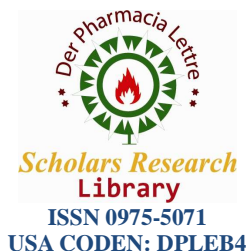




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Antihypertensive effect of methanolic extract from the leaves of *Hibiscus Sabdariffa* L. in rats

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ABSTRACT

The aim of this study was to investigate the antihypertensive effects of methanol extract of *H. sabdariffa* leaves (MEHS) in rats. Hypertension was induced in the rats by adding 8% NaCl in their diet (salt-loading) for 6 weeks. The rats were randomly divided into five groups of 5 rats each. Group 1 was the normotensive control group and was fed with normal rat chow and water ad libitum; groups 2 and 3 were the hypertensive and positive control groups respectively while groups 4 and 5 were the experimental groups. Group 2 was given normal rat chow and water while group 3 was treated with 30 mg/kg captopril. Groups 4 and 5 were treated (p.o) with 200 and 400 mg/kg b. wt. of MEHS respectively per day for 4 weeks. The LD₅₀ of MEHS was greater than 2000 mg/kg. Qualitative phytochemical studies revealed the presence of saponins, tannins, flavonoids, phenols, steroids, triterpenoids, and fixed oils. The MEHS significantly reduced ($p < 0.05$) blood pressure and heart rate in hypertensive rats in a dose-dependent manner. The blood pressure reduction was associated with a reduction in serum lipid peroxidation product, as well as with an increase in serum antioxidant enzyme activities in hypertensive rats. The effects of MEHS on blood pressure and oxidative stress markers were similar to those of captopril. The results suggest that the MEHS possesses significant antihypertensive effect against salt-induced hypertension in rats. The antihypertensive effect appears to be mediated by a reduction in serum oxidative stress.

Keywords: *Hibiscus sabdariffa*, hypertension, antioxidant, methanol leaf extract, rats.

INTRODUCTION

The occurrence of cardiovascular diseases (CVD), including hypertension, is on the increase globally. It remains a major public health challenge in developed as well as developing countries because of its impact on the population morbidity and mortality [1]. Cardiovascular diseases have emerged as an important health problem in Nigeria [2]. Hypertension has been implicated as a major risk factor, and if it is better controlled can lead to a drastic reduction in annual mortality rate from CVD in Nigeria [3]. Epidemiological studies demonstrate that prevalence of hypertension is increasing rapidly among Nigerian urban populations. Current evidence suggests that more than two-fifths of the Nigerian urban adult population has hypertension with the prevalence lower but on the increase in rural populations [2, 4].

The pathogenesis of hypertension is multifactorial in nature, for example, oxidative stress [5], increased activity of renin-angiotensin-aldosterone system (RAAS), endothelin (ET) system, kallikrein-kinin system, sympathetic nervous system, and genetic influence have been specified [6]. Interestingly, hypertension is identified as the most modifiable risk factor in prevention of many chronic diseases [7].

The treatment of hypertension mainly relies on synthetic medicines, although new antihypertensive drugs with improved efficacy have been introduced to the market, and they still possess serious side effects. Furthermore, 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 importance to discover natural therapeutics for prevention and management of hypertension. Recently, attention has focused on herbal preparations which are traditionally used as therapeutic agents in the prevention and management of CVD [8, 9]. These herbs are cheaper, much easier to obtain with fewer side-effects than synthetic drugs [10]. One of the medicinal plants that may have a great prospect as an antihypertensive drug is *Hibiscus sabdariffa* L. (HS).

H. sabdariffa Linn (family: Malvaceae) is one of the emerging plants of interest in the management of hypertension. It is a medicinal plant, commonly known as “Roselle”, “Rozelle”, “Indian sorrel”, “Flor de jamaica”, “Sour tea” or “Kerkrade” and so on. It 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 [11]. The plant is widely grown in tropics like Central and West Africa, South East Asia, Jamaica and Central America [12].

Previous studies on the antihypertensive properties of the plant have focused on the calyx [13, 14], petal [15, 16], and seed extracts [17]. The plant extracts have also been reported capable of relaxing vascular smooth muscle by inhibition Ca^{2+} influx [18, 19], activation of endothelial pathway of nitric oxide/cGMP (Ajay *et al.*, 2007), and as an inhibitor of angiotensin converting enzyme (ACE) [14, 20, 21]. According to previous reports, *H. sabdariffa* leaf extracts have been found to possess many biological properties such as hypoglycemic [22], hypolipidemic [23], anti-inflammatory [24], antioxidant [25, 26], anticancer [27], and estrogenic-like effects [28].

Interestingly, *H. sabdariffa* has gained attention for its antihypertensive activity. Regrettably, only the calyces of *H. sabdariffa* are widely used in the management of hypertension and the leaves are usually ignored and discarded around the world, except in Africa countries, where it is consumed as vegetables [25]. Despite the documented uses of various parts of *H. sabdariffa* in the management of hypertension, there still exists paucity of reports in the literature regarding the antihypertensive effect of *H. sabdariffa* leaves. Most of the antihypertensive effects of *H. sabdariffa* have been focused on the calyx, petal, and seed. Very little attention has been paid to the leaves especially on their antihypertensive activities. Thus, this study was designed to investigate the antihypertensive effects of methanolic leaf extract of *H. sabdariffa* (MEHS) in rats. This is with a view to providing a scientific justification or otherwise for the possible use of the plant’s leaves in the management, control and treatment of hypertension.

MATERIALS AND METHODS

Drugs, chemical, and reagents

All chemical, drugs, and reagents used in this investigation were of analytical grade. Methanol was purchased from Sigma-Aldrich Chemical Company (St. Louis, Missouri, USA). All test drugs and reagents were freshly prepared before use. Captopril (ACE-inhibitor) was used as the reference anti-hypertensive drug. The water used was glass distilled.

Preparation of drugs

Captopril: Captopril (Globela Pharma PVT. Ltd, India) was purchased from Godal Pharmacy in Abakaliki, Nigeria. Captopril was reconstituted in sterile distilled water to give required dose of 30 mg/kg/2 ml b. wt. and was administered orally [29].

Sodium pentobarbital (Nembutal): Sodium pentobarbital (Embassy Pharmaceutical and Chemicals Ltd., Nigeria) was purchased from Danax Pharmacy, Ibadan, Nigeria. The animals were anaesthetized by intraperitoneal injection of sodium pentobarbital at the dose of 100 mg/kg b. wt. [30].

Experimental animals

Inbred adult male Wistar rats weighing between (273.6 ± 3.24 g) obtained from the Animal Unit, College of Medicine, University of Nigeria Enugu Campus, Enugu, Nigeria were used for the experiment. They were maintained under standard laboratory conditions (12 h light, 12 h dark schedule) and were fed with commercially formulated rat's pellets (Pfizer Livestock Feeds PLC, Enugu, Nigeria) and tap water ad libitum. The animals were allowed to acclimatize for two weeks to the new laboratory environment after which they were divided into groups prior to the commencement of the experiment. During the two weeks, the rats were subjected to human handling 5min/day to prevent stress induced hypertension during the experiments. Excess feeds and water were removed and replaced daily.

Ethical approval

The experimental procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by the National Institute of Health [31]. Ethical approval was obtained from the Institutional Animal Ethics and Care Committee of the University with reference number (NHREC/05/01/2508B-FWA00002458-IRB00002323).

Plant collection and authentication

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

Extraction and preparation of methanol extract

The leaves were air-dried and milled to fine powder. A powdered dried leaf (1000g) was weighed and cold-macerated in 2.5 L of 80% methanol and shaken vigorously at interval for 48 h in a dark room environment. After this extraction period, the extracted solution was filtered through Whatman No. 1 filter paper (Whatman international Ltd; Maidstone, England) to obtain a pure filtrate (methanol leaf extract, *MEHS*). The filtered extract was concentrated in a rotary evaporator (BÜCHI, Vacuum Controller, V-800) at 40°C under a reduced pressure for 3 h. The resulting residue which weighed 132.4 g (recovery 13.2%) was later stored under 4°C before use. The dried *MEHS* was reconstituted in distilled water to give the required doses of 200 and 400 mg/kg/2 ml b. wt., respectively. The dosages were prepared fresh on the day of experiments prior to administration to the rats by oral gavage.

Preliminary phytochemical screening

Freshly prepared *MEHS* was subjected to various qualitative phytochemical tests, to identify the secondary metabolites present in the leaves. The screening involves detection of saponins, tannins, alkaloids, flavonoids, phenols, triterpenoids, steroids, anthraquinones, and fixed oils using standard phytochemical procedures and tests [32].

Acute toxicity test

The acute oral toxicity test of crude *MEHS* was determined according to Organization for Economic Co-operation and Development guidelines [33], limit test procedure; with starting dose of 2000 mg/kg b. wt. was adopted. Starting dose of 2000 mg/kg (*p.o.*) of each was given to 5 animals after 3 – 4 h of fasting. After administration of extract, food was withdrawn for further 3 - 4 h. Animals were observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 72 h for any physical signs of toxicity such as writhing, gasping, diarrhea, palpitation, decreased respiratory rate and mortality.

Induction of experimental hypertension

Hypertension was induced by salt-loading rats with 8% sodium chloride diet for 6 weeks according to the method described by Dahl [34] and Mojiminiyi *et al.*, [35]. Weekly systolic (SBP), diastolic (DBP), mean arterial pressure (MAP), and heart rate (HR) were measured and recorded. Rats with SBP and DBP above 140 and 100 mm Hg respectively after three consistent readings were considered hypertensive.

Experimental design and animal grouping

Age matched normotensive (NTR) and hypertensive rats (HTR) were randomly divided into five groups of 5 rats each. Group 1 and 2 served as normotensive (negative) and hypertensive (control) respectively and received distilled water (2 ml/kg/day, *p.o.*). Group 3 served as hypertensive (positive) control group and received captopril (30

mg/kg/day). Group 4 and 5 served as treatment groups and were administered with MEHS at graded doses (200 and 400 mg/kg/day, *p.o.*) respectively. Administration commenced by the 7th week of salt loading. Treatment lasted for four weeks ($W_0 - W_4$) and was carried out between 08.00 am and 09.00 am daily by oral gavage.

Determination of blood pressure parameters

A commercially available automated computerized tail-cuff blood pressure monitor, the CODA II™ NIBP recording system (Kent Scientific Corporation, Connecticut, USA) was used to record the SBP, DBP, MAP, and, HR in rats. Rats were placed in restraining holders with a nose cone to calm the animals. The restrainers were placed on a heating pad ($32 \pm 2^\circ\text{C}$) to warm the rat's tail and maintain blood flow to the tail. Animals were placed in the restrainers for at least 5 mins before monitoring the blood pressure and average of three consistent readings were taken for each rat.

Protocol for blood sampling and biochemical assay

After the last measurement of blood pressure, the rats were fasted for 12 h and anaesthetized with sodium pentobarbital (100 mg/kg b. wt., *i.p.*) and fasting blood samples were collected through cardiac puncture into labeled sterile plain tubes for biochemical serum analyses. Blood samples for sera preparation was allowed to clot at room temperature for 1h, and then centrifuged at 3000rpm for 10 mins. The clear serum was collected with Pasteur pipette into clean, dry sample bottles and stored in refrigerator under 4°C until required. All analyses were completed within 24 h of sample collection.

Evaluation of serum SOD, CAT, GSH-P_x and MDA

Superoxide dismutase (SOD) activity and the malondialdehyde (MDA) level were estimated in the serum using assay kits according to the instructions of the manufacturer (Randox Laboratories Ltd., UK). Catalase (CAT) and Glutathione peroxidase (GSH-P_x) activities were estimated using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cloud Clone, USA) as described by manufacturer.

Statistical analysis

All the data are expressed as mean \pm SEM. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) followed by Student Newman-Keul's *post hoc* test using Graph pad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA). Values of $p < 0.05$ were considered significant.

RESULTS

Percentage yield of extract

The percentage yield of the crude MEHS was 13.2% (w/w).

Preliminary phytochemical screening

Preliminary phytochemical analysis of MEHS qualitatively revealed the presence of saponins, tannins, flavonoids, phenols, steroids, triterpenoids, and fixed oil (Table 1).

Table 1: Qualitative phytochemical analysis of methanol extract of *H. sabdariffa* (MEHS)

Parameters	MEHS
Saponins	+
Tannins	+
Alkaloids	-
Flavonoids	+
Phenols	+
Triterpenoids	+
Steroids	+
Anthraquinones	-
Fixed oil	+

Key words: MEHS - Methanol extract, (+) = presence of the compound, (-) = compound not detected

Acute toxicity test

The rats treated with 2000 mg/kg dose did not show any drug induced physical signs of toxicity during the whole experimental period and no mortality was recorded after 72 h. Behavioral changes observed included transient dullness and weakness. These early symptoms subsequently disappeared after few minutes.

Changes in blood pressure and heart rate during induction

In the first six weeks of high salt diet there was a progressive increase ($p < 0.05$) in mean SBP, DBP, MAP, and HR when compared to the normotensive group (Tables 2, 3, 4, and 5) respectively. Daily consumption of (8% NaCl) diet for six weeks caused significant increase ($p < 0.05$ vs. control group) in SBP from 120.8 ± 2.03 to 199.6 ± 1.53 mmHg, (Table 2); DBP from 84.2 ± 1.79 to 142.8 ± 2.69 mmHg, (Table 3); MAP from 96.2 ± 2.05 to 159.6 ± 4.32 mmHg, (Table 4); and HR from 369.3 ± 2.48 to 481.4 ± 4.90 beats/min, (Table 5). Towards the end of induction (5th to 6th week), there was no significant increase or decrease in SBP, DBP, MAP, and HR in all the high salt groups.

Table 2: Mean systolic blood pressure (SBP) during induction

Systolic Blood Pressure (mm Hg)					
Group	Baseline	2 nd week	4 th week	5 th week	6 th week
1	119.7 ± 2.00	122.4 ± 1.78	121.3 ± 3.64	120.0 ± 3.76	120.8 ± 2.03
2	121.2 ± 0.76	$142.2 \pm 2.24^{*#}$	$158.4 \pm 4.50^{*#}$	$190.8 \pm 2.58^{*#}$	$192.0 \pm 0.84^{*#}$
3	124.4 ± 2.37	$140.5 \pm 3.75^{*#}$	$154.0 \pm 0.74^{*#}$	$188.2 \pm 5.40^{*#}$	$190.8 \pm 2.87^{*#}$
4	120.7 ± 3.04	134.8 ± 2.69	$160.5 \pm 1.58^{*#}$	$187.6 \pm 3.25^{*#}$	$189.3 \pm 3.75^{*#}$
5	122.4 ± 0.34	$140.6 \pm 1.54^{*#}$	$158.0 \pm 1.58^{*#}$	$197.2 \pm 2.24^{*#}$	$199.6 \pm 1.53^{*#}$

Values are expressed as mean \pm SEM ($n=5$ in each group). $^{*}p < 0.05$ vs. normal control group; $^{#}p < 0.05$ vs. corresponding baseline values; Group 1 = normal control rats, Group 2-5 = rats that received (8% NaCl) diet for 6 weeks.

Table 3: Mean diastolic blood pressure (DBP) during induction

Diastolic Blood Pressure (mm Hg)					
Group	Baseline	2 nd week	4 th week	5 th week	6 th week
1	83.2 ± 2.25	85.0 ± 1.67	82.6 ± 2.85	83.8 ± 1.87	84.2 ± 1.79
2	84.7 ± 1.63	$113.2 \pm 0.36^{*#}$	$122.3 \pm 0.60^{*#}$	$138.2 \pm 0.74^{*#}$	$140.9 \pm 3.05^{*#}$
3	87.2 ± 1.24	$100.5 \pm 1.24^{*#}$	$129.4 \pm 3.73^{*#}$	$141.6 \pm 2.40^{*#}$	$142.8 \pm 2.69^{*#}$
4	84.5 ± 1.52	89.4 ± 0.90	$125.0 \pm 2.55^{*#}$	$142.4 \pm 1.49^{*#}$	$140.5 \pm 3.50^{*#}$
5	83.4 ± 2.39	$90.6 \pm 1.72^{*#}$	$118.2 \pm 0.92^{*#}$	$135.6 \pm 2.62^{*#}$	$138.7 \pm 2.76^{*#}$

Values are expressed as mean \pm SEM ($n=5$ in each group). $^{*}p < 0.05$ vs. normal control group; $^{#}p < 0.05$ vs. corresponding baseline values; Group 1 = normal control rats, Group 2-5 = rats that received (8% NaCl) diet for 6 weeks.

Table 4: Mean arterial blood pressure (MAP) during induction

Mean Arterial Blood Pressure (mm Hg)					
Group	Baseline	2 nd week	4 th week	5 th week	6 th week
1	95.8 ± 1.64	97.0 ± 4.18	95.4 ± 2.95	95.8 ± 3.09	96.2 ± 2.05
2	96.0 ± 2.61	$128.2 \pm 2.67^{*#}$	$143.8 \pm 3.60^{*#}$	$156.2 \pm 2.34^{*#}$	$159.6 \pm 4.32^{*#}$
3	94.2 ± 3.24	$119.7 \pm 1.24^{*#}$	$141.3 \pm 5.06^{*#}$	$150.2 \pm 5.40^{*#}$	$152.7 \pm 1.56^{*#}$
4	92.2 ± 1.53	$105.4 \pm 3.66^{*#}$	$137.2 \pm 2.55^{*#}$	$154.8 \pm 3.37^{*#}$	$153.0 \pm 3.91^{*#}$
5	95.4 ± 2.30	$124.6 \pm 1.72^{*#}$	$142.0 \pm 0.92^{*#}$	$156.2 \pm 7.08^{*#}$	$157.4 \pm 1.45^{*#}$

Values are expressed as mean \pm SEM ($n=5$ in each group). $^{*}p < 0.05$ vs. normal control group; $^{#}p < 0.05$ vs. corresponding baseline values; Group 1 = normal control rats, Groups 2-5 = rats that received (8% NaCl) diet for 6 weeks.

Table 5: Mean heart rate (HR) during induction

Heart Rate (beats/min)					
Group	Baseline	2 nd week	4 th week	5 th week	6 th week
1	368.5 ± 3.29	370.5 ± 2.03	372.9 ± 5.02	370.5 ± 3.82	369.3 ± 2.48
2	373.0 ± 7.63	$426.2 \pm 4.26^{*#}$	$440.7 \pm 2.76^{*#}$	$475.6 \pm 2.34^{*#}$	$478.0 \pm 3.32^{*#}$
3	368.2 ± 3.24	$438.5 \pm 1.24^{*#}$	$450.4 \pm 1.88^{*#}$	$478.1 \pm 2.53^{*#}$	$480.6 \pm 2.55^{*#}$
4	370.6 ± 2.30	$432.8 \pm 2.05^{*#}$	$446.6 \pm 2.50^{*#}$	$477.8 \pm 4.86^{*#}$	$476.1 \pm 2.85^{*#}$
5	376.4 ± 5.31	$429.6 \pm 3.72^{*#}$	$450.2 \pm 3.92^{*#}$	$480.7 \pm 3.08^{*#}$	$481.4 \pm 4.90^{*#}$

Values are expressed as mean \pm SEM ($n=5$ in each group). $^{*}p < 0.05$ vs. normal control group; $^{#}p < 0.05$ vs. corresponding baseline values; Group 1 = normal control rats, Group 2-5 = rats that received (8% NaCl) diet for 6 weeks.

Effects of MEHS and captopril on blood pressure and heart rate in salt-induced hypertensive rats

The effects of MEHS and captopril on SBP, DBP, MAP, and HR in hypertensive rats are presented in Figures (1, 2, 3, and, 4) respectively. At baseline, there was no significant difference in SBP, DBP, MAP, and, HR among experimental groups. Daily administration of salt diet for six weeks significantly increase ($p < 0.05$) SBP (198.3 ± 2.42 mmHg), (Figure 1); DBP (140.3 ± 3.54 mmHg), (Figure 2), MAP (158.7 ± 2.53 mmHg), (Figure 3); and HR (476.8 ± 5.87 beats/min), (Figure 4) compared to the control group. Treatment with MESH significantly reduced ($p < 0.05$) SBP, DBP, MAP and HR compared to the vehicle group. Treatment with captopril (30 mg/kg/day)

significantly decreased ($p < 0.05$) SBP, DBP, MAP, and HR in hypertensive rats compared to the untreated hypertensive group. The mean values of SBP, DBP, MAP, and HR in all the treatment groups after four weeks of treatment were significantly reduced ($p < 0.01$) when compared with their respective baseline values.

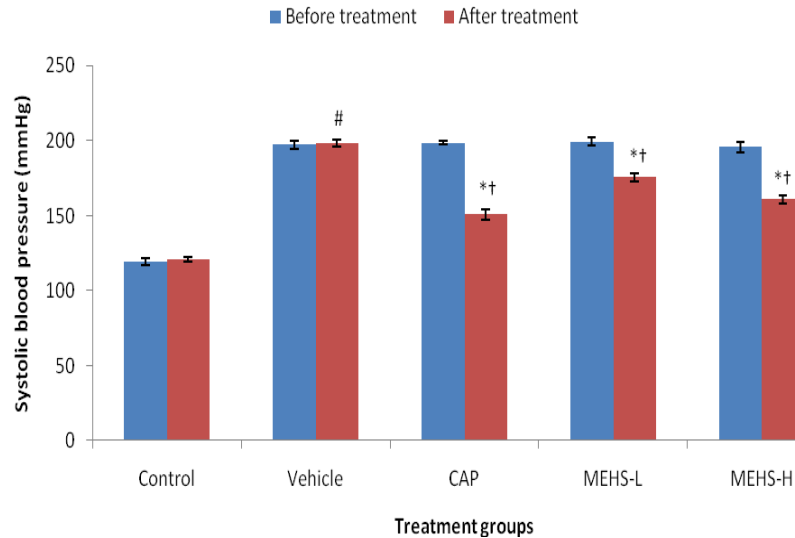


Figure 1: Effect of MEHS and captopril on systolic blood pressure (SBP) in salt-induced hypertensive rats. Values are expressed as mean \pm SEM (n=5 in each group). [#] $p < 0.05$ vs. control group; ^{*} $p < 0.01$ vs. corresponding baseline values; [†] $p < 0.05$ vs. vehicle group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), and CAP = hypertensive rats that received captopril (30 mg/kg/day).

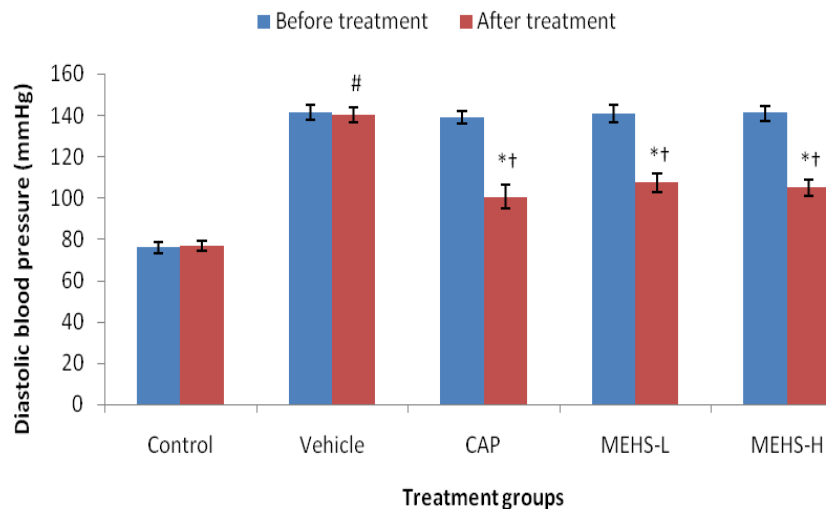


Figure 2: Effect of MEHS and captopril on diastolic blood pressure (DBP) in salt-induced hypertensive rats. Values are expressed as mean \pm SEM (n=5 in each group). [#] $p < 0.05$ vs. control group; $p < 0.01$ vs. corresponding baseline values; [†] $p < 0.05$ vs. vehicle group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), and CAP = hypertensive rats that received captopril (30 mg/kg/day).

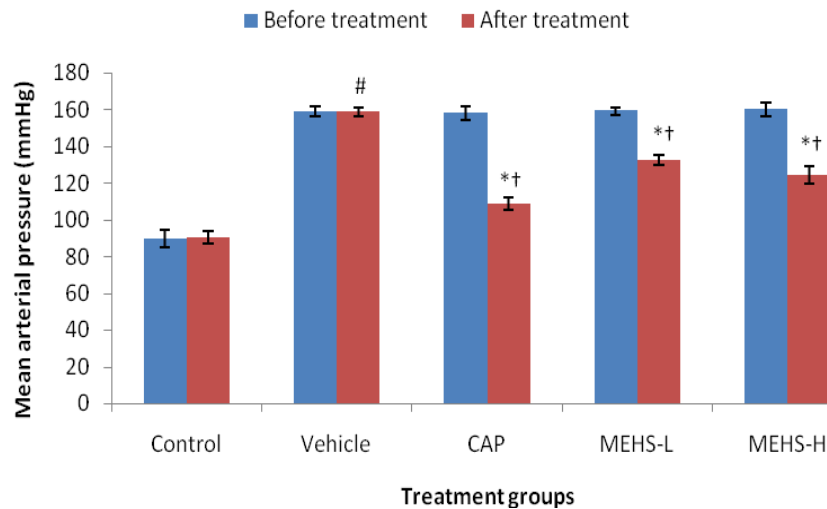


Figure 3: Effect of MEHS and captopril on mean arterial blood pressure (MAP) in salt-induced hypertensive rats. Values are expressed as mean \pm SEM (n=5 in each group). [#] $p < 0.05$ compared to control group; ^{*} $p < 0.01$ compared to baseline values; [†] $p < 0.05$ compared to untreated hypertensive group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), and CAP = hypertensive rats that received captopril (30 mg/kg/day).

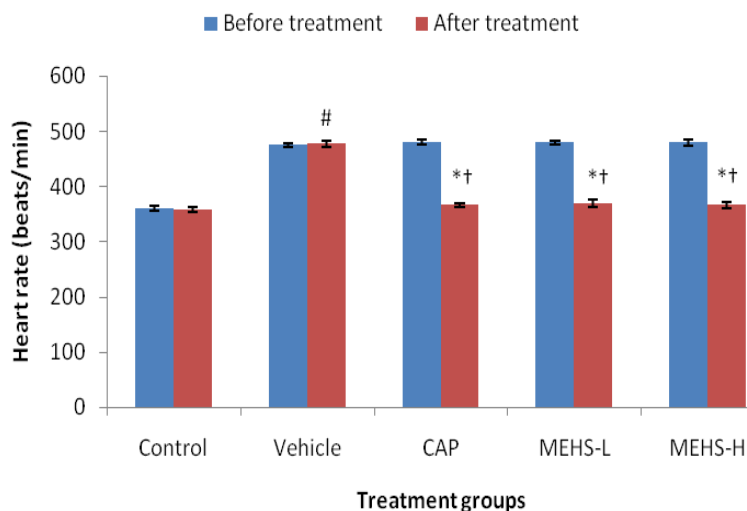


Figure 4: Effect of MEHS and captopril on heart rate (HR) in salt-induced hypertensive rats. Values are expressed as mean \pm SEM (n=5 in each group). [#] $p < 0.05$ vs. control group; ^{*} $p < 0.01$ vs. corresponding baseline values; [†] $p < 0.05$ vs. vehicle group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), and CAP = hypertensive rats that received captopril (30 mg/kg/day).

Antioxidant activity of MEHS in hypertensive rats

The effects of MEHS and captopril on serum contents of SOD, CAT, GSH-Px, and MDA in hypertensive rats are presented in Figures (5, 6, 7, and 8) respectively. Daily consumption of 8% NaCl in diet for six weeks caused significant decrease ($p < 0.05$) in serum activity of SOD (12.4 ± 3.47 U/mL, Figure 5); CAT (132.5 ± 14.53 mIU/mL, Figure 6); and GSH-Px (12.6 ± 8.36 ng/mL, Figure 7) compared to control group. However, a significant increase ($p < 0.05$) in MDA (183.7 ± 5.89 ng/mL, Figure 8) was observed when compared with the control group. Treatment with MEHS significantly increased ($p < 0.05$) the serum levels of SOD, CAT, and GSH-Px compared to vehicle group. Similarly, MEHS-treated groups showed significantly lower ($p < 0.05$) MDA level when compared with the vehicle group. The effects of MEHS on the serum activities/levels of the estimated antioxidant indices appeared to

be dose-dependent. Captopril also significantly increased ($p < 0.05$) serum SOD, CAT, and GSH-Px levels, but reduced ($p < 0.05$) MDA content in the hypertensive rats compared to vehicle group.

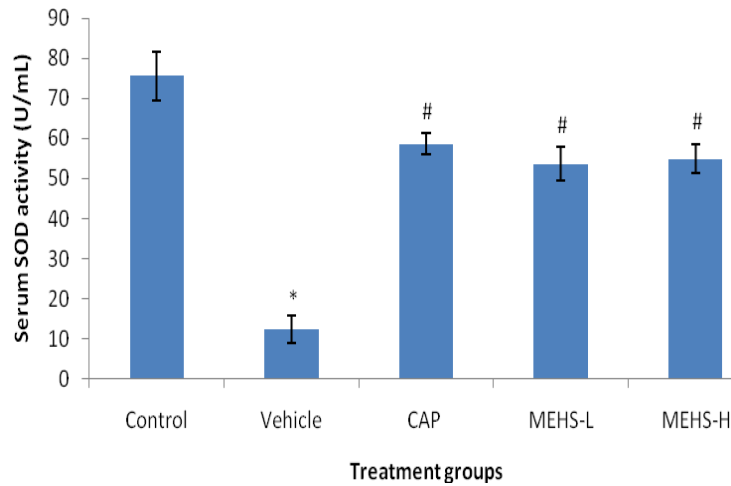


Figure 5: Serum superoxide dismutase (SOD) activity in salt-induced hypertensive rats treated with MEHS and captopril. Values are expressed as mean \pm SEM (n=5 in each group). * $p < 0.05$ vs. control group; # $p < 0.05$ vs. vehicle group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), and CAP = hypertensive rats that received captopril (30 mg/kg/day).

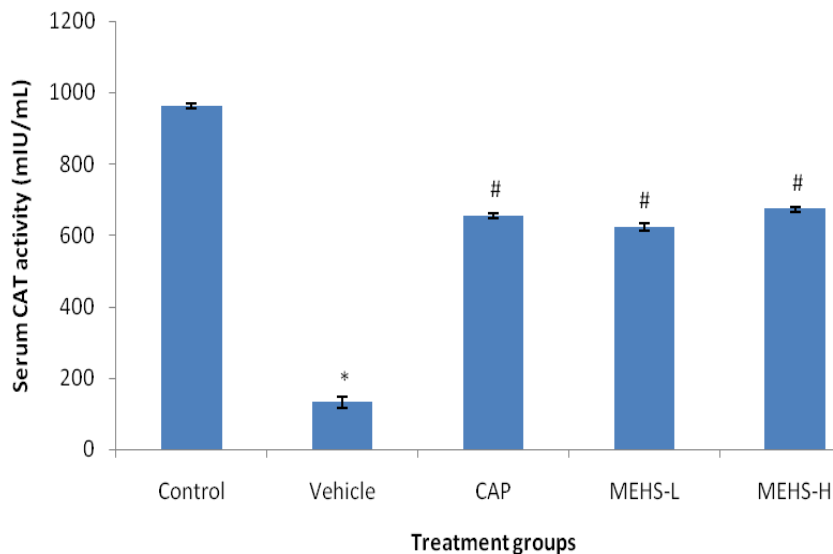


Figure 6: Serum catalase (CAT) activity in salt-induced hypertensive rats treated with MEHS and captopril. Values are expressed as mean \pm SEM (n=5 in each group). * $p < 0.05$ vs. control group; # $p < 0.05$ vs. vehicle group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), CAP = hypertensive rats that received captopril (30 mg/kg/day).

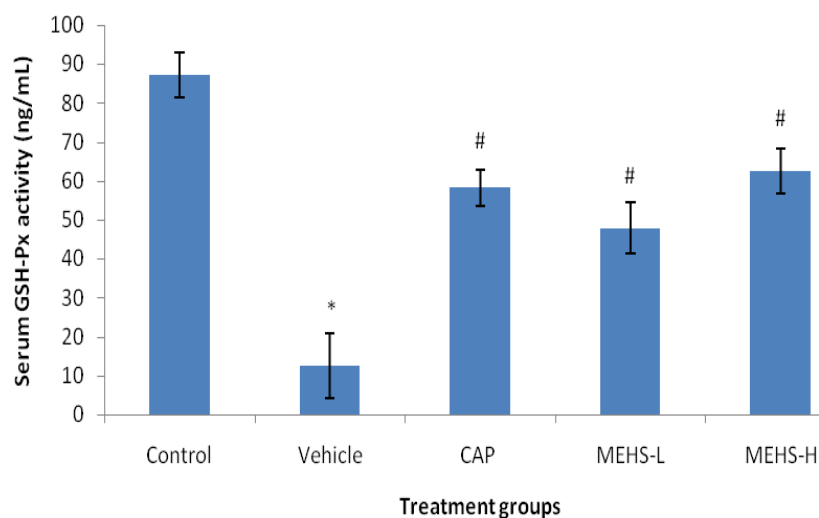


Figure 7: Serum glutathione peroxidase (GSH-Px) activity in salt-induced hypertensive rats treated with MEHS and captopril. Values are expressed as mean \pm SEM (n=5 in each group). * p <0.05 vs. control group; # p <0.05 vs. vehicle group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), and CAP = hypertensive rats that received captopril (30 mg/kg/day).

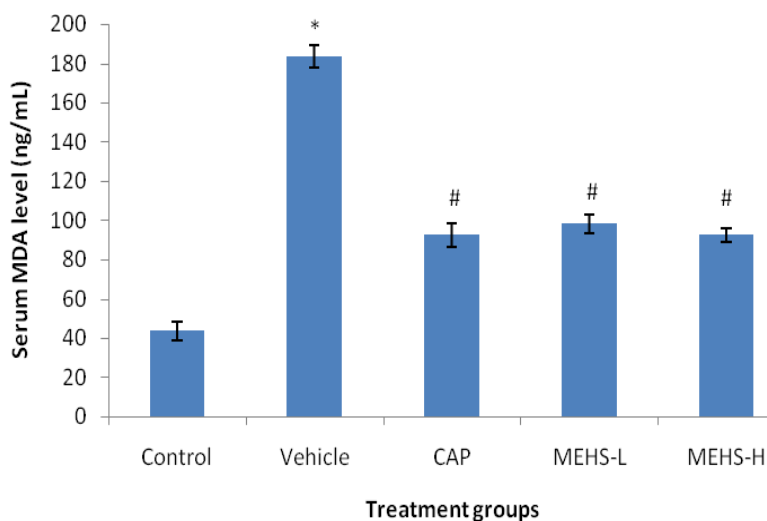


Figure 8: Serum malondialdehyde (MDA) activity in salt-induced hypertensive rats treated with MEHS and captopril. Values are expressed as mean \pm SEM (n=5 in each group). * p <0.05 vs. control group; # p <0.05 vs. vehicle group; Control = normal control rats, vehicle = untreated hypertensive rats, MEHS-L = hypertensive rat that received MEHS (200 mg/kg/day), MEHS-H = hypertensive rat that received MEHS (400 mg/kg/day), CAP = hypertensive rats that received captopril (30 mg/kg/day).

DISCUSSION

The use of non-pharmacological agents for the prevention and management of CVD, including hypertension has been increasing recently. In this study, the anti-hypertensive effects of the methanol leaf extract of *H. sabdariffa* (MEHS) was investigated using salt-induced hypertensive model in rats. The results obtained from this study have demonstrated that MEHS possessed significant antihypertensive effect against salt-induced hypertension in rats. To our knowledge, this is the first report to show that MEHS can ameliorate the development of salt-induced hypertension in rat model.

The results of the toxicity study suggest that MEHS has a wide margin of safety and thus administration as done in folk medicine may not have any immediate adverse effects. Our findings were consistent with those of other studies, which reported that lethal dose (LD₅₀) was greater than 5000 mg/kg body weight [36, 37].

In this study, we demonstrated that salt loading with (8% NaCl) *via* diet significantly increased blood pressure in Wistar rats, in agreement with accumulated reports in Sprague-Dawley rats [35, 38], and Wistar rats [39, 40]. However, other previous studies found no significant change in blood pressure with salt loading in Sprague-Dawley rats [41, 42]. These disagreements may reflect differences in the time frame of salt loading or in the route of administration (with fluid or food intake).

Our findings showed that chronic consumption of sodium chloride diet caused hypertension and also induced other abnormalities in rats, including oxidative stress in experimental rats. MEHS ameliorates blood pressure alterations and increased oxidative stress. Both MEHS and captopril reversed the abnormalities in hypertensive rats. It is noteworthy that MEHS produced a significant reduction in blood pressure and heart rate of hypertensive rats, suggesting that MEHS possesses antihypertensive and negative chronotropic effects against salt-induced hypertension in rat. Maximum decrease in blood pressure was observed at the dose of 400 mg/kg body weight. The available research data show that the MEHS is efficient as antihypertensive agent by significantly preventing the increase of blood pressure in hypertensive rats. Thus, the results showed that graded doses of MEHS have significant blood pressure lowering effect in hypertensive rats in a dose response manner.

Activation of oxidative stress markers were attenuated in hypertensive rats after MEHS treatment. Therefore, it is likely that the anti-oxidative stress properties are one mechanism by which MEHS reduces blood pressure in this rat model of hypertension. The results of this study suggest that salt loading induces hypertension via oxidative stress, since it elicits lipid peroxidation and reduced the serum activities of antioxidant enzymes in the hypertensive rats. This is indicated by the increase in the serum activities of SOD, CAT, GSH-Px, and markedly reduced MDA serum level following treatment with MEHS which was overwhelmed due to oxidative stress induced by salt. The result suggests the extract has an antioxidant effect and may contribute to blood pressure reduction. The antioxidant effects of *H. sabdariffa* have been previously reported both *in vitro* [43, 44] and *in vivo* [25, 44] experimental studies. This finding concurs with Mohd-Esa *et al.*, [43] and the Mossalam *et al.*, [44] studies, which suggested that the antioxidant effect of *H. sabdariffa* contributed towards its antihypertensive effect. The antioxidant activity of the extract is due to its strong scavenging effect on reactive oxygen species (ROS) and free radicals [25, 44].

The effect of MEHS in this study was comparable to that of captopril, which is an ACE inhibitor. We chose captopril for this study because it has antioxidant effects and *H. sabdariffa* was reported to possess ACE inhibitor properties [14, 21]. The captopril-induced reduction of blood pressure in this study was associated with a reduction in MDA with concomitant increase in the serum activities of antioxidant enzymes, which indicated that captopril has antioxidant properties. The antioxidant effect of an ACE inhibitor has been previously reported [45, 46]. These findings suggest that MEHS and captopril could have antihypertensive effects on reducing blood pressure on salt-induced hypertension in rats and that this effect appears to be mediated by a reduction in serum oxidative stress.

The phytochemical constituents responsible for the potent antihypertensive activity of MEHS have not yet been identified. However, in the preliminary phytochemical screening on MEHS, we detected the presence of saponins, tannins, flavonoids, triterpenoids, steroids, phenols, and fixed oil. It has also been reported that, the flavonoids like flavonol and anthocyanins have been isolated from the leaves [47]. Flavonoids are among the antihypertensive materials for which anti-hypertensive efficacy has been extensively confirmed [48, 49]. It is suggested that, these active compounds would be able to exhibit substantial antioxidant capacity and counteract with the deteriorating effects of reactive oxidants in the serum [26, 50]. In view of the hypertension model used, active secondary metabolites detected in MEHS could either act singly or in synergy with one other. These findings therefore, suggest that, the antihypertensive activities of the leaf extract may be due to the presence of phytochemical constituents.

CONCLUSION

Our findings suggest that the MEHS possesses significant antihypertensive effect against salt-induced hypertension in rats. The antihypertensive effects might be possibly mediated via anti-oxidative stress properties. Thus, MEHS might be beneficial for controlling hypertension.

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Authors' contributions

This work was carried out in collaboration between all authors. Author MEB, DCN and EEI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors EEB, JNO and SFAD managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Competing interests

Authors have declared that no competing interests exist

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