ANTIHYPERTENSIVE EFFECT OF *Harungana madagascariensis* LAM. EX POIR. ON SODIUM FLUORIDE-INDUCED HYPERTENSION AND ASSOCIATED CARDIO-RENAL DYSFUNCTIONS

[1]Gbadamosi I. T., [1]Okolo N. A., [2]Oyagbemi A. A., [2]*Ajibade T. O., [3]Omobowale T. O.

1Department of Botany, University of Ibadan, Nigeria.
2Department of Veterinary Physiology and Biochemistry University of Ibadan.
3Department of Veterinary Medicine, University of Ibadan.
*E-mail of corresponding author: toajibade@gmail.com; Tel: +2348181941873

ABSTRACT

Hypertension is an important risk factor for cardiovascular diseases, which are major causes of mortality globally. This study evaluated the antihypertensive effects of *Harungana madagascariensis* on sodium fluoride-induced hypertension. Twenty-five rats were randomly divided into five groups (A-E). Group A (control), received normal food and water with no treatment; Groups B to E were exposed to 300 ppm sodium fluoride (NaF). In addition, Group C was treated with 10 mg/kg enalapril, while Groups D and E were treated with 100 mg/kg and 200 mg/kg of *H. madagascariensis* extract, respectively. The result showed that there was a significant decrease in the systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) of the rats treated with 200 mg/kg of *H. madagascariensis* compared with rats exposed to NaF alone. Also significant increase was observed in the activities of the enzymatic and non-enzymatic antioxidants in the cardiac and renal tissues of rats treated with 100 mg/kg and 200 mg/kg of *H. madagascariensis* extract similar to enalapril, compared with rats exposed to NaF alone. However, serum nitric oxide (NO) decreased significantly in rats exposed to NaF alone compared with rats that received 200 mg/kg *H. madagascariensis* as treatment. Blood urea nitrogen (BUN) and creatinine increased significantly in rats exposed to NaF alone compared with rats that received 200 mg/kg *H. madagascariensis* as treatment. The histopathology of the cardiac tissue of the rat exposed to NaF alone showed mild hydropic and vacuolar degeneration of cardiomyocytes, while the renal tissue showed foci of markedly flattened tubular epithelial cells and congestion of interstitial blood vessels. The aforementioned lesions were ameliorated in rats treated with 200 mg/kg *H. Madagascariensis*. In conclusion, *H. madagascariensis* had potent antihypertensive effect in rats. The effect was probably mediated via the alleviation of oxidative stress in cardiac and renal tissues.

Key words: *Harungana madagascariensis*, Antioxidant activity, Oxidative Stress, Hypotensive effect, Sodium fluoride.

INTRODUCTION

Hypertension in mammals is an abnormally elevated blood flow rate in the vasculature characterised by increased exertion of force by blood on a unit area of blood vessel. Hypertension strains the heart, injure blood vessels, increases the risk of heart attack, stroke, kidney problems and if untreated may cause death (Dayanand *et al.*, 2015). Globally hypertension is an important risk factor for cardiovascular diseases, with people in sub-Saharan Africa more affected and likely to suffer one or more hypertension related diseases (Ogah, 2012). Indeed, it has already been projected that up to three quarters of the world's hypertensive population will be in economically- developing countries, such as Nigeria, by the year 2025 (Kearney *et al.*, 2005). Hypertension may result from several aetiologies, including renal artery stenosis, pheochromocytoma, excessive adrenal aldosterone production (Carretero and Oparil, 2000), and inadvertent exposures to certain toxicants including lead, cadmium, organochlorine pesticides and polycyclic biphenyls (Kahn and Trasande, 2018). Similarly, drugs including steroids, sympathomimetic amines, immunosuppressive agents, nonsteroidal anti-inflammatory agents (NSAIDs), antidepressants and erythropoietin have been reported to induce hypertension or worsen pre-existing hypertension (Gyamlani and Geraci, 2007).

Sodium fluoride (NaF) is an environmental pollutant found in drinking water and is commonly included in tooth paste for its purported usefulness in the prevention of dental decay resulting from excessive acid formation and bacterial infection (Sadhukhan *et al.*, 2016). Unfortunately, high concentrations of fluoride ion are deleterious to mammalian health and have

been associated with interference with bone mineralisation and defects that are generally irreversible on the skeleton, teeth, and soft tissues (Suska, 2002). For instance, chronic fluoride exposure causes polycystic and some other kidney diseases and high exposure to sodium fluoride has also been reported to cause nervous system degenerations in animals (Barbier *et al.*, 2010). Moreover, reports by various authors have suggested the induction of severe myocardial damage and disturbances of homeostasis in vital organs of mammals following prolonged exposure to sodium chloride (Ghosh *et al.*, 2016). The deleterious effects of sodium fluoride are mediated via the induction of oxidative stress in various organs and tissues (Das *et al.*, 2008), with consequent induction of varying degrees of abnormalities ranging from reduced functional capacities to end stage organ damage especially in the brain, heart, kidney and liver. Oxidative stress, which results from an imbalance between the formation and neutralization of pro-oxidants, has been implicated as an important modulator in the pathogenesis of hypertension (Farias *et al.*, 2017).

Despite a diversity of pharmacological agents to treat hypertension, suboptimal control remains a significant problem in as many as 43% of patients with hypertension (Ayah *et al.*, 2013). Consequently, especially in the last two to three decades, various medicinal plants have been and are being investigated for their potential beneficial effects on the management of hypertension because they are generally considered to be safe and usually not associated with the common side effects including headache, excessive urinary excretion of potassium, fatigue, cough and diarrhoea (Tabassum and Ahmad, 2011).

Harungana madagascariensis Lam. ex Poir commonly known as the dragon's blood tree, orange-milk tree or haronga, belongs to the family 'Hypericaceae' and the genus is monotypic being the sole member of the genus Harungana (Iwalewa *et al.*, 2008). It is an herbal plant used traditionally in the treatment of many aliments such as malaria, jaundice, typhoid, asthma and ulcers (Moronkola *et al.,* 2018). Documented scientific studies of *H. madagascariensis* indicate isolation of compounds like anthrones, anthraquinones, xanthones, flavonoids, and essential oils (Simeon *et al.*, 2007). *H. madagascariensis* is a component of Jubi Formula, an herbal preparation which was found to restore the haematocrit and haemoglobin concentration in anaemic conditions and is a potential substitute for blood transfusion (Erah *et al.*, 2003). Biapa *et al.* (2013) reported that *H. madagascariensis* could protect the red blood cells' membrane through its antioxidative properties. Moreover, Tom *et al.* (2018) reported potent vasodilatory activity for the stem bark of *H. madagascariensis* in isolated rat aorta. Furthermore, *H. madagascariensis* extract, reportedly, normalizes the activities of antioxidant enzymes such as superoxide dismutase and catalase in oxidative stress mediated organ specific injuries (Biapa *et al.*, 2013). Therefore, this study was designed to investigate the modulatory role of *H. madagascariensis* in sodium fluoride-induced cardiovascular and renal dysfunctions in rats, with the purpose of obtaining more information on its potential benefit and probable development into a scientifically validated, safe and potent alternative to the currently available orthodox antihypertensive drugs.

MATERIALS AND METHODS
Collection, Identification and Preparation of Plant Sample
The stem bark of *H. madagascariensis* was collected from Moniya, a village within Akinyele Local Government Area, Ibadan with longitude 3.9152° E and latitude 7.5249° N. The plant was identified at the University of Ibadan Herbarium (UIH) with voucher number UIH 22455. Thereafter, the stem bark was cut into small pieces, dried at room temperature and milled to a coarse powder. Two kilogram (2 Kg) of the stem bark was soaked for about 48 hours in 2 L of water – methanol mixture (1:1 ratio) and then the suspension was filtered. Thereafter, the filtrate was concentrated using a rotary evaporator, as previously described by Njar (1993).

Animals
Male wistar rats (7 weeks old) were used for this study. They were kept in plastic cages under controlled light cycle (12 h light/12 h dark) and were allowed to acclimatize for two weeks at the animal house unit of the Department of Veterinary Physiology and Biochemistry, University of Ibadan, prior to further experiment.

The rats were allowed free access to water and commercial pelletized rat feed *ad libitum*. The animals were humanely cared for according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. During the experiment, ethics regulations were followed in accordance with the United States national and institutional guidelines for the protection of the animal's welfare (PHS, 1996). Ethical approval was obtained from the University of Ibadan Animal Care Use and Research Ethics Committee (UI-ACUREC/App/2015/011).

Experimental Design

Twenty five rats were divided into 5 groups, of five rats each, as follows: Group A (control), which received normal food and water with no treatment; Group B received normal food and water containing 300 ppm sodium fluoride (NaF); Group C was exposed to water containing 300 ppm sodium fluoride and 10 mg/kg body weight of enalapril was administered as treatment; Group D and Group E were also exposed to 300 ppm sodium fluoride but received 100 mg/kg body weight and 200 mg/kg body weight of *H. madagascariensis* extract respectively. The experiment lasted for seven consecutive days.

Determination of Blood Pressure

Indirect blood pressure parameters (systolic, diastolic and mean blood pressure) were determined without anesthesia, by tail plethysmography using an electrosphygnomanometer (CODA, Kent Scientific, USA).

Sample Collection

Blood sample (3 ml of blood) was collected from the retro-orbital venous plexus of the animals into plain sample bottles before they were humanely sacrificed by cervical dislocation. The blood was centrifuged at 4, 000 x rpm for 15 min to obtain the serum. The kidney and hearts were harvested and immediately placed on ice, rinsed, and homogenized in aqueous potassium phosphate buffer (0.1 M, pH 7.4) and the homogenate centrifuged at 10, 000 x rpm (4 ºC) for 10 min to obtain the supernatant fraction.

Chemicals and Reagents

Reagents and chemicals used in this study included Biuret's reagent, Greiss reagent, phosphoric acid, sodium hydroxide, N 1-Naphthyl ethylenediamine, sulphanilamide, distilled water, AST reagents kit, phosphate buffered saline, creatinine reagent, copper sulphate, trichloroacetic, reduced glutathione (GSH), thiobarbituric acid, ammonium ferrous sulphate, glacial acetic acid, potassium iodide, Ellman's reagent (DTNB), ethanol, urea reagent.

Biochemical Assays

The protein concentration of the various samples was determined using biuret method as described by Gornal *et al.* (1949). Briefly, 0.1 ml of serum was placed into a test tube, 2.9 ml distilled water was added and 3 ml of working biuret reagent was also added. The tubes were placed in a water bath at 37 oC for 10 min. Thereafter the mixture was allowed to cool and reading was done with a spectrometer (SP600) at 540 nm wavelength.

Production of nitric oxide (NO) was evaluated in the heart and kidney tissues using Griess reagent as described by Olaleye *et al.* (2007). This method involved the addition of N-(1naphthyl) ethylenediamine and sulfanilic acid to prepare the Griess Reagent, and the mixture of 100 µl of Griess Reagent, 300 µl of the nitrite-containing sample and 2.6 ml of deionized water in a cuvette. Incubation of the mixture was carried out for 30 min at room temperature and a reference sample was prepared by the addition of 100 µl of Griess Reagent and 2.9 ml of deionized water without adding the sample. The absorbance of was measured at 548 nm wavelength. Myeloperoxidase was determined according to the method of Xia and Zweier (1997). Reduced glutathione level was evaluated through the method of Ellman (1959), which involved addition of 0.5 ml of 4% sulfosalicylic acid to 0.5 ml of post mitochondrial fraction of sample and centrifugation for 5 min at 4,000 rpm. The supernatant (0.5 ml) was mixed with 4.5 ml of Ellman's reagent and absorbance was read at 412 nm wavelength. Glutathione S-transferase activity was determined as described by Habig *et al.* (1974). Glutathione peroxidase activity was measured according to the method of Buetler *et al.* (1963). A solution containing 0.5 ml potassium phosphate buffer, 0.1 ml sodium azide,

0.2 ml reduced glutathione solution, 0.1 ml hydrogen peroxide, 0.5 ml post mitochondrial fraction of sample and 0.6 ml of distilled water was prepared. The mixture was incubated at 37 °C for 5 min and 0.5 ml of trichloro acetic acid was added. Centrifugation was done at 4,000 rpm for 5 min. The supernatant (1 ml) was added to K_2HPO_4 (2 ml) and Ellman's reagent (1 ml). The absorbance of the mixture was read at 412 nm wavelength. Protein carbonyls (PC) were measured using the method of Reznick and Packer (1994). The post mitochondrial fraction of samples (1 ml) were added to two test tubes; one containing 4 ml of 2,4-Dinitrophenylhydrazine and 2.5 M hydrochloric acid whereas the other contained 4 ml of hydrochloric acid. The tubes were incubated at room temperature for 1 hour and vortexed every 15 min. Thereafter, 5 ml of trichloro acetic acid was added and the tubes were placed on ice for 10 min. Centrifugation was then done for 5 min and the supernatants obtained after centrifugation were carefully removed. The precipitates were dissolved in guanidine hydrochloride solution and are left for 10 min at 37 °C and vortexed.

The malondialdehyde (MDA) level, a lipid peroxidation index, was determined spectrophotometrically as described by Draper and Hadley (1990). 1.6 ml of Tris-KCl, 0.5 ml of 30 % TCA, 0.4 ml of samples, 0.5 ml of 0.75% thiobarbituric acid (in 0.2 M HCl) were mixed. Incubation was done at 80 °C for 45 min. The mixture was cooled on ice and centrifuged at 4,000 rpm for 15 min. The absorbance was measured against a blank (distilled water) at 532 nm. Ascorbic acid (vitamin C) determination was performed as described by Jacques-Silva *et al.* (2001). A solution containing 4.5 mg/ml dinitrophenyl hydrazine and 0.6 mg/ml thiourea in a 9 mol/L H_2SO_4 solution was prepared. Also a second solution was prepared with the addition of 0.075 mg/ml $CuSO_4$. An aliquot of the sample in a final volume of 1 ml of the solution was incubated for 3 hr at 38 °C, then 1 ml H_2SO_4 65% (v/v) was added to the medium.

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of the auto-oxidation of epinephrine at pH 7.2 at 30 OC as described by Misra and Fridovich (1972). This involved dissolving 100 mg of epinephrine in 100 ml distilled water acidified with 0.5 ml concentrated hydrochloric acid. 10 μl of post mitochondrial fraction of samples was added to 2.5 ml 0.05 M carbonate buffer, and 300 μl of 0.3 mM adrenaline was added. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

Histopathology

The heart and kidney of each animal were harvested for routine histopathology. The organs were fixed in 10% formalin, and then dehydrated with grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the samples in 2 changes of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin-embedded, 5-6 μm thick, transverse sections of the heart and kidney were cut using a rotary microtome and mounted on glass slides. The slides were stained with haematoxylin and eosin (H&E). Stained sections of control and treated rats were examined under the light microscope (Olympus CH Japan) for alterations (Drury *et al.*, 1976).

Statistical Analysis

All values were expressed as mean ± standard deviation (SD). The test of significance between two groups was estimated by Student's t-test. One-way Analysis of Variance (ANOVA) with Tukey's post-hoc test using Graph pad prism 6.0 was also performed with p-values <0.05 considered statistically significant.

RESULTS

The result of the effect of *H. madagascariensis* extract on the blood pressure parameters of rats exposed to sodium fluoride is presented in table 1. The extract significantly (p<0.05) reduced the analysed blood pressure parameters of rats compared with the rats exposed to NaF without extract treatment (Group B) (Table 1). Also, there was significant increase in the kidney/body weight ratio in the negative control (Group B) when compared with other groups (Table 2). The markers of oxidative stress- malondialdehyde (MDA) and protein carbonyl (PC) increased significantly in the negative control (Group B) compared with the control and other treated groups, but reduced glutathione (GSH) and protein thiol (PT) decreased significantly (Tables 3

and 4). Markers of oxidative stress increased in the sodium fluoride alone treated group (Group B) when compared with the control and other treated groups, but reduced glutathione (GSH) and protein thiol (PT) decreased significantly (Tables 5 and 6). Serum nitric oxide (NO) decreased significantly in rats exposed to sodium fluoride alone compared with rats that received 200 mg/kg of *H. madagascariensis* extract (Figure 1). The hydrogen peroxide level increased significantly in cardiac and renal tissues of rats exposed to NaF alone compared with rats treated with *H. Madagascariensis* extract (Figures 2 and 3). Blood urea nitrogen (BUN) and creatinine increased significantly in rats exposed to sodium fluoride alone compared with rats that received 200 mg/kg

H. madagascariensis as treatment (Table 7).

The histopathology of the cardiac tissue of the rat exposed to sodium fluoride showed mild hydropic/vacuolar degeneration of cardiomyocytes whereas in other groups, cardiomyocytes appear either normal with no visible lesion or with mild infiltration of inflammatory cells. The renal tissue of the exposed rats showed few foci of marked flattening of tubular epithelial cells. There is marked congestion of interstitial blood vessels and glomerular capillary tufts whereas the control and the extract treated groups showed normal glomeruli and tubules.

Table 1: Effect of *H. madagascariensis* extract on Blood Pressure Parameters of Sodium Fluoride-Induced Hypertensive Rats

Groups	SBP (mmHg)	DBP (mmHg)	MAP(mmHg)
A	132.20 ± 4.15[b]	102.00 ± 5.57[b]	115.00 ± 4.36[b]
B	169.00 ± 10.68[a]	148.2 ± 8.76[a]	155.2 ± 9.36[a]
C	155.80 ± 6.30[ab]	140.60 ± 3.91[a]	148.20 ± 3.27[a]
D	123.00 ± 10.70[b]	105.00 ± 11.34[b]	106.80 ± 11.39[b]
E	124.20 ± 25.01[b]	116.80 ± 14.67[b]	121.60 ± 14.67[b]

Values are expressed as mean ± SD, n= 5. SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, MAP= Mean Arterial Pressure. Means with the same letters down the column are not significantly different at P<0.05. Group A (Control), group B (Negative control), group C (Induced + standard drug), group D (Induced + 100 mg/kg of *H. madagascariensis* extract) and group E (Induced + 200 mg/kg of *H. madagascariensis* extract)

Table 2: Effect of *H. madagascariensis* extract on Organ Weight in Sodium Fluoride-Induced Hypertensive Rats

Weight	A	B	C	D	E
Body weight (g)	112.5 ± 5.00[b]	117.5 ± 5.00[a]	126 ± 5.48[a]	128 ± 8.37[a]	140 ± 10.00[a]
Heart (g)	0.525 ± 0.050[b]	0.500 ± 0.082[a]	0.360 ± 0.089[b]	0.500 ± 0.071[a]	0.533 ± 0.058[a]
Kidney (g)	0.825 ± 0.123[b]	0.875 ± 0.171[a]	0.820 ± 0.110[b]	0.880 ± 0.110[a]	0.967 ± 0.153[a]
Heart/Body weight (g/g)	0.0047 ± 0.0002[b]	0.0043 ± 0.0009[a]	0.0028 ± 0.0006[ab]	0.0039 ± 0.0008[a]	0.0038 ± 0.0007[a]
Kidney/Body weight (g/g)	0.0073 ± 0.0012[b]	0.0075 ± 0.0015[a]	0.0065 ± 0.0010[b]	0.0069 ± 0.0011[b]	0.0070 ± 0.0016[ab]

Values are expressed as mean ± standard deviation; n=5; g = gram. Means with the same letters down the column are not significantly different at P<0.05. Group A (control), group B (negative control), group C (induced + standard drug), group D (induced + 100 mg/kg of *H. madagascariensis* extract) and group E (induced + 200 mg/kg of *H. madagascariensis* extract)

Table 3: Effect of _H. madagascariensis_ extract on Enzymatic Antioxidants of Cardiac Tissue in Sodium Fluoride-Induced Hypertensive Rats

Enzymes	A	B	C	D	E
GST(μmol/min/ mg protein)	0.55 ± 0.42[b]	0.95 ± 0.51[a]	0.71 ± 0.67[b]	0.74 ± 0.56[ab]	0.69 ± 0.47[b]
SOD(μmol/L)	15.53 ± 3.21[b]	19.97 ± 4.31[a]	17.93 ±1.49[b]	18.49 ± 0.99[ab]	18.54 ± 1.31[ab]
GPx (units/ mg protein)	175.50 ± 26.09[b]	222.8 ± 40.94[a]	209.9 ± 2.87[b]	215.00 ±7.45[ab]	211.90 ± 14.69[b]

Values are expressed as mean ± standard deviation, n=5; means with the same letters down the column are not significantly different at P<0.05. GST-Glutathione S-Transferase, GPx-Glutathione Peroxidase and SOD-Superoxide Dismutase. Group A (control), group B (negative control), group C (induced + standard drug), group D (induced + 100 mg/kg of _H. madagascariensis_ extract) and group E (induced + 200 mg/kg of _H. madagascariensis_ extract).

Table 4: Effect of _H. madagascariensis_ extract on Enzymatic Antioxidants of Renal Tissue in Sodium Fluoride-Induced Hypertensive Rats

Enzymes	A	B	C	D	E
GST	1.49 ± 0.25[b]	1.65 ± 0.36[a]	1.36 ± 0.23[ab]	1.49 ± 0.29[b]	1.42 ± 0.51[b]
SOD	7.31 ± 0.81[b]	8.44 ± 1.67[a]	7.13 ± 0.68[b]	7.66 ± 1.50[b]	7.89 ± 1.54[ab]
GPx	84.5 ± 12.58[b]	97.32 ± 18.87[a]	89.05 ± 8.61[b]	92.56 ± 12.90[ab]	94.15 ± 12.09[ab]

Values are expressed as mean ± standard deviation, n=5; means with the same letters down the column are not significantly different at P<0.05. GST-Glutathione-S-Transferase (μmol/min/mg protein), GPx-Glutathione Peroxidase (nmol/mgHg) and SOD-Superoxide Dismutase (units/mg protein). Group A (control), group B (negative control), group C (induced + standard drug), group D (induced + 100 mg/kg of _H. madagascariensis_ extract) and group E (induced + 200 mg/kg of _H. madagascariensis_ extract).

Table 5: Effect of _H. madagascariensis_ extract on Non-Enzymatic Antioxidants of Cardiac Tissue in Sodium Fluoride-Induced Hypertensive Rats

Enzymes	A	B	C	D	E
GSH	88.66 ± 6.18[b]	71.20 ± 6.61[a]	90.82 ± 4.77[b]	86.24 ± 8.02[b]	87.83 ± 5.55[b]
MDA	6.22 ± 1.13[b]	8.05 ± 2.50[a]	7.55 ± 1.50[a]	7.37 ± 1.15[ab]	6.61 ± 4.21[b]
PC	6034.59 ± 1859.99[b]	8486.27 ± 2173.16[a]	7222.01 ± 4210.10[ab]	6902.72 ± 4555.32[b]	6686.12 ± 2587.86[b]
PT	38.01 ± 4.68[b]	34.19 ± 19.06[a]	40.94 ± 20.03[ab]	39.82 ± 18.15[b]	40.70±18.32[b]

Values are expressed as mean ± standard deviation, n=5. Means with the same letters down the column are not significantly different at P<0.05. GSH-Reduced Glutathione, MDA-Malondialdehyde, PT-Protein Thiol and PC-Protein Carbonyl. Group A (control), group B (negative control), group C (induced + standard drug), group D (induced + 100 mg/kg of _H. madagascariensis_ extract) and group E (induced + 200 mg/kg of _H. madagascariensis_ extract) GSH= Reduced Glutathione (μmol/mgprotein); MDA = Malondialdehyde (nmol/mg protein); PC = Protein Carbonyl (nmol/mg protein); PT= Protein Thiol = (μmol/mg protein).

Table 6: Effect of *H. madagascariensis* extract on Non-Enzymatic Antioxidants of Renal Tissue in Sodium Fluoride -Induced Hypertensive Rats

Enzymes	A	B	C	D	E
GSH	99.26 ± 6.84[b]	88.40 ± 28.83[a]	96.84 ± 5.39[ab]	99.91±11.57[b]	98.56±16.95[b]
MDA	6.01 ± 3.36[b]	8.49 ± 8.64[a]	5.31 ± 4.32[b]	4.02 ± 3.53[ab]	5.62 ± 8.72b[a]
PC	9472.57 ± 3380.60[b]	10368.3 ± 4096.62[a]	9654.58 ± 3326.48[b]	9036.71 ± 5480.52[b]	9478.34 ± 4559.98[b]
PT	99.81 ± 17.1[b]	90.07 ± 34.35[a]	115.30 ± 33.20[b]	111.10 ± 15.4[ab]	115.7 ± 25.49[ab]

Values are expressed as mean ± standard deviation, n=5. Means with the same letters down the column are not significantly different at P<0.05. Group A (control), group B (negative control), group C (induced + standard drug), group D (induced + 100 mg/kg of *H. madagascariensis* extract) and group E (induced + 200 mg/kg of *H. madagascariensis* extract). GSH= Reduced Glutathione (µmol/mg protein); MDA = Malondialdehyde (nmol/mg protein); PC = Protein Carbonyl (nmol/mg protein); PT= Protein Thiol = (µmol/mg protein).

Table 7: Effect of *H. madagascariensis* Extract on Analysed Markers of Kidney Function in Sodium Fluoride-Induced Hypertensive Rats

Markers	A	B	C	D	E
BUN (mmol/dl)	16.93 ± 0.40[b]	17.68 ± 1.52[a]	15.98 ± 0.88[ab]	17.43 ± 0.69[a]	16.75±0.44[b]
CREATININE (mmol/dl)	0.78 ± 0.05[b]	0.88 ± 0.15[a]	0.68 ± 0.08[ab]	0.78 ± 0.05[b]	0.75 ± 0.06[b]

Values are expressed as mean ± standard deviation, n=5. Group A (control), group B (negative control), group C (induced + standard drug), group D (induced + 100 mg/kg of *H. madagascariensis* extract) and group E (induced + 200 mg/kg of *H. madagascariensis* extract). BUN = Blood Urea Nitrogen.
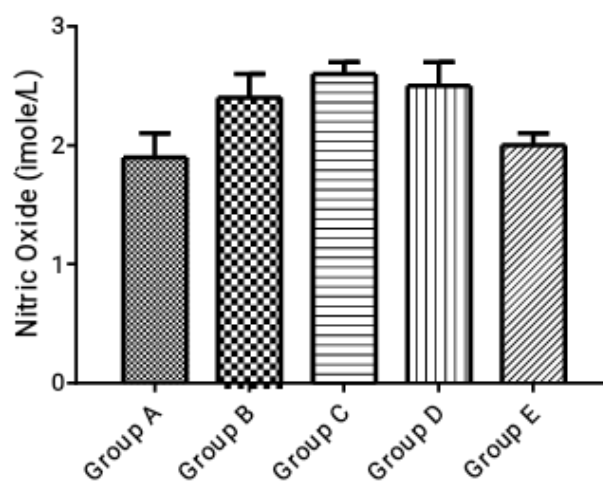
Figure 1: The effect of *H. Madagascariensis* on Serum Nitric Oxide (NO) of Sodium Fluoride Exposed Rats
Group A (control), group B (negative control), Group C (induced + standard drug), Group D (induced + 100 mg/kg of *H. madagascariensis* extract) and Group E (induced + 200 mg/kg of *H. madagascariensis* extract)
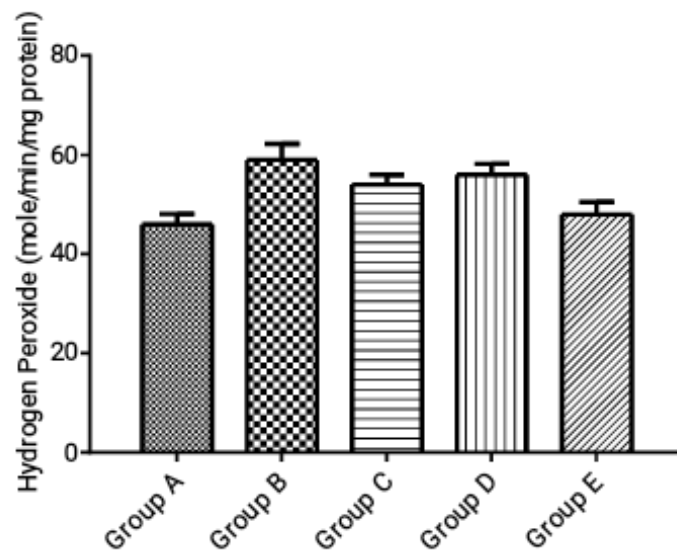
Fig 3: The effect of *H. madagascariensis* extract on the Renal Tissue of Sodium Fluoride Exposed Rats
Group A (control), group B (negative control), Group C (induced + standard drug), Group D (induced + 100 mg/kg of *H. madagascariensis* extract) and Group E (induced + 200 mg/kg of *H. madagascariensis* extract)
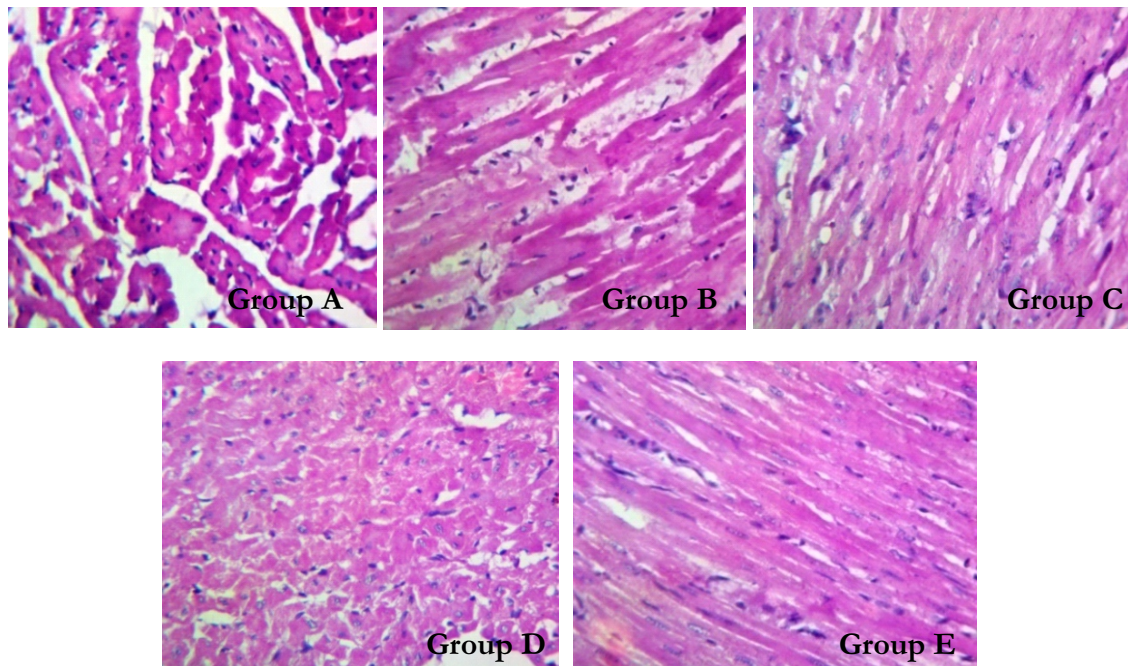

Figure 4: Effect of *H. madagascariensis* extract on Histology of Cardiac Tissue of Sodium Fluoride-Induced Hypertensive Rats
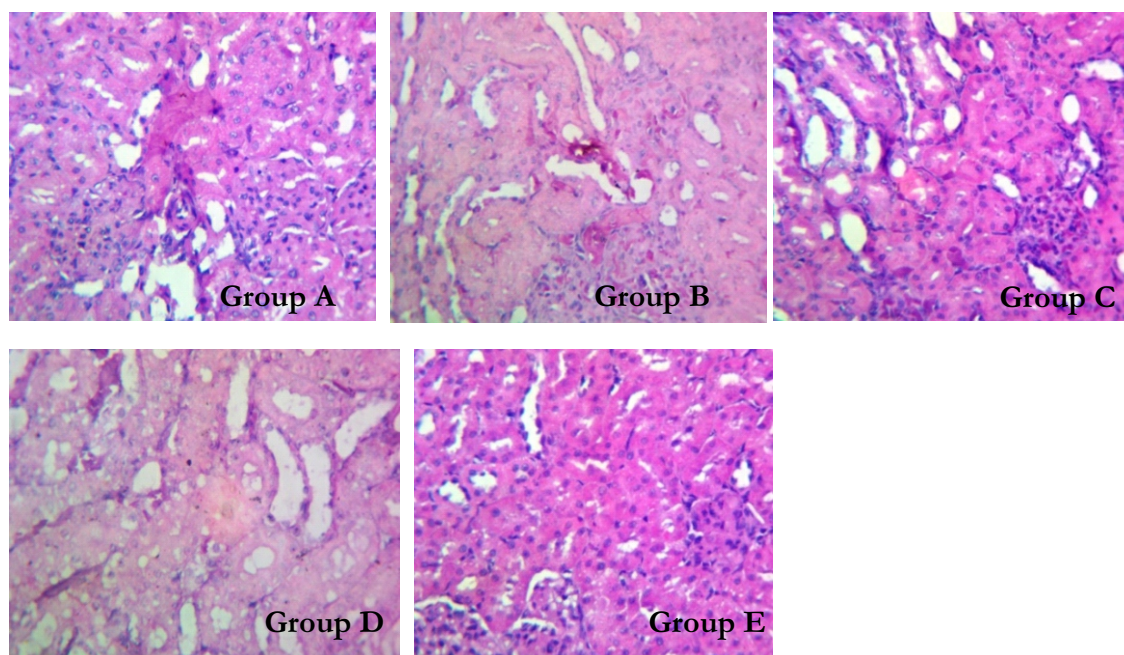
Figure 5: Effect of *H. madagascariensis* extract on Histology of **Renal Tissue** of Sodium Fluoride-Induced Hypertensive Rats

DISCUSSION

In this study, there was a significant difference between the systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) of the normotensive rats and the hypertensive rats in relation to the *H. madagascariensis* stem bark extract treated groups. The reduction of the SBP, DBP and MAP suggest an antihypertensive effect for the plant extract (Mojiminiyi *et al*., 2007). There was significant increase in the kidney/body weight ratio in the negative control when compared with other groups. This is not in agreement with the work of Oyagbemi *et al.* (2016) who reported a significant decrease in the kidney/body ratio of rats exposed to sodium fluoride (NaF). This may be as a result of the level of exposure or the positive effect exhibited by the extract of *H. madagascariensis* stem bark when compared with the treated group and control. Sodium fluoride is known to target the kidneys which serve as the primary organs involved in its excretion and retention (Song *et al*., 2014). It is documented that fluoride salt induces toxic effects in target organs such as the liver and kidney (Santoyo-Sanchez *et al*., 2013). The increase in the kidney/body weight of rats observed in this study may be due to the hypertensive effect caused by the exposure to sodium fluoride but were ameliorated by the administration of enalapril and

the extract of *H. madagascariensis* stem bark. Furthermore, this study revealed significant changes in the activities of the enzymatic and non enzymatic antioxidants in the cardiac and renal tissues. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) protect cells from toxic reactive oxygen species (Mates *et al.,* 1999). The presence of endogenous GSH and antioxidant enzymes such as CAT, GPx and GST has been suggested to be responsible for the elimination of hydrogen peroxide (H_2O_2) and other toxic substances in biological systems (Sharma *et al*., 2012). The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free H_2O_2 to water. Peroxidase possesses high affinity for and can remove H_2O_2 even when it is present in low concentration, and SOD activity enhances the spontaneous dismutation of superoxide radicals to H_2O_2 (Kohen and Nyska, 2002). In addition, the GPx and GST are key enzymes that take part in maintaining glutathione homeostasis in the tissues. GPx and GST as antioxidant enzymes work together with GSH in the decomposition of H_2O_2 and other organic hydro peroxides (Zhou *et al*., 2016). Decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of the enzyme, which has been reported to occur in

diabetes and hypertension (Sozmen *et al.*, 2001) as a result of depletion owing to excessive use of these enzymes to mop up the hypertension-induced free radical generation. In this study, the observed increased activity of GST, GPx and SOD may be due to the activations caused by the reactive oxygen species.

Also in this study, the markers of oxidative stress malondialdehyde (MDA) and protein carbonyl (PC) increased significantly in the positive control compared with treatment groups, but reduced glutathione (GSH) and protein thiol (PT) decreased significantly. Observation in this study corroborates the earlier report of Nwanjo *et al.* (2007) who reported increase in plasma MDA level, which is a marker of lipid peroxidation due to oxidative stress in sodium fluoride (NaF)-induced hypertension. The elevated MDA levels observed in this study may be due to the production of superoxide, peroxyl, and hydroxyl radicals as earlier suggested by Abdel-Wahhab *et al.* (2017). Increased peroxidation of membrane lipids is one of principal consequences of oxidative damage produced by NaF exposure (Ameeramja *et al.*, 2018). Elevated levels of protein carbonyl are believed to be caused by increased oxidation of protein due to oxidative stress (Yaidikar and Thakur, 2015). Reduced glutathione (GSH) is present at high concentrations in all mammalian cells, especially in the renal cells, hepatocytes, and erythrocytes (Sehirli *et al.,* 2008). GSH is one of the most important endogenous intracellular antioxidants. It plays the role of a sulfhydryl (SH) group provider for direct scavenging reactions. The decrease in the protein thiol is indicative of enhanced protein oxidation in the renal tissue which might be associated with systemic oxidative stress (Manna and Jain, 2015).

Also in this study, there was a significant decrease in the serum nitric oxide (NO) of the positive control group when compared to the treated groups. The reduced NO bioavailability has been documented to precipitate hypertension (Chalupsky *et al.*, 2015). Human hypertension is associated in a decrease in nitric oxide (NO) bioavailability and an increase in oxidative stress (Touyz and Schiffrin, 2004). Nitric oxide is released by the endothelium and causes vascular

relaxation (Rafieian-Kopaei *et al.*, 2014). The significant increase in blood urea nitrogen (BUN) and creatinine in the sodium fluoride-exposed rats may be due to increased protein catabolism resulting from sodium fluoride-induced systemic oxidative damage. This suggests extensive glomerular damage and tubular epithelial cells damage which may also reduce the rate of creatinine clearance from the kidneys and equally its retention in the blood circulation (Seelhammer *et al.*, 2016).

CONCLUSION

Harungana madagascariensis extract showed hypotensive and antioxidant effects in sodium fluoride-induced hypertensive rats. The plant might be useful in the management of hypertension caused by oxidative stress. However, more clinical studies in human are still required to further validate its efficacy.

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