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Attenuation of salt-induced hypertension by aqueous calyx extract of *Hibiscus Sabdariffa*

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Summary: The aqueous calyx extract of *Hibiscus sabdariffa* (HS) has a folk reputation as an antihypertensive agent. On account of its antioxidant properties and probably high K^+ concentration, we hypothesized that HS may attenuate the development of salt-induced hypertension. Sprague-Dawley rats (n=8 each) were treated for 12 weeks as follows: control (normal diet + water), salt-loaded (8% salt diet + water), HS (normal diet + 6mg/ml HS), salt+HS (8% salt diet + 6mg/ml HS) and furosemide (normal diet+ 0.25mg/Kg furosemide). Their blood pressure and heart rates were measured and responses to noradrenalin and acetylcholine (0.01mg/kg respectively) were estimated. The cationic concentration of 6mg/ml HS was determined. The Na^+ and K^+ concentrations of 6mg/ml HS were 3.6 and 840mmol/l respectively. The mean arterial pressure (MAP±SEM; mmHg) of salt loaded rats (184.6±29.8) was significantly higher than control (113.2±3.0; $P<0.05$), HS (90.0±7.4; $P<0.001$) salt+HS (119.4±8.9; $P<0.05$) and furosemide (94.9±11.5; $P<0.01$). The MAP of salt+HS and control rats did not differ significantly and the effect of HS was comparable to furosemide. The pressor response to noradrenalin or vasodilator response to acetylcholine remained similar in all groups. These results suggest that HS attenuated the development of salt-induced hypertension and this attenuation may be associated with its high K^+ content or high potassium: sodium ratio and not with altered pressor/depressor response to noradrenalin or acetylcholine. Also the effects of HS and furosemide on blood pressure are comparable.

Keywords*: Hibiscus sabdariffa* calyx, salt-induced hypertension, anti-hypertensive effect, High dietary K^+, K^+:Na^+

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INTRODUCTION

Hypertension, especially salt sensitive hypertension, is more common in the black race than in Caucasians (Luft and Weinberger, 1997; Fields et al., 2004; O'Shaughnessy and Karet, 2004). An animal model for salt sensitive hypertension may be obtained through the feeding of inbred rats with 8% salt diet (Sofola et al., 2002; Mojiminiyi et al., 2007). The aqueous calyx extract of *Hibiscus sabdariffa* (HS) is consumed as a local soft drink in Northern Nigeria. Its folk reputation as an antihypertensive agent in Nigeria has been validated by several studies in animals (Odigie, Ettarh and Adigun, 2003; Mojiminiyi et al., 2007) and man (Haji Faraji and Haji Tarkhani, 1999; Herrera-Arellano et al., 2004; Herrera-Arellano et al., 2007). HS has also been reported to have diuretic properties (Mojiminiyi, 2000; Alorcon-Alonzo et al., 2012).

The development of hypertension may be associated with oxidative stress (Touyz and Briones, 2011). Also hypertension may be ameliorated or prevented by the consumption of plant/fruit based diets rich in potassium (Appel et al., 2006; Androgue and Madias, 2007). Since HS has antioxidant properties (Ajiboye, et al., 2011; Peng, et al., 2011; Frank et al., 2012), and like other plants, may be rich in potassium, we hypothesized that it may be able to prevent or ameliorate the development of salt-induced hypertension. Consequently this study was carried out to test this hypothesis. We also tested the plausible notion that the blood pressure lowering effect of HS may be associated with a dampening of the pressor effect of noradrenalin or enhancement of the depressor/vasodilator effect of acetylcholine.

MATERIALS AND METHODS

Extract preparation and measurement of cationic composition

A random sampling of sachets of HS soft drink obtained from sellers revealed a concentration of about 6mg/ml. *Hibiscus sabdariffa* calyx was identified as previously described (Mojiminiyi, et al., 2007). 10g of the dry calyces was boiled in 1 litre of water at 100^{o}C for about 2 hours and the cold decoction was filtered using a white handkerchief and then with size one Whatman filter paper. The filtrate was evaporated to dryness in an aeration oven at 60^{o}C. The dry powder was used to prepare an HS concentration of 6mg/ml and its cationic content measured by flame photometry.

Induction of hypertension, administration of HS and standard drug

The experiments were done in line with guidelines on use of animals for experiments as issued by the Physiological Society of Nigeria and Physiological Society, London. Hypertension was induced by salt-loading rats with 8% sodium chloride diet (Sofola et al., 2002; Mojiminiyi et al., 2007). HS was also administered while furosemide served as standard drug. Weanling in bred Sprague-Dawley rats (n=8 each; weight 112-140g) were treated for 12 weeks by administering the following: control (normal diet + water), salt-loaded (8% salt diet + water), HS (normal diet + 6mg/ml HS) salt+HS (8% salt diet + 6mg/ml HS) and furosemide (normal diet + 0.25mg/kg furosemide). The normal diet contained 0.3% salt.

Measurement of blood pressure, heart rate and administration of noradrenalin and acetylcholine

At the end of 12 weeks the rats were anaesthetised with a mixture of 25% urethane and 1% chloralose given intraperitoneally at a dose of 5ml/kg body weight and the blood pressure and heart rate measured as described earlier (Mojiminiyi, et al., 2007). It is briefly explained here. The trachea was cannulated to improve ventilation and the femoral artery and vein were cannulated for the measurement of blood pressure and administration of drugs respectively. Immediately after the cannulation of the femoral artery, 0.2 ml of heparinized saline was injected into it to prevent intravascular coagulation. The arterial cannula was then coupled to a pressure transducer (Statham P23D) which had been previously calibrated. This was in turn connected to a model 7D Grass polygraph (Grass Instruments, Quincy, MA, USA) for the recording of blood pressure and heart rate. The animals were allowed about 30 minutes for the stabilization of the blood pressure before starting the experiments. The initial blood pressure and heart rate were then taken. About 30 minutes later, noradrenalin (0.05mg/Kg) was administered through the femoral vein and the responses recorded. After the blood pressure had returned to basal levels, acetylcholine (0.05mg/Kg) was similarly administered and the responses recorded. The systolic, diastolic and pulse pressures were measured from the recordings and the mean arterial pressure (MAP) was calculated as sum of diastolic blood pressure and 1/3 pulse pressure (Adeneye et al., 2006; Mojiminiyi, et al., 2007). Heart rates were obtained by counting the arterial pulses for 15s and then multiplied by four to convert to beats/min (Adeneye et al., 2006; Mojiminiyi, et al., 2007).

Statistical Analysis

Results are presented as mean ± S.E.M. They were analysed using one way ANOVA and a post hoc Tukey Kramer multiple comparison test. $P<0.05$ was taken as statistically significant.

RESULTS

Percentage yield

The percentage yield of the extract was 58% w/w.

Cationic composition

The Na^+ and K^+ concentrations of the 6mg/ml HS administered is shown in Table 1. The Na^+ concentration was low whilst the K^+ concentration is high i.e. the potassium;sodium ratio is high.

Table 1. Cationic composition of 6mg/ml aqueous extract of *Hibiscus sabdariffa* (HS)

Cation	Concentration (mmol/l)
Potassium	840
Sodium	3.6

Table 2. The mean arterial pressure (MAP) of control rats and those given 8% Salt, aqueous extract of *Hibiscus sabdariffa* (HS; 6mg/ml), Salt+HS and 0.25mg/Kg Furosemide

Group	Mean Arterial Pressure (mmHg)
Control	113.2 ± 3.0
Salt	184.6 ± 29.8*
HS	90.0 ± 7.4***
Salt+HS	119.4 ± 8.9#
Furosemide	94.9 ± 11.5**

Values are mean ± SEM (n=8 each) * =P<0.05 versus control, ** =P< 0.01 versus salt, *** =p<0.001 versus salt, #=p<0.05 versus salt

Blood pressure and Heart rate of rats

The mean arterial pressure (MAP) of salt loaded rats was significantly higher than control, HS, salt+HS, and furosemide groups (Table 2). The MAP of HS, salt+HS and furosemide groups did not differ significantly from each other. A similar trend to this was observed for systolic (Fig. 1) and diastolic (Fig. 2) pressures of the different rat groups when they were compared. The Heart rate of salt loaded rats was significantly ($p<0.05$) higher than that of control rats only

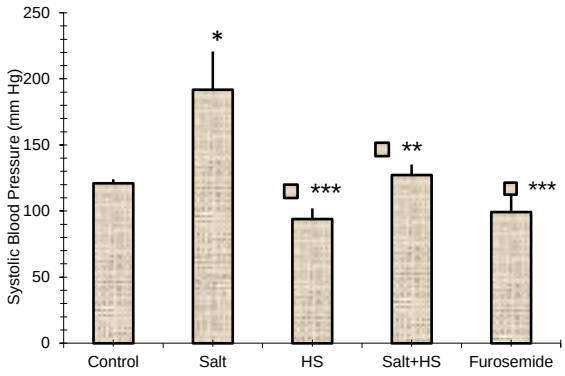

Fig. 1. The systolic blood pressure of control rats and those given salt, HS, salt+HS and Furosemide. Values are presented as mean and SEM (n=8 each). HS: *Hibiscus sabdariffa* extract. * =p<0.05 vs control, **= p<0.05 vs salt *** =p<0.001 vs salt.

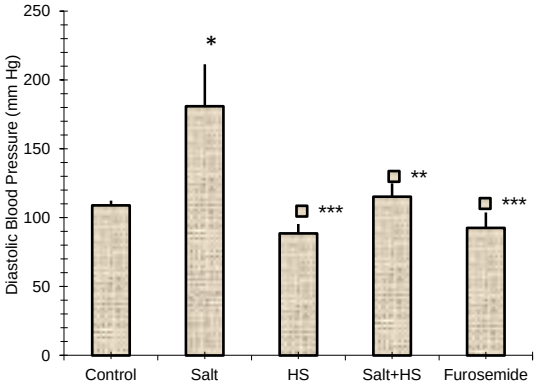

Fig. 2. The diastolic blood pressure of control rats and those given salt, HS, salt+HS and Furosemide. Values are presented as mean ± SEM (n=8 each). HS: *Hibiscus sabdariffa* extract. * =p<0.05 vs control, **=p<0.05 vs salt, *** =p<0.01 vs salt

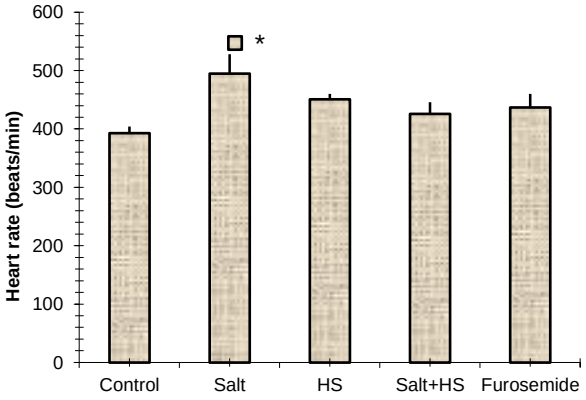

Fig. 3. The Heart rate of control rats and those given salt, HS, salt+HS and Furosemide. Values are presented as mean ± SEM (n=8 each). HS: *Hibiscus sabdariffa* extract. * = p<0.05 vs control.

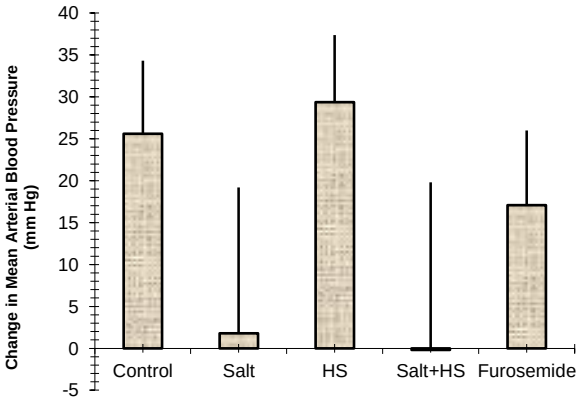

Fig. 4. Changes in mean arterial blood pressure (MAP) in response to the administration of Noradrenalin in control rats and those given salt, HS, salt+HS and Furosemide. Values are presented as mean ± SEM (n=8 each). HS: *Hibiscus sabdariffa* extract.

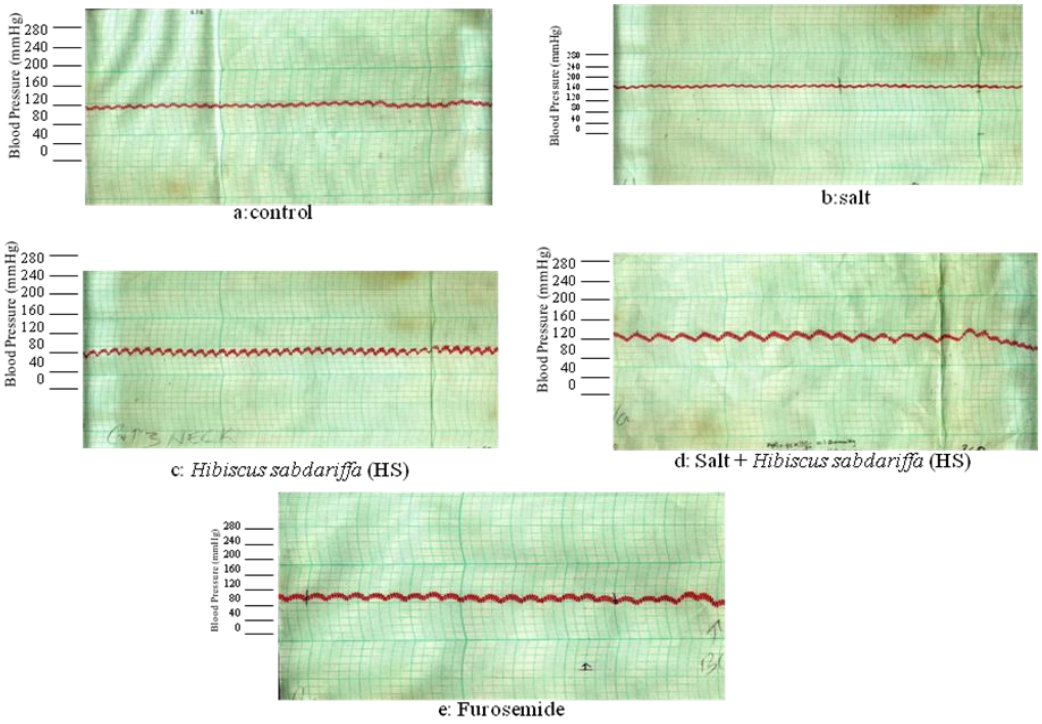

Fig. 5. Typical blood pressure tracings of control rats (a) and those chronically given salt (b), HS (c) salt + HS (d) and furosemide (e).

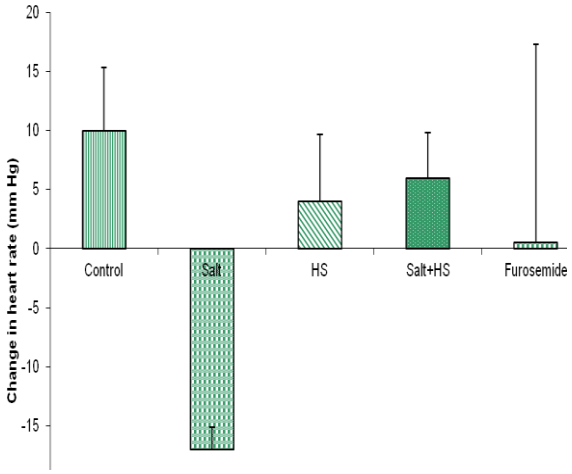

Fig. 6: Changes in heart rate in response to the administration of Noradrenalin in rats given salt, HS, salt+HS and Furosemide. Values are presented as Mean and SEM (n=8 each). HS: *Hibiscus sabdariffa* extract.

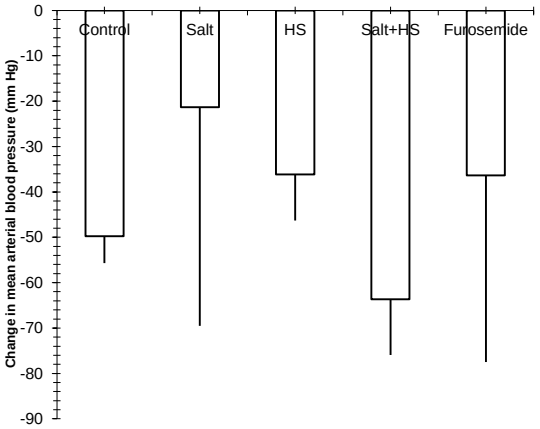

Fig. 7: Changes in Mean arterial blood pressure in response to the administration of Acetylcholine in control rats and those given salt, HS, salt+HS and Furosemide. Values are presented as mean ± SEM (n=8 each). HS: *Hibiscus sabdariffa* extract.

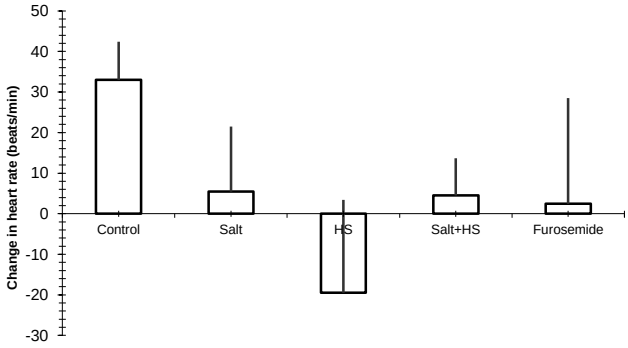

Fig. 8. Changes in heart rate in response to administration of Acetylcholine in control rats and those given salt, HS, salt+HS and Furosemide. Values are presented as mean and SEM (n=8 each). HS: *Hibiscus sabdariffa* extract.

(Fig. 3). The typical tracings of the blood pressures of the different rat groups are presented in Figure 5.

Blood pressure and Heart rate responses to noradrenalin and acetylcholine

The responses to noradrenalin (Figs. 4 and 6) or acetylcholine (Figs. 7 and 8) did not differ significantly in all groups. An overall comparison of the blood pressure and heart rate data for the HS and furosemide groups (Table 2 and Figs. 1-8) shows a striking similarity between the two. The data showed no significant difference from each other.

DISCUSSION

The major new finding in this study is that HS attenuated the development of salt-induced hypertension in rats. This attenuation of hypertension may not be due to altered pressor response to noradrenalin or altered depressor response to acetylcholine. Instead it may be due to the high K^+ concentration of HS or high potassium: sodium ratio. To our knowledge, this is the first report to show that the aqueous calyx extract of *Hibiscus sabdariffa* can attenuate the development salt induced hypertension. Hence the hypothesis that HS may attenuate the development of hypertension, as propounded in this study, may be accepted.

Dietary salt-loading of inbred rats produces a useful animal model for studying salt-sensitive hypertension (Sofola et al., 2002; Mojiminiyi et al., 2007). The present findings confirm this notion since the rats used in this study became hypertensive following dietary salt-loading. Essential hypertension in black Africans and black Africans in the Diaspora is largely salt-sensitive (Luft and Weinberger, 1997; Fields et al., 2004; O'Shaughnessy and Karet, 2004) i.e. the hypertension is associated with excess salt consumption. Extrapolation of the findings of this study to humans may indicate that HS may be useful in attenuating or preventing the development salt-sensitive hypertension in black Africans. It will be interesting to investigate this. However studies in other non-negroid races have shown that HS lowers blood pressure in hypertensive human subjects (Haji Faraji and Haji Tarkhani, 1999; Herrera-Arellano et al., 2004; Herrera-Arellano et al., 2007).

Information on the cationic composition of HS is scanty. Hence this study sought to address this problem. Our results (Table 1) suggest that HS is high in K^+ and low in Na^+ i.e. its potassium:sodium ratio is high. Its efficacy in attenuating salt-induced hypertension, as seen this study, may be associated with its high K^+ concentration or high potassium:sodium ratio. This is because previous works such as the Dietary Approach to Stop Hypertension (DASH) study have shown that the ability of plant/fruit based diets to prevent

hypertension is associated with the high potassium concentration (Morris et al., 1999; Appel et al., 2006) or high potassium:sodium ratio (Androgue and Madias, 2007) of such diets. Indeed the diet of early man, which was largely fruit/plant-based, had these features and so hypertension was rare or non-existent because the kidneys could easily conserve the little sodium and eliminate the surfeit of potassium (O'Shaughnessy and Karet, 2004). Furthermore, Kempner's rice-fruit diet (Kempner, 1945), one of the earliest effective treatment approaches to hypertension was also low in sodium but rich in potassium. The high concentration of potassium in HS may act similarly to a high-potassium diet which has been shown to stimulate the sodium pump and open potassium channels (Amberg et al., 2003; Haddy, Vanhoutte and Feletou, 2006). These effects result in the hyperpolarization of the endothelial cells which ultimately spreads to the vascular smooth muscle (VSM) producing a fall in VSM intracellular calcium leading to vasodilatation (Amberg et al., 2003; Haddy, Vanhoutte and Feletou, 2006) and a fall in blood pressure.

The higher heart rate of salt loaded rats compared to control rats (Fig. 3) is suggestive of sympathetic activation and implies that the elevated blood pressure may be associated with increased activation of the sympathetic nervous system. In view of the association of the activation of the sympathetic nervous system with the ontogeny of hypertension (Simms et al., 2009), it may be interesting to investigate if the attenuation of hypertension by HS is dependent on the sympathetic nervous system. Again evidence from previous studies suggests that a diet rich in potassium may act on the central nervous system by stimulating the neuronal sodium pump and inhibiting the renin-angiotensin system (Shah and Jandhyala, 1991; Lichtstein and Rosen, 2001; Meneton et al, 2005). These effects inhibit the sympathetic nervous system thereby producing a fall in blood pressure (Shah and Jandhyala, 1991; Lichtstein and Rosen, 2001; Meneton et al, 2005). It is conceivable that HS, on account of its high potassium concentration, might have attenuated hypertension through this mechanism.

Apart from its high potassium concentration or its high potassium:sodium ratio, the antioxidant effects of HS (Ajiboye, et al., 2011; Peng, et al., 2011; Frank et al., 2012) may also have contributed to its ability to attenuate hypertension presumably by reducing the oxidative stress associated with hypertension (Touyz and Briones, 2011). HS is rich in polyphenols, flavonoids and anthocyanins (Lin, Chen and Wang, 2011) and these may account for its antioxidant actions.

The striking similarity of blood pressure and heart rate data for HS and furosemide groups and the lack of significant difference between them suggest that they may be acting in a similar fashion and that the effects of HS and furosemide on blood pressure are comparable. Furosemide is a standard diuretic and HS has been reported to have diuretic properties (Mojiminiyi, 2000; Alorcon-Alonzo et al., 2012).

Earlier studies (Sofola et al., 2002; Mojiminiyi et al., 2007) carried out dietary salt-loading for 4-8weeks to induce hypertension. These studies focused on the developmental stages of hypertension. The period for dietary salt loading was extended to twelve weeks in the present study in order to cover the developmental phase as well as the chronic phase of hypertension.

The present study suggesting the attenuation of hypertension by HS is a follow up to our earlier work (Mojiminiyi et al., 2007) that showed that acute parenteral administration of HS lowered blood pressure in hypertensive rats. In that study, the rats were first made hypertensive via dietary salt loading or nitric oxide sythase inhibition and then HS was given intravenously. In the present study both the pressor agent (salt) and HS were administered concurrently.

In conclusion the findings of the present study suggest that HS attenuated the development of hypertension in salt-loaded rats. This action may be associated with the high K^+ concentration of HS or its high potassium: sodium ratio and not with a lowering of the pressor response to noradrenalin or enhanced depressor/vasodilator response to acetylcholine. Also, the effects of HS and furosemide on blood pressure are comparable.

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REFERENCES

Adeneye, A.A., Ajagbonna, O.P., Mojiminiyi, F.B.O., Odigie, I.P., Ojobor, P.D., Etarrh, R.R., Adeneye, A.K. (2006). The hypotensive mechanisms for the aqueous stem bark extract of *Musanga cecropioides* in Sprague-Dawley rats. J. Ethnopharmacol. 106: 203–207.

Ajiboye, T.O., Salawu, N.A., Yakubu, M.T., Oladiji, A.T., Akanji, M.A., Okogun, J.I. (2011). Antioxidant and drug detoxification potentials of Hibiscus sabdariffa anthocyanin extract. Drug Chem. Toxicol. 34: 109-115.

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: 751-756.

Amberg, G.C., Bonev, A.D., Rossow, C.F., Nelson MT, Santana LF. (2003). Modulation of the

molecular composition of large conductance, Ca2+ activated K+ channels in vascular smooth muscle during hypertension. J. Clin. Invest.112:717-724.

Androgue, H.J., Madias, N.E. (2007). Sodium and Potassium in the Pathogenesis of Hypertension. N. Eng. J. Med. 356: 1966-1978.

Appel, L.J., Brands, M. W., Daniels, S. R., Karanja, N., Elmer, P. J., Sacks, F. M. (2006). Dietary Approaches to Prevent and Treat Hypertension: A Scientific Statement From the American Heart Association. Hypertension 47: 296-308.

Fields, L.E., Burt, V.L., Cutler, J.A., Hughes, J., Roccella, E.J., Sorlie, P. (2004). The burden of adult hypertension in the United States 1999 to 2000: a rising tide. Hypertension 44:398 – 404.

Frank, T., Netzel, G., Kammerer, D.R., Carle, R., Kler, A., Kriesl, E., Bitsch, I., Bitsch, R., Netzel, M. (2012). Consumption of Hibiscus sabdariffa L. aqueous extract and its impact on systemic antioxidant potential in healthy subjects. J. Sci. Food Agric. 92:2207-2218.

Haddy, F.J., Vanhoutte, P.M., Feletou, M. (2006). Role of potassium in regulating blood flow and blood pressure. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290:R546-R552.

Haji Faraji, M., Haji Tarkhani, A. (1999). The effect of sour tea (Hibiscus sabdariffa) on essential hypertension. J. Ethnopharmacol. 65: 231–236.

Herrera-Arellano, A., Flores-Romero, S., Chavez-Soto, M.A., Tortoriello, J. (2004). Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial. Phytomedicine, 11: 375–382.

Herrera-Arellano, A., Miranda-Sanchez, 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: 6–12.

Kempner, W. Compensation of renal metabolic dysfunction: treatment of kidney disease and hypertensive vascular disease with rice diet. (1945). N. C. Med. J. 6:61-87.

Lichtstein, D., Rosen, H. (2001). Endogenous digitalis-like Na+, K+-ATPase inhibitors and brain function. Neurochem. Res. 26:971-978.

Lin, H.H., Chen, J.H., Wang, C.J. (2011). Chemopreventive properties and molecular mechanisms of the bioactive compounds in Hibiscus sabdariffa Linne. Curr. Med. Chem. 18(8):1245-1254.

Luft, F.C. and Weinberger, M.H. (1997). Heterogeneous responses to changes in dietary salt intake: the salt-sensitivity paradigm. Am. J. Clin. Nutr. 65(2 Suppl):612S-617S.

Meneton, P., Jeunemaitre, X., de Wardener, H.E., MacGregor, G.A. (2005). Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. Physiol. Rev. 85:679-715.

Mojiminiyi, F.B.O., 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: 292-297.

Mojiminiyi, F.B.O., 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.

Morris, R.C. Jr., Sebastian, A., Forman, A.,Tanaka, M., Schmidlin, O. (1999). Normotensive salt sensitivity: effects of race and dietary potassium. Hypertension 33:18-23.

Odigie, P.I., Ettarh, R.R., Adigun, S. A. (2003). Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-IC hypertensive rats. J. Ethnopharmacol. 86: 181-185.

O'Shaughnessy, K.M., Karet, F.E. (2004). Salt handling and hypertension. J. Clin. Invest. 113:1075-1081.

Peng, C.H., Chyau, C.C., Chan, K.C., Chan, T.H., Wang, C.J., Huang, C.N. (2011). Hibiscus sabdariffa polyphenolic extract inhibits hyperglycemia, hyperlipidemia,
and glycation-oxidative stress while improving insulin resistance. J. Agric. Food Chem. 59: 9901-9909.

Shah, J., Jandhyala, B.S. (1991) Role of Na+, K+-ATPase in the centrally mediated hypotensive effects of potassium in anaesthetized rats. J. Hypertens. 9:167-170.

Simms, A. E., Paton, J. F. R., Pickering, A. E., Allen, A. M. (2009). Amplified respiratory–sympathetic coupling in the spontaneously hypertensive rat: does it contribute to hypertension? J. Physiol. 587:597–610.

Sofola, O.A., Knill, A., Hainsworth, R., Drinkhill, M. (2002). Change in endothelial function in mesenteric arteries of Sprague-Dawley rats fed a high salt diet. J. Physiol. 543:255-260.

Touyz, R.M., Briones, A.M. (2011). Reactive oxygen species and vascular biology: implications in human hypertension. Hypertension Res. 34(1):5-14.