

AMELIORATIVE EFFECT OF AQUEOUS EXTRACT OF *HIBISCUS SABDARIFFA* (ROSELLE) ON SALT-INDUCED HYPERTENSION IN WISTAR RATS

Balogun, Morufu Eytayo^{1,2,*}; Besong, Elizabeth Enohnyaket¹; Obimma, Jacinta Nkechi¹; Iyare, Eghosa E.²; Nwachukwu, Daniel Chukwu².

¹Department of Physiology, Faculty of Basic Medical Sciences, Ebonyi State University, Ebonyi State, Abakaliki 480214, Nigeria

²Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Enugu 400241, Nigeria

Email address: *marufbalogun@yahoo.com

Abstract

Hibiscus sabdariffa L., Malvaceae, was reported for its culinary and therapeutic uses, especially in alternative medicine. The aqueous leaf extract of *H. sabdariffa* (AEHS) was evaluated for its ameliorative potentials on salt-induced hypertensive rats using an *in vivo* assay. Hypertension was induced experimentally in Groups 2 to 5 by placing the rats on high-sodium diet containing 8% sodium chloride for 6 weeks. Rats in Group 1 were orally treated with distilled water (2 mL/kg) only as normal control group. Groups 2 to 5 were treated with 2 mL/kg of distilled water (hypertensive control group), and 100, 200, and 400 mg/kg of AEHS (test groups), respectively, once daily for 6 weeks during hypertension induction. Blood pressure, heart rate and antioxidant parameters were determined. Serum content levels of total cholesterol (TC), triglycerides (TAG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and high-density lipoprotein cholesterol (HDL-C) were also assessed. Treatment with AEHS significantly ($P < 0.05$) reduced systolic (SBP), diastolic (DBP), mean arterial blood pressure (MAP) and heart rate (HR) in comparison to hypertensive control group. The extract also exhibited a significant ($P < 0.05$) reduction in serum levels of TC, TAG, LDL-C, VLDL-C and malondialdehyde (MDA) while a significant ($P < 0.05$) increase in serum activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_x), and HDL-C concentration were observed. The results suggest that AEHS offered an ameliorative action against salt-induced hypertension in Wistar rats. The ameliorative effect might be mediated via antihyperlipidemic and antioxidative mechanisms.

Keywords: *Hibiscus sabdariffa*, salt-induced hypertension, anti-oxidative, serum lipid profile, rats.

Introduction

Worldwide annually, an estimate of 17 million premature deaths are recorded resulting from cardiovascular diseases (CVD) with hypertension being the leading CVD accounting for 55% of all documented cases [1]. In developed as well as developing countries, high blood pressure (BP) remains a major public health challenge because of its impact on the population mortality and morbidity [2]. The roles sodium as an important nutrient in the body cannot be over emphasized most especially in the proper functioning of muscles and nerves of the body. It also plays crucial roles in auto regulation of water and maintenance of body fluid balance [3].

Consumption of high-sodium diet presents a major challenge to the kidneys to excrete large amounts of salt intake. One of the principal organ systems vulnerable to the devastating effects of excessive dietary sodium intake is the cardiovascular system. Previous reports in laboratory animal studies showed that high dietary salt intake predisposes to elevated BP [3,4]. On the other hand, hypertension and hyperlipidemia are associated with oxidative stress and have been indicated in the pathogenesis of several chronic diseases including renal disease, stroke, heart failure and increase incidence of CVD [5,6].

Lifestyle modifications, physical exercises, low dietary salt consumption, intake of healthy diets are some common issues associated with reducing the risk of CVD including raised BP. However, at a critical stage drugs are indispensable. Although new antihypertensive orthodox drugs with improved efficacy have been introduced to the market, they still possess side effects. Moreover, because of limited resources, synthetic drug treatment may not be affordable to the majority of hypertensive patients in developing countries such as Nigeria. Therefore, it is of great significance to discover natural therapeutics with little or no side effects for prevention, treatment and management of hypertension.

In recent years, there has been growing interest on herbal preparations which are traditionally used as therapeutic agents in the prevention, treatment and management of hypertension [7,8]. Several lines of evidence indicate that naturally occurring medicinal plants possess antihypertensive activity in

laboratory animal studies [9,10]. One of the herbal plants that may have a great prospect as an antihypertensive agent is *Hibiscus sabdariffa*.

Hibiscus sabdariffa L. (family: Malvaceae) is one of the emerging plants of interest used for hypertension treatment, management and prevention. It is a medicinal plant, commonly known as *Roselle*, *Red sorrel*, *Hibiscus* in English and Arabic as *Karkadeh* [11,12]. *Hibiscus sabdariffa* is popularly consumed in Nigeria as a refreshing drink called *Zobo*. In Nigeria, different tribal groups have their indigenous names as: *Yakuwa* in Hausa, *Amukan* in Yoruba and *Okworo ozo* in Igbo [13]. It is an annual shrub which grows up to an altitude of about 0.5 to 3.0 m, with a strong tap-root and cultivated for its leaf, fleshy calyx, seed or fibre [14]. It has been widely used in Nigerian traditional medicine as an antihypertensive, aphrodisiac, digestive, diuretic, stomachic and tonic agent [15,16].

Previous scientific studies on the antihypertensive activities of the plant have focused on the calyx [17,18], petal [19,20], seed [21] and leaf extracts [4]. According to previous findings in animal experiments, *H. sabdariffa* leaf extracts have been shown to possess many biological potencies such as hypoglycemic [22], hypolipidemic [23], antioxidative [24], anti-inflammatory [25] and anticancer effects [26]. Meanwhile, the aqueous leaf extract of *H. sabdariffa* has a powerful folk reputation as a non-pharmacological antihypertensive agent. However, there still exists paucity of report in literature regarding the antihypertensive activity of aqueous extract of *H. sabdariffa* leaves. Thus, the present study aimed to investigate the possible ameliorative effect of the aqueous leaf extract of *H. sabdariffa* (AEHS) on salt-induced hypertension in Wistar rats. Furthermore, the possible antihypertensive mechanisms of action in hypertensive rats were also investigated.

Materials and Methods

Chemicals and reagents

Commercially obtained laboratory chemicals and reagents used in this investigation were of analytical grades and were purchased from Sigma-Aldrich Chemical Company (St. Louis Missouri, USA). Sodium chloride (NaCl) salt was purchased from Polypharm Private Limited (Mumbai, India). Distilled

water was procured from Human Physiology Department Laboratory, Ebonyi State University, Abakaliki, Nigeria.

Drug preparation

Sodium pentobarbital (Nembutal): Sodium pentobarbital (Embassy Pharmaceutical and Chemicals Ltd, Nigeria) was purchased from Godal Pharmacy, Abakaliki, Nigeria. The rats were anaesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 100 mg/kg body weight (5 mL/kg) as described by us [4].

Animals

Weanling adult male (10–12 weeks old) Wistar rats (inbred) weighing (196.50 ± 2.93 g) were used for the study. The animals were obtained from the College of Medicine, Central Animal Unit, University of Nigeria, Enugu Campus, Nigeria. They were maintained on standard rat pellets (Vital Feeds Nig Ltd) and water *ad libitum* and were kept under standard laboratory conditions (12 h light-dark cycle at 18–26 °C and relative humidity of 30% to 70%). The animals were acclimatized 1 week before the experiment commenced.

Ethical clearance

This study was carried out following the ethical approval by the College of Medicine ethics committee for animal experimentation of University of Nigeria, Nsukka (Ethic No. NHREC/05/01/2508B-FWA00002458-1RB00002323) and animal handling was according to accepted guidelines by the National Institute of Health for care and use of laboratory animals [27].

Collection of plant materials and identification

The fresh leaves of *H. sabdariffa* were collected from Gboro farm settlement in Iseyin Local Government Area of Oyo State, Nigeria in the month of August. The plant specimen was identified and authenticated by Mr. K. A. Adeniji in the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen (FHI. 110315) has been earlier deposited at the Herbarium of the institute.

Preparation of aqueous leaf extract

Fresh leaves of *H. sabdariffa* were air-dried and pulverized into fine powder using a suitable grinder. A powdered dried leaf (500 g) was weighed and macerated in 4000 mL of hot distilled water at 96 °C for 2 h and the mixture was vacuum-filtered through Whatman No. 1 filter paper. The extracted solution was concentrated using a vacuum rotary evaporator (Eyla N-1000, Japan) maintained at 45 °C. The resulting residue which weighed 52.5 g (recovery 10.5%) was stored at –22 °C before use. Prior to oral administration of the extract, the required dose of 100, 200 and 400 mg/kg body weight was obtained after the extract was reconstituted in distilled water.

Qualitative phytochemical screening

Freshly prepared crude AEHS was screened for the presence of secondary constituents (such as saponins, tannins, flavonoids, anthraquinones, alkaloids, phenols and steroids) using standard phytochemical procedures and tests previously described by Harborne [28].

Acute oral toxicity studies

The “fixed dose” method of Organization for Economic Cooperation and Development (2008) guideline No. 425 was adopted to assess the acute oral toxicity of AEHS in adult Wistar rats [29]. The method was commenced with an initial dose of 5000 mg/kg body weight after overnight dietary deprivation. The animals were observed for general behavioural, autonomic and neurological behaviour during the course of the experiment.

Experimental study design and treatment

Following 1 week adaptation, the rats were allotted into 5 different groups each consisting of 5 rats. Hypertension was induced experimentally in Groups 2 to 5 by salt-loading the rats with high-sodium diet containing 8% NaCl throughout the experimental period. Group 1 (normal control) received 2 mL/kg of distilled water only. Group 2 (hypertensive control) received distilled water (2 mL/kg) during modelling. Groups 3, 4 and 5 (test groups) received 100, 200 and 400 mg/kg body weight (5 mL/kg body weight) of AEHS respectively. All the groups were treated for 6 weeks concurrently with hypertension induction between

08:00 a.m. and 09:00 a.m., once a day by oral gavage.

Induction of experimental hypertension

In this study, hypertension was induced experimentally using salt-induced hypertension model previously described by Sofola et al [30] and Balogun et al [4]. In this method, the rats were placed on high-sodium diet with 8% NaCl for a period of 6 weeks. The high salt chow was prepared as previously described by Sofola et al [30]. The normal rat chow contained 0.3% NaCl by weight of sodium.

Measurement of blood pressure parameters

Blood pressure and heart rate of conscious rats were determined and documented at the onset of the experiment and weekly. A non-invasive method was used to measure systolic (SBP), diastolic (DBP), mean arterial blood pressure (MAP) and heart rate (HR) using an automated computerized tail-cuff blood pressure monitor, the CODA II™ NIBP recording system (Kent Scientific Corporation, USA), according to instructions of the manufacturer.

Protocol for blood sampling and biochemical assay

At the end of the experimental period, all the rats were fasted for 12 h and anaesthetized with intraperitoneal injection of sodium pentobarbital at a dose of 100 mg/kg body weight (5 mL/kg body weight). Fasting blood samples were collected through cardiac puncture technique into labeled sterile plain tubes for biochemical serum estimation. Blood samples for sera preparation was allowed to clot at room temperature for 1 h, and then centrifuged at 3000 x g for 10 mins at 4 °C. The clear serum was collected with Pasteur pipette into clean, dry sample bottles and stored in refrigerator under 4 °C until required. All biochemical analyses were completed within 24 h of sample collection.

Assay of antioxidant and enzymatic activities

Serum contents of superoxide dismutase (SOD) activity and the malondialdehyde (MDA) were estimated using assay kits according to instructions of the manufacturer (Randox Laboratories Ltd., UK). Serum activities of catalase (CAT) and glutathione peroxidase (GPx) were evaluated using a commercially available enzyme-linked

immunosorbent assay (ELISA) kits (Cloud Clone, USA) as described by manufacturer.

Serum lipid profile assessment

The total cholesterol (TC), triglycerides (TAG) and high-density lipoprotein-cholesterol (HDL-C) contents in the serum were estimated by enzymatic calorimetric methods using standard commercial assay kits (Randox Laboratories Ltd., UK), according to instructions of the manufacturer. The serum very low-density lipoprotein cholesterol (VLDL-C) was calculated by dividing the serum TAG by 5 [31]. Serum low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald et al [31] and Warnick et al [32] using the formula: LDL-C (mg/dL) = TC - (HDL-C) - (VLDL-C).

Statistical analysis

All the data are expressed as mean ± SEM. Data were analyzed using One-way analysis of variance (ANOVA). Bonferroni post-hoc test using GraphPad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA) was used to compare the means. Values of $P < 0.05$ were established to be statistically significant.

Results

Extract yield

The aqueous extraction of 500 g of *H. sabdariffa* leaf powder yielded 10.5% (w/w) dark brown semisolid extract.

Preliminary qualitative phytochemical studies

The result of the preliminary qualitative phytochemical analysis of AEHS revealed the presence of saponins, tannins, flavonoids, alkaloids, phenols and steroids.

Acute oral toxicity studies

All the rats remained alive and showed no visible signs of drug induced toxicity even with the highest administered dose. There were no abnormal signs, changes in body weight and behaviour in the AEHS-treated rats throughout the period of observation compared to normal control rats. Based on these observations, the median lethal dose was established to be greater than 5000 mg/kg body weight.

Effect of AEHS on blood pressure and heart rate of salt-induced hypertensive rats

The effects of AEHS on blood pressure and heart rate are presented in Tables 1. Hypertensive control group showed significant ($P < 0.05$) increase in SBP, DBP, MAP and HR as compared to the normal control group. The severity of salt loading-induced hypertension was significantly ($P < 0.05$) reduced by treatment with AEHS in comparison to the hypertensive control group. However, in AEHS-treated groups, maximum inhibition was observed at a dose of 400 mg/kg body weight of AEHS. The effects of AEHS on SBP, DBP, MAP and HR were dose-dependent ($P < 0.05$).

Effect of AEHS on antioxidant activities of SOD, CAT, GPx and MDA level of salt-induced hypertensive rats

The effects of AEHS on serum contents of oxidative stress biological markers (SOD, CAT, GPx and MDA) of salt-induced hypertensive rats are reported in Table 2. The SOD, CAT and GPx activities were significantly ($P < 0.05$) reduced in the hypertensive control group compared with the normal control group. However, serum concentration of MDA was significantly ($P < 0.05$) elevated in the hypertensive control group in comparison to normal control group. On the other hand, serum activities of SOD, CAT and GPx were significantly ($P < 0.05$) improved by treatment with AEHS compared to hypertensive control group. In contrast, AEHS-treated groups showed significant ($P < 0.05$) reduction in serum MDA level when compared to the hypertensive control group. The AEHS produced a significant ($P < 0.05$) and dose-dependent ($P < 0.05$) effect on the serum contents of the evaluated antioxidant parameters.

Effect of AEHS on serum lipid profile of salt-induced hypertensive rats

The effects of AEHS on serum levels of TC, TAG, HDL-C, LDL-C and VLDL-C are shown in Table 3. Daily consumption of high sodium chloride diet significantly ($P < 0.05$) increased serum concentrations of TC, TAG, LDL-C and VLDL-C in the hypertensive control group with concomitant decrease in HDL-C level when compared to the normal control group. However, the AEHS-treated groups showed a significant ($P < 0.05$) and dose

dependent ($P < 0.05$) reduction in serum levels of TC, TAG, LDL-C and VLDL-C compared to the hypertensive control group. In contrast, a significant ($P < 0.05$) and dose dependent ($P < 0.05$) increase in serum HDL-C level was observed in groups treated with AEHS in comparison to the hypertensive control group.

Discussion

There is growing interest on the identification and evaluation of drugs from natural sources as an alternative therapeutic approach in the prevention and management of CVD including elevated BP. The present investigation employed salt-induced hypertension model to investigate the possible ameliorative potential of AEHS. This model represents some of the most common etiologies of hypertension in humans, and demonstrated that AEHS possesses antihypertensive activity against salt loading-induced hypertension in rats. An extensive literature survey showed that, this study is the first to demonstrate that AEHS is capable of attenuating the development of salt-induced hypertension in rats. The results of oral toxicity studies suggest that AEHS is virtually non-toxic and thus may not have any immediate adverse effects as done in folk medicine. Our findings were in agreement with other previous reports, which showed that lethal dose (LD_{50}) was greater than 5000 mg/kg body weight [33,34].

According to several researchers, the pathogenic effect of dietary salt-induced hypertension have been established to include increased BP (SBP, DBP and MAP), and HR, reduced serum antioxidative enzymes activities, SOD, CAT, GPx with concomitant increased in MDA concentration and alteration of serum lipid contents [35,36]. Our results showed that in salt-induced hypertensive rats, the AEHS-treated groups (100, 200 and 400 mg/kg body weight) produced a remarkable reduction in SBP, DBP, MAP and HR in comparison to the hypertensive control group. Maximum reduction in BP and HR were observed at 400 mg/kg dose which produced significant antihypertensive effects in hypertensive rats. However, the mechanisms behind these antihypertensive effects of AEHS in salt-induced hypertensive rats have not yet been unravelled. The observed decrease in BP and HR may be due to a reduction in oxidative stress and

lipid contents as evidenced from the serum total antioxidant and lipid profile status. This is an indication that AEHS contains some phytochemical compounds that reduce the BP and HR which had been increased by salt-loading with high salt diet.

According to previous studies, the possible mechanisms by which high salt diets induced hypertension could be due to increase in the level of circulating sodium which cause cells to release water due to osmotic pressure which raises the pressure on blood vessel walls [37,38]. On the other hand, it could be possibly due to over generation of superoxide anions and other free radicals due to activation of NADPH oxidase may overwhelm the antioxidant capability and cause imbalances between oxidant and antioxidant status which may result in oxidative stress [39,40].

Previous reports in animal experiments have implicated oxidative stress in pathogenesis of salt-induced hypertension [4,36]. It was observed in the current study that salt-induced hypertension *via* oxidative stress was induced by salt-loading the rats with high salt diet, since it elicits lipid peroxidation and affects antioxidative enzyme activities in the serum. This is demonstrated by the increase in the level of total antioxidant and activities of SOD, CAT, GPx, and remarkably attenuated the increase in MDA content following treatment with AEHS which was overwhelmed by salt-induced oxidative stress. Our findings are consistent with several reports both *in vitro* [24], and *in vivo* [41] which have shown that AEHS had potent antioxidant effect. The antioxidant activity of AEHS is due to its strong scavenging effect on reactive oxygen species and free radicals [41,42]. Antioxidants have been reported to play crucial role in the attenuation of development of salt-induced hypertension [4,36]. Significant amounts of antioxidant active compounds have been reported to be present in the leaves of *H. sabdariffa* [40]. The active compounds flavonoids, alkaloids and phenols which are present in the *H. sabdariffa* leaves have been reported by Chen et al. [43] and Chiu et al. [40] as antioxidant materials. Therefore, it is suggested that the antioxidant potential of this leaf extract, which offers a first line of defence against salt-induced hypertension by lowering serum oxidative stress, may be attributed to its antihypertensive activity.

An anomaly in lipids and lipoprotein metabolism has been also been implicated in the pathogenesis of hypertension [10]. High BP and hyperlipidemia are so common in both human and animal models of hypertension and many have argued that the raised BP itself may play a role in disrupting lipid metabolism, resulting in abnormalities. Findings from this study showed a decrease in the serum levels of TC, TAG, LDL-C and VLDL-C and enhanced HDL-C in salt-induced hypertensive rats after treatment with AEHS. These observations suggest the possible antihyperlipidemic potential of this leaf extract. High serum concentration of TC and LDL-C are major predisposing risk factors for CVD, whereas enhanced HDL-C is associated with a decrease in CVD risk. Accumulating evidence show that an increase in HDL-C is associated with a reduction in coronary risk factors [44] and most of the antihypertensive medications that reduce TC also reduce HDL-C [45]. However, in the present study the AEHS markedly reduced serum TC, TAG, LDL-C and VLDL-C and enhanced the HDL-C significantly. This is an indication that AEHS contains some biological compounds that reduce serum TC and enhanced HDL-C which had been disrupted by salt-loading with high salt diet.

According to our findings, AEHS has been shown to contain secondary metabolites such as flavonoids, alkaloids and phenols which are among the antihypertensive materials known to possess hypolipidemic [23], antioxidant [24], and anti-inflammatory activities [25]. It is presumed that these biological compounds can enhance activities of antioxidant enzymes and attenuate the damaging effects of free radicals in the serum [40,46]. Nevertheless, it is pertinent that in this study, the AEHS reduced BP and HR, enhanced serum HDL-C and improved the activities antioxidant enzymes, all of which could be due to the presence of these biological compounds or some other mechanisms yet to be established. However, further studies are still required to elucidate the exact mechanism of action and isolate the active ingredients in the leaves responsible for the antihypertensive effects of AEHS so as to offer new alternatives for the clinical management of hypertension. In conclusion, our findings suggest that AEHS possesses significant ameliorative effects against salt-induced hypertension in Wistar rats. The observed

ameliorative effect might be possibly due to its antihyperlipidemic and antioxidative potencies. Findings from this study may have favorable application in the management of hypertension associated with salt-induced hypertension.

References

1. World Health Organization. A global brief on hypertension: silent killer, global public health crisis: World Health Day 2013. World Health Organization; 2013.
2. Guo X, Zou L, Zhang X, Zheng L, Sun Z. Prehypertension : A Meta analysis of the epidemiology, risk factors, and predictors of progression. *Tex Heart Inst J* 2011; 38(6): 643-652.
3. Gomes PM, Sá RW, Aguiar GL, Paes MH, Alzamora AC, Lima WG, et al. Chronic high-sodium diet intake after weaning lead to neurogenic hypertension in adult Wistar rats. *Sci Rep* 2017;7(1):5655.
4. Balogun ME, Nwachukwu DC, Iyare EE, Besong EE, Obimma JN, Djobissie SF. Antihypertensive effect of methanolic extract from the leaves of *Hibiscus Sabdariffa* L. in rats. *Der Pharmacia Lettre* 2016; 8 (19):473-484.
5. He FJ, MacGregor GA. Salt, blood pressure and cardiovascular disease. *Curr opin Cardiol* 2007; 22(4):298-305.
6. Delahaye F. Should we eat less salt?. *Arch Cardiovasc Dis* 2013; 106(5):324-332.
7. Anwar MA, Al Disi SS, Eid AH. Anti-hypertensive herbs and their mechanisms of action: part II. *Front Pharmacol* 2016; 7:50.
8. Bassey Etim CA, Uzoma I, Uzoigwe J, Chinemerem N, Mercy A, Ogochukwu N, et al. Ameliorative Effect of Aqueous Extract of *Tetracarpidium Conophorum* (African Walnut) on Salt Induced Hypertensive Wistar Rats. *Am J Hypertens* 2018; 5(1): 1-7.
9. Amat N, Amat R, Abdureyim S, Hoxur P, Osman Z, Mamut D, et al. Aqueous extract of *dioscorea opposita thunb.* normalizes the hypertension in 2K1C hypertensive rats. *BMC complement Altern Med* 2014; 14(1):14-36.
10. Uche OK, Osakpolor FA. Kolaviron Attenuates Elevation in Blood Pressure and Ameliorates Dyslipidemia in Salt-Induced Hypertensive Sprague-Dawley Rats. *Afr J Biomed Res* 2018; 21(2):219-224.
11. Ross IA. Medicinal plants of the world: Chemical constituents, traditional and modern medicinal uses (Vol. 1). Humana Press Inc. 2003.
12. Ali BH, Wabel NA, Blunden G. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: a review. *Phytother Res* 2005; 19(5):369-375.
13. Williams CN, Chew WY, Rajaratnam JH. Tree and field crops of the wetter regions of the tropics. Longman Group Ltd.; 1980.
14. Dalziel JM. The useful plants of west tropical Africa. The useful plants of West Tropical Africa. The Crown Agents, London 1937; 314-315.
15. Morton JF. *Roselle*. In: *fruits of warm climate*, (CF Dowling (ed). Media, Inc. Greensboro, NCP. 1987; 281-286.
16. Olaleye MT. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *J Med Plants Res* 2007; 1(1): 009-013.
17. Nwachukwu DC, Aneke EI, Obika LF, Nwachukwu NZ. Effects of aqueous extract of *Hibiscus sabdariffa* on the renin-angiotensin-aldosterone system of Nigerians with mild to moderate essential hypertension: A comparative study with lisinopril. *Indian J. Pharmacol* 2015; 47(5): 540-545.
18. Abdel-Rahman RF, Hessin AF, Abdelbaset M, Ogaly HA, Abd-Elsalam RM, Hassan SM. Antihypertensive effects of roselle-olive combination in L-NAME-induced hypertensive rats. *Oxid Med Cell Longev* 2017; 2017.
19. Aguwa CN, Ndu OO, Nwanma CC, Udeogaranya PO, Akwara NO. Verification of the folkloric diuretic claim of *Hibiscus sabdariffa* L. petal extract. *Nig J Pharm Res* 2004; 3(1):1-8.
20. El-Mahmoudy A, El-Mageid AA, Abdelaleem A. Pharmacodynamics evaluation of *Hibiscus sabdariffa* extract for mechanisms underlying its antihypertensive action:

- Pharmacological and Biochemical aspects. *J Physiol Pharmacol Adv* 2014; 4 (6): 379-388.
21. Bako IG, Mabrouk MA, Maje IM, Buraimoh AA, Abubakar MS. Hypotensive effect of aqueous seed extract of *Hibiscus sabdariffa* linn (Malvaceae) on normotensive cat. *Intern J Animal Veterinary Adv* 2010; 2(1):5-8.
 22. Sachdewa A, Nigam R, Khemani LD. Hypoglycemic effect of *Hibiscus rosa sinensis* L. leaf extract in glucose and streptozotocin induced hyperglycemic rats. *Indian J Exp Biol* 2001; 39, 284–286.
 23. Kuriyan R, Kumar DR, Rajendran R, Kurpad AV. An evaluation of the hypolipidemic effect of an extract of *Hibiscus Sabdariffa* leaves in hyperlipidemic Indians: A double blind, placebo controlled trial. *BMC Complement Altern Med* 2010; 10, 27–34.
 24. Wang J, Cao X, Jiang H, Qi Y, Chin K, Yue Y. Antioxidant activity of leaf extracts from different *Hibiscus sabdariffa* accessions and simultaneous determination five major antioxidant compounds by LC-Q-TOF-MS. *Molecules* 2014;19(12):21226-21238.
 25. Meraiyebu AB, Olaniyan OT, Eneze C, Anjorin YD, Dare JB. Anti-inflammatory activity of methanolic extract of *Hibiscus sabdariffa* on carrageenan induced inflammation in wistar rat. *Int J Pharmaceutical Sci Invention* 2013; 2:22-24.
 26. Chiu CT, Chen JH, Chou FP, Lin HH. *Hibiscus sabdariffa* leaf extract inhibits human prostate cancer cell invasion via down-regulation of Akt/NF-kB/MMP-9 pathway. *Nutrients* 2015a; 7(7):5065-5087.
 27. National Research Council (US). Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. 8th ed. Washington, DC: National Academies Press (US); 2011.
 28. Harborne AJ. *Phytochemical methods: a guide to modern techniques of plant analysis*. 3rd ed. Netherlands: Springer; 1998.
 29. Organization for Economic Cooperation and Development. OECD guidelines for testing of chemicals, Section 4; Test No. 425: acute oral toxicity—up-and-down procedure (UDP). (2008-10-03) [2017-11-12].
 30. Sofola OA, Knill A, Hainsworth R, Drinkhill M. Change in endothelial function in mesenteric arteries of Sprague-Dawley rats fed a high salt diet. *J Physiol* 2002; 543(1):255-260.
 31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18(6):499-502.
 32. Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem* 1990; 36(1):15-19.
 33. Gaya IB, Mohammad OM, Suleiman AM, Maje MI, Adekunle AB. Toxicological and lactogenic studies on the seeds of *Hibiscus sabdariffa* Linn (Malvaceae) extract on serum prolactin levels of albino wistar rats. *Internet J Endocrinol* 2009; 5(2):1-6.
 34. Ndu OO, Nworu CS, Ehiemere CO, Ndukwe NC, Ochiogu IS. Herb–drug interaction between the extract of *Hibiscus sabdariffa* L. and hydrochlorothiazide in experimental animals. *J Med Food* 2011;14(6):640-644.
 35. Tian N, Thrasher KD, Gundy PD, Hughson MD, Manning Jr RD. Antioxidant treatment prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension. *Hypertensn* 2005;45(5):934-939.
 36. Saidu Y, Bilbis LS, Muhammad SA, Nasir MA. Serum Lipid Profile and Antioxidant Status of Salt-induced Hypertensive Rats Treated with an Antioxidants Rich Nutraceutical. *Cameroon J Exp Biol* 2012;8(1):47-54.
 37. Barker M. Phenotypic Expression of Hypertension in Rodent Models through Dietary Manipulation. *Hypertensn* 2002; 40, 83-89.
 38. Mojiminiyi FB, Audu Z, Etuk EU, Ajagbonna OP. Attenuation of salt-induced hypertension by aqueous calyx extract of *Hibiscus sabdariffa*. *Nig J Physiol Sci* 2012; 27(2):195-200.

39. Briones AM, Touyz RM. Oxidative stress and hypertension: Current concepts. *Curr Hypertens Rep* 2010; 12: 135-142.
40. Chiu CT, Hsuan SW, Lin HH, Hsu CC, Chou FP, Chen JH. Hibiscus sabdariffa leaf polyphenolic extract induces human melanoma cell death, apoptosis, and autophagy. *J Food Sci* 2015b; 80(3):H649-658.
41. Joshua PE, Ogara AL, Nkwocha CC, Nduka FO, Okoli PC, Okafor JO, et al. Hepatocurative effect of aqueous extract of Hibiscus sabdariffa on some antioxidants and haematological indices of acetaminophen-challenged Wistar albino rats. *Afr J Pharm Pharmacol* 2017;11(20):250-259.
42. Mohd-Esa N, Hern FS, Ismail A, Yee CL. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. *Food Chem* 2010;122(4):1055-60.
43. Chen JH, Wang CJ, Wang CP, Sheu JY, Lin CL, Lin HH. Hibiscus sabdariffa leaf polyphenolic extract inhibits LDL oxidation and foam cell formation involving up-regulation of LXR α /ABCA₁ pathway. *Food Chem* 2013; 141(1):397-406.
44. Kalam SB, Bhupendra S, Dheeraj G, Bhola N. Assessment of Serum Lipid Profile among Hypertensive patients in Uttarakhand. *Santosh Univ J Health Sci* 2016; 2(2):122-124.
45. Mora S, Glynn RJ, Ridker PM. High-density lipoprotein cholesterol, size, particle number, and residual vascular risk after potent statin therapy. *Circulation* 2013; 128(11):1189–1197.
46. Cassidy A, O'Reilly EJ, Kay C, Sampson L, Franz M, Forman JP, et al. Habitual intake of flavonoid subclasses and incident hypertension in adults. *Am J Clin Nutr* 2011; 93(2):338-347.

Table 1. Effects of AEHS on blood pressure (SBP, DBP and MAP), and heart rate of salt-induced hypertensive rats

Group (beats/min)	Treatment and dose	SBP (mm Hg)	DBP	MAP (mm Hg)	HR (mm Hg)
1 (Control) 433.20 ± 3.69	Distilled water (2 mL/kg)	121.60 ± 1.91		87.50 ± 2.48	101.30 ± 2.53
2 (Hypertensive control) 459.30 ± 2.31*	NaCl (8%) + Distilled water (2 mL/kg)	159.50 ± 2.76*		119.60 ± 3.05*	152.60 ± 1.76*
3 (Test) 447.50 ± 2.07 [#]	NaCl (8%) + AEHS (100 mg/kg)	130.70 ± 0.50 [#]		99.30 ± 0.73 [#]	130.20 ± 0.80 [#]
4 (Test) 439.60 ± 1.58 [#]	NaCl (8%) + AEHS (200 mg/kg)	123.00 ± 3.87 [#]		90.20 ± 2.90 [#]	114.90 ± 3.03 [#]
5 (Test) 432.10 ± 3.55 [#]	NaCl (8%) + AEHS (400 mg/kg)	118.90 ± 0.36 [#]		84.70 ± 1.08 [#]	98.50 ± 2.64 [#]

All values are expressed as mean ± standard error of mean (n = 5 in each group). *P < 0.05, vs control group, [#]P < 0.05, vs hypertensive control group. NaCl: sodium chloride salt; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial blood pressure; HR: heart rate; AEHS: aqueous leaf extract of *Hibiscus sabdariffa*.

Table 2: Effects of AEHS on serum activities of SOD, GPx and CAT and MDA level of salt-induced hypertensive rats

Group	Treatment and dose	SOD (U/mL)	MDA (ng/mL)	CAT	GPx (mIU/mL)
1 (Control)	Distilled water (2 mL/kg)	82.30 ± 4.50	87.30 ± 3.58	958.50 ± 27.35	48.90 ± 4.21
2 (Hypertensive control)	NaCl (8%) + Distilled water (2 mL/kg)	23.70 ± 6.49*	126.80 ± 5.63*	526.30 ± 14.80*	19.60 ± 8.34*
3 (Test)	NaCl (8%) + AEHS (100 mg/kg)	63.50 ± 5.63 [#]	91.00 ± 1.89 [#]	943.70 ± 10.76 [#]	43.00 ± 3.72 [#]
4 (Test)	NaCl (8%) + AEHS (200 mg/kg)	72.60 ± 3.87 [#]	83.20 ± 4.56 [#]	952.60 ± 23.09 [#]	47.90 ± 6.07 [#]
5 (Test)	NaCl (8%) + AEHS (400 mg/kg)	79.80 ± 7.24 [#]	79.30 ± 2.77 [#]	961.80 ± 18.73 [#]	52.70 ± 9.63 [#]

All values are expressed as mean ± standard error of mean (n = 5 in each group). *P < 0.05, vs control group, [#]P < 0.05, vs hypertensive control group. NaCl: sodium chloride salt; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; MDA: malondialdehyde; AEHS: aqueous leaf extract of *Hibiscus sabdariffa*.

Table 3: Effects of AEHS on serum lipid profile of salt-induced hypertensive rats

Group	Treatment and dose	TC	TAG	HDL-C	LDL-C
VLDL-C		(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)
1 (Control)	Distilled water (2 mL/kg)	143.70 ± 5.02	127.30 ± 3.08	64.90 ± 2.25	53.30 ± 5.03
					25.50 ± 2.01
2 (Hypertensive control)	NaCl (8%) + Distilled water (2 mL/kg)	268.10 ± 3.78*	432.80 ± 5.53*	47.30 ± 5.08*	134.20 ± 6.21*
					86.60 ± 0.78*
3 (Test)	NaCl (8%) + AEHS (100 mg/kg)	169.30 ± 2.47 [#]	148.50 ± 1.87 [#]	53.90 ± 3.69 [#]	85.70 ± 3.76 [#]
					29.70 ± 3.62 [#]
4 (Test)	NaCl (8%) + AEHS (200 mg/kg)	152.50 ± 3.96 [#]	136.10 ± 4.73 [#]	61.30 ± 2.41 [#]	64.00 ± 4.60 [#]
					27.20 ± 4.90 [#]
5 (Test)	NaCl (8%) + AEHS (400 mg/kg)	147.60 ± 4.38 [#]	124.30 ± 2.11 [#]	67.80 ± 6.44 [#]	54.90 ± 3.83 [#]
					24.90 ± 1.73 [#]

All values are expressed as mean ± standard error of mean (n = 5 in each group). *P < 0.05, vs control group, [#]P < 0.05, vs hypertensive control group. NaCl: sodium chloride salt; TC: total cholesterol; TAG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; AEHS: aqueous leaf extract of *Hibiscus sabdariffa*.