract increased NO levels in pretreated hypertensive rats as compared to non-treated hypertensive group as well as compared to sham group. Consistent with our results, Ajay et al. (2007) found that HS extract has a vasodilator effect in the isolated aortic rings of hypertensive rats and that these effects are probably mediated through the endothelium-derived nitric oxide-cGMP-relaxant pathway. Alarcón-Alonso et al. (2012) stated that quercetin found in HS had effect on the vascular endothelium causing NO release.

In the present study HS extract produced non-significant change in serum Na^+ and did not correct serum hypokalemia in 2K1C pretreated hypertensive rats compared with non-treated hypertensive group with significant decrease in serum K^+ as compared to sham group. Herrera-Arellano et al. (2007) found that HS extract demonstrated a tendency to reduce serum Na^+ concentrations which is not significant compared to hypertensive patients (from 139.09 to 137.35, P = 0.07), while K^+ level was not modified. On the contrary, other investigators have demonstrated that HS presents natriuretic, potassium sparing and diuretic effects (Alarcón-Alonso et al. 2012; Jiménez-Ferrer et al., 2012)..

In the present work, hypertensive rats pretreated with HS extract showed significant increase in aortic antioxidant parameters, SOD and GSH compared to non- treated hypertensive group. This suggests an antioxidant effect of HS extract which is in agreement with other studies, where Wang et al. (2000) found that hibiscus anthocyanin, a pigment extract from the calyx of HS, significantly reduced oxidative stress in rat hepatocytes in vivo. Also, Gauthaman et al. (2006) reported that hibiscus extract in a dose of 250 mg/kg augments endogenous antioxidant compounds (SOD, GSH and catalase) in rat heart and prevents myocardium injury. Also, Ekor et al. (2010) found that HS extract prevented cholesterol-induced depletion of enzymatic (SOD, catalase) and non-enzymatic (GSH, vitamin C) antioxidants in animals.

The antioxidant effect of HS extract was explained by Crawford et al. (1998) where they found various antioxidant constituents in the calyx of HS,

such as anthocyanins, flavonoids, protocatechuic acid and polyphenolṣ.

Thus the mechanisms of the antihypertensive effect of HS extract observed in this study could be attributed to its negative chronotropic effect, increased production of NO and antioxidant effects. Other mechanisms related to blood pressure-lowering effect of HS extract have been explored by others are due to the fact that HS is a vasorelaxant via action on calcium channels (Owolabi et al., 1995) or via cholinergic or histaminergic actions (Adegunloye et al., 1996), its diuretic effect (Mojiminiyi et al., 2000) and its ability to inhibit angiotensin converting enzyme through the presence of anthocyanins (Herrera-Arellano et al., 2007). Ojeda et al. (2010) found that, anthocyanins delphinidin-3-O-sambubioside (1) and cyanidin-3-O-sambubioside (2), are constituents responsible of the angiotensin converting enzyme activity of the aqueous extract of HS and that these compounds inhibit the enzyme activity by competing with the substrate for the active site.

5. CONCLUSION

The present study showed that HS extract has a prophylactic effect and prevents the development of hypertension in the 2KIC animal model of hypertension which may be attributed to its negative chrontropic, NO preserving and its antioxidant effects and is not related to effect on serum electrolytes.

6. REFERENCES

Adegunloye, B.J., Omoniyi, J.O., Owolabi, O.A., Ajagbonna, O.P., Sofola, O.A., Coker, H.A., 1996. Mechanisms of the blood pressure lowering effect of the calyx extract of Hibiscus sabdariffa in rats. Afr. J. Med. Med. Sci 25(3), 235-8.

Ajay, M., Chai, H.J., Mustafa, A.M., Gilani, A.H., Mustafa, M.R., 2007. Mechanisms of the anti-hypertensive effect of Hibiscus sabdariffa L. calyces. J. Ethnopharmacol 109(3), 388-93.

Alarcón-Alonso, J., Zamilpa, A., Aguilar, F.A., Herrera-Ruiz, M., Tortoriello, J., Jimenez-Ferrer, E., 2012. Pharmacological characterization of the diuretic effect of Hibiscus sabdariffa Linn (Malvaceae) extract. J. Ethnopharmacol 139(3), 751-6.

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., Fu, P.C., 1974. Enzymatic determination of total serum cholesterol. Clin. Chem 20, 470-475.

Basso, N., Terragno, N.A., 2001. History about the discovery of the renin-angiotensin system. Hypertension 38 (6), 1246–9.

Bearnes, C.D., Eltherington L.G., 1964. Drug dosage in laboratory animals. A hand book 2nd ed., University of California Press Parkly and Los Angeles.

Beutler, E., Duran, O., Kelly, M.B., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med 61, 882-888.

Burden, D.T., Balber, L.C., Natoff, I.L., 1979. A simple method for recording left ventricular pressure and heart rate in cat. Pharmacol. Ther 5, 99-102.

Canadian Council on Animal Care (CCAC), 1993. Guide to the Care and Use of Experimental Animals, Volume 1 (2nd Edition), Canada.

Cangiano, J.L., Rodriguez-Sargnt, C., Martinez-Maldonado, M., 1979. Effects of antihypertensive treatment on systolic blood pressure and renin in experimental hypertension in rats. J. Pharmacol. Exp. Ther 208(2), 310-3.

Chan, M., Sorter, D., Ko, W.W., 1987. Effects of morphine on cardiovascular responses to acute myocardial infarction in rats. Br.J.Pharmacol 90, 537-43.

Crawford, R.S., Kirk, E.A., Rosenfeld, M.E., LeBoeuf, R.C., Chait, A., 1998. Dietary antioxidants inhibit development of fatty streak lesions in the LDL receptor-deficient mouse. Arterioscler. Thromb. Vasc. Biol 18, 1506-1513.

Douglas, J.R. Jr., Johnson, E.M. Jr., Heist, J.F., Marshall, G.R., Needleman P., 1976. Is the peripheral sympatho-adrenal nervous system necessary for renal hypertension? J. Pharmacol. Exp. Ther 196(1), 35-43.

Ekor, M., Adesanoye, O.A., Udo, I.E., Adegoke, O.A., Raji, J, Farombi, E.O. 2010. Hibiscus sabdariffa ethanolic extract protects against dyslipidemia and oxidative stress induced by chronic cholesterol administration in rabbits. Afr. J. Med. Med. Sci 39 (Suppl), 161-70.

Erlund, I., Koli, R., Alfthan, G., Marniemi, J., Puukka, P., Mustonen, P., Mattila, P., Jula, A., 2008. Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. Am. J. Clin. Nutr 87, 323–31.

Farnworth, N.R., Bunyapraphatsara, N., 1992. Thai Medicinal Plants. Prachachon Press, Bangkok, pp. 163–166.

Fassati, P., Prencipe, L., 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin.Chem 28, 2077-2080.

Friedewald, W.T., Levy, R.I., Frederickson, D.S., 1972. Estimation of the concentration of low- density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18, 499-502.

Gaet, N., 1999. Hibiscus sabdariffa LINN. In: Ivan, A. (Ed.), Medicinal Plants of the World. Human Press, New York, pp. 165–170.

Gauthaman, K.K., Saleem, M.T., Thanislas, P.T., Prabhu, V.V., Krishnamoorthy, K.K., Devaraj, N.S., Somasundaram, J.S., 2006. Cardioprotective effect of the Hibiscus rosa sinensis flowers in an oxidative stress model of myocardial ischemic reperfusion injury in rat. BMC Complement. Altern. Med 6, 32.

Gosain, S., Ircchiaya, R., Sharma, P.C., Thareja, S., Kalra, A., Deep, A., Bhardwaj, T.R., 2010. Hypolipidemic effect of ethanolic extract from the leaves of Hibiscus sabdariffa L. in hyperlipidemic rats. Acta Pol. Pharm 67(2), 179-84.

Guo, Y.M., Zhu, X.N., Pan, J.Y., 2000. Nitric oxide changes aortic function in rats with renal hypertension. Sheng Li Xue Bao 52(3), 243-6.

Haji Faraji, M., Haji Tarkhani, A., 1999. The effects of sour tea (Hibiscus sabdariffa) on essential hypertension. J. Ethnoparmacol 65, 231-236.

Herrera-Arellano, A., Miranda-Sánchez, J., Avila-Castro, P., Herrera-Alvarez, S., Jiménez-Ferrer, J.E., Zamilpa, A., Román-Ramos, R., Ponce-Monter, H., Tortoriello, J., 2007. Clinical effects produced by a standardized herbal medicinal product of Hibiscus sabdariffa on patients with hypertension. A randomized, double-blind, lisinopril-controlled clinical trial. Planta Med 73(1), 6-12.

Hexeberg, S., Hexeberg, E., Willumsen, N., Berge, R.K., 1994. A study on lipid metabolism in heart and liver of cholesterol- and pectin-fed rats. Br. J. Nutr 71(2), 181-92.

Iversen, B.M., Morkrid, L., Ofstad, J., 1983. Afferent arteriolar diameter in DOCA-salt and two-kidney one-clip hypertensive rats. Am. J. Physiol 245, F755–F762.

Iwamoto, K., Watanabe, J., Atsumi, F., 1987. Effects of urethane anesthesia and age on organ blood flow in rats measured by hydrogen gas clearance method. J. Pharmacobiodyn 10(6), 280-4.

Jiménez-Ferrer, E., Alarcón-Alonso, J., Aguilar-Rojas, A., Zamilpa, A., Jiménez-Ferrer, C.I., Tortoriello, J., Herrera-Ruiz, M., 2012. Diuretic effect of compounds from Hibiscus sabdariffa by modulation of the aldosterone activity. Planta Med 78(18), 1893-8.

Krishna, G.G., 1994. Role of potassium in the pathogenesis of hypertension. Am. J. Med. Sci 307 (Suppl.1), S21-5.

Kumar, V., Singh, P., Chander, R., Mahdi, F., Singh, S., Singh, R., Khanna, A.K., Saxena, J.K., Mahdi, A.A., Singh, V.K., 2009. Hypolipidemic activity of Hibiscus rosa sinensis root in rats. Indian J. Biochem. Biophys 46(6), 507-10.

Kuriyan, R., Kumar, D.R. R.R., Kurpad, A.V., 2010. An evaluation of the hypolipidemic effect of an extract of Hibiscus Sabdariffa leaves in hyperlipidemic Indians: a double blind, placebo controlled trial. MC Complementary and Alternative Medicine 10, 27.

Laursen, J.B., Somers, M., Kurz, S., Warnholtz, A., McCann, L., Freeman, B., Tarpey, M., Fukai, T., Harrison, D., 2001. Endothelial regulation of vasomotion in Apo(E)-deficient mice implications for interactions between peroxynitrite and tetrahydrobiopterin. Circ. Res 103, 1282-1288.

Lin, T.L., Lin, H.H., Chen, C.C., Lin, M.C., Chou, M.C., Wang, C.J., 2007. Hibiscus sabdariffa extract reduces serum cholesterol in men and women. Nutrition Research 27, 140-145.

Lopes-Virella, M.F., Stone, P., Ellis, S., Colwell, J.A., 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem 23, 882-884.

McKay, D.L., Chen, C.Y., Saltzman, E., Blumberg, J.B., 2010. Hibiscus sabdariffa L. tea (tisane) lowers blood pressure in prehypertensive and mildly hypertensive adults. J. Nutr 140(2), 298-303.

Mojiminiyi, F.B., Adegunloye, B.J., Egbeniyi, Y.A., Okolo, R.U. 2000. An investigation of the diuretic effect of an aqueous extract of the petals of Hibiscus sabdariffa. J. Med. Med. Sci 2, 77–80.

Mojiminiyi, F.B., Dikko, M., Muhammad, B.Y., Ojobor, P.D., Ajagbonna, O.P., Okolo, R.U., Igbokwe, U.V., Mojiminiyi, U.E., Fagbemi, M.A., Bello, S.O., Anga, T.J., 2007. Antihypertensive effect of an aqueous extract of the calyx of Hibiscus sabdariffa. Fitoterapia 78(4), 292-7.

Montgomery, H.A.C., Dymock, J.F., 1961. Colorimetric determination of nitrite in water. Analyst 86, 414-416

Mozaffari-Khosravi, H., Jalali-Khanabadi, B.A., Afkhami-Ardekani, M., Fatehi, F., Noori-Shadkam, M., 2009. The effects of sour tea (Hibiscus sabdariffa) on hypertension in patients with type II diabetes. J. Hum. Hypertens 23, 48–54.

Nishikimi, M., Appaji, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Bioph. Res. Common 46, 849-854.

Obiefuna, P.C.M., Owolabi, O.A., Adegunloye, B.J., Obiefuna, I.P., Sofola, O.A., 1993. The petal extract of Hibiscus sabdariffa produces relaxation of isolated rat aorta. International Journal of Pharmacognosy 32, 69–74.

Odigie, I.P., Ettarh, R.R., Adigun, S.A., 2003. Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. Journal of Ethnopharmacology 86:181–185.

Ojeda, D.; Jiménez-Ferrer, E.; Zamilpa, A.; Herrera-Arellano, A.; Tortoriello, J. and Alvarez, L., 2010: Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3-O-sambubiosides from Hibiscus sabdariffa. J. Ethnopharmacol., 127(1):7-10.

Onyenekwe, P.C., Ajani, E.O., Ameh, D.A., Gamaniel, K.S., 1999. Antihypertensive effect of roselle (Hibiscus sabdariffa) calyx infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in Wistar rats. Cell Biochemistry and Function 17, 199–206.

Oparil, S., Zaman, M.A., Calhoun, D.A., 2003. Pathogenesis of hypertension. Ann. Intern. Med 139, 761–776.

Othman, H.K., Mahmud, A.M.R., 2012. Effects of Potassium and Magnesium on Some Hemodynamic and Renal Function Related Parameters in 2k1c Hypertensive Rats. Journal of Pharmacy 2(6), 33-42.

Owolabi, O.A., Adegunloye, B.J., Ajagbona, O.P., Sofola, O.A., Obiefuna, P.C.M., 1995. Mechanism of relaxant effect mediated by an aqueous extract of Hibiscus sabdariffa petals in isolated rat aorta. Pharm. Biol 33, 210–4.

Redon, J., Oliva, M.R., Tormos, C., Giner, V., Chaves, J., Iradi, A., Saez, G.T., 2003. Antioxidant activities and oxidative stress byproducts in human hypertension. Hypertension 41, 1096–1101.

Schiffrin, L., 2002. Beyond blood pressure: the endothelium and atherosclerosis progression. Am. J. Hypertens 15(10 Pt 2), 115S-122S.

Schroeder,E.B.,Laio, D.,Chambless, L.E., Prineas, R.J., Evan, G.W., Heiss G., 2003. Hypertension,blood pressure, and heart rate variability:the atherosclerosis risk in communities (ARIC) study.Hypertension 42, 1106-1111.

Seto, S., Kaku, T., Kuramochi, M., Hashiba, K., Okajima, S., 1991. Changes in sodium-22 turnover and total body potassium in two-kidney, one-clip renovascular hypertension. Jpn Circ. J 55(5), 524-33.

Slama, M., Susic, D., Frohlich, E.D., 2002. Prevention of hypertension. Curr. Opin. Cardiol 17(5), 531-6.

Sugiyama, T., Xi, D., Graham-Maar, R.C., Inoue, K., Kobayashi, K., Stettler N., 2007. Dietary and Lifestyle Factors Associated with Blood Pressure among U.S. Adolescents. Journal of Adolescent Health 40, 166-172.

Touyz, R.M., 2004. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension 44, 248–252.

Vaziri, N.D., Liang, K., Ding, Y., 1999. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. Kidney Int 56, 1392–1398.

Wang, C.J., Wang, J.M., Lin, W.L., Chu, C.Y., Chou, F.P., Tseng, T.H., 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide (t-BHT) induced hepatic toxicity in rats. Food and Chemical Toxicology 38, 411–416.

Wang, H.X., Ng, T.B., 1999. Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sci 65(25), 2663-77.

Wiesel, P., Mazzolai, L., Nussberger, J., Pedrazzini, T., 1997. Two-kidney, one clip and one-kidney, one clip hypertension in mice. Hypertension 29(4), 1025-30.

Wu, T., Fu, J., Yang, Y., Zhang, L., Han, J., 2009. The effects of phytosterols/stanols on blood lipid profiles: a systematic review with meta-analysis. Asia Pac. J. Clin. Nutr 18, 179–186.