Original Research Article

Annona muricata L. leaves or *Curcuma longa* L. rhizomes ameliorates oxidative stress associated with hypertension in uninephrectomized Wistar rats daily loaded with sodium chloride

ABSTRACT

Aims: Oxidative stress sequel to hypertension exacerbates the clinical condition and accelerates associated organopathies, therefore prevention is important. Traditionally in Nigeria, hypertension is treated with *Annona muricata* L. leaves or *Curcuma longa* L. rhizomes, two medicinal plants with antioxidant properties.

Study design: This study assessed the effect of these plants on hypertension-induced oxidative stress in uninephrectomized Wistar rats daily loaded with sodium chloride (1%).

Place and Duration of Study: Department of Veterinary Pharmacology and Toxicology Experimental Animal House, University of Ibadan, Nigeria, between August and November 2017.

Methodology: Hypertensive rats were treated with methanol extracts of the plants for 42days. Two other groups of hypertensive rats were treated with lisinopril or chlorothiazide. Blood pressure was monitored by non-invasive tail plethysmography using an electro-sphygmomanometer. Oxidative stress markers were determined in blood and tissue (heart, kidney and liver); GPX, GST, GSH, SOD, MDA and NO.

Results: Treatment of uninephrectomized rats with *A. muricata* or *C. longa* significantly ($p<0.0001$) decreased blood pressure and MDA, while elevating enzymatic and non-enzymatic antioxidant defense mechanisms of GST, GSH, GPx and SOD, comparable to normotensive rats. NO, the ubiquitous molecule required for basal vascular tone, myocardial contractility regulation and platelet adhesion prevention, was restored in the extract-treated rats. However, hypertensive untreated rats showed evidence of oxidative damages with significant increase in MDA, especially in the heart and liver, with decreases in the antioxidant defense system.

Conclusion: Results of this study justified the traditional use of *A. muricata* or *C. longa* for management of hypertension in Nigeria and showed that the extracts ameliorated oxidative damage that accompanied hypertension, thus also preventing complications of hypertension.

Keywords: Annona muricata, Curcuma longa, hypertension, oxidative stress markers

1. INTRODUCTION

Oxidative stress, precipitated by excessive production of reactive oxygen species (ROS) which has overwhelmed the antioxidant defense mechanisms, has been implicated in pathophysiological conditions that affect cardiovascular system such as hypercholesterolemia, diabetes and hypertension [1,2,3]. In animal models, oxidative stress has been demonstrated in spontaneous hypertension [4], renovascular hypertension [5], deoxycorticosterone acetate-salt model [6] and obesity-related hypertension [7].

Spontaneous hypertension in rats can be significantly decreased by reducing superoxide radicals which can be achieved by infusion of superoxide dismutase (SOD) [8].

In humans, hypertension is also considered as a state of oxidative stress that can contribute to the development of atherosclerosis [9] and other hypertension-induced organ damages [10]. Evaluation of antioxidant activities and lipid peroxidation byproducts in hypertensive subjects show an excessive amount of ROS and a decrease in the mechanism of antioxidant activity in both blood as well as in several other cellular systems [11,12], including vascular wall cells [13]. The instability of critical non-lipid macromolecules as another consequence of the overproduction of ROS may have important consequences on cellular functions. More recent management strategy for hypertension targets alleviation of oxidative stress, thus more research are geared towards antihypertensive drug candidates with capacity to reverse and or prevent development of oxidative stress in hypertensive patients.

A major source been explored are natural antioxidants from plants species which have protective effect against oxygen ion derived from free radicals involved in the development of many diseases such as arthritis, cardiovascular disorders, cancer and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases [14]. Phytochemicals such as flavonoids, polyphenols, vitamin C and E and carotenoids as antioxidants have been reported to protect the body system against reactive oxygen species [15,16]. Various efforts are now concentrated on many herbal plant extracts because of their antioxidant effects [17].

This study is focused on two plants traditionally used for management of hypertension in Nigeria and are proven to have antioxidant properties [18,19,20,21,22]. The plants are widely grown in Nigeria and readily available. These medicinal plants; *Annona muricata* L. and *Curcuma longa* L. have a long history of use in African Traditional medicine for treatment of several ailments including diabetes and cancer [18,23,24,25,26]. These medicinal plants are well reported to be traditionally used in Nigeria for treatment of hypertension [27,28,29,30]. This study therefore seeks to evaluate treatment outcome on oxidative stress status of hypertensive Wistar rats treated with the extracts of *Annona muricata* leaves or *Curcuma longa* rhizomes.

2. MATERIAL AND METHODS

2.1 Plant collection and extract preparation

Fresh leaves of *Annona muricata* were harvested from a private orchard in Asaba, Delta State, Nigeria and rhizomes of *Curcuma longa* were purchased from Bodija Market, Ibadan, Nigeria. The plants were identified and voucher specimen deposited at Department of Botany, University of Ibadan (Voucher-Numbers UIH-22593 and UIH-22595). The rhizomes were chopped and air dried, while the leaves were air dried and pulverized. The plant materials were extracted by cold macerated in methanol (96%) for 72 hours. The filtrate decanted was concentrated using a rotary evaporator (BUCHI R-210, Switzerland) and the extract obtained was stored at 4^0C. Fresh extract was reconstituted daily for dosing.

2.2 Experimental Animals

Seventy male Wistar rats weighing 140-180g were obtained from and housed at the Experimental Animal unit of the Department of Veterinary Pharmacology and Toxicology, University of Ibadan. They were allowed free access to standard rat

pellets and fresh water *ad libitum*. The rats were acclimatized for two weeks before commencement of the experiment. All experiments and protocols described in the study were in accordance with the recommendation for animal care and use by University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/11/2017/054) which follow internationally acceptable best practices for experimental animal care and use as adapted from the European Community and US guidelines.

2.3 Experimental protocol

The rats were randomly divided into ten groups with 7 rats in each group. Group A rats were maintained as normal healthy rats (Normotensive control), while hypertension was induced in groups B-J by unilateral nephrectomy and daily loading with sodium chloride (1%) for 42 days. Group B rats remained hypertensive and untreated throughout the study. Groups C and D rats were hypertensive rats treated with lisinopril or hydrochlorothiazide (standard antihypertensives). Groups E, F and G were hypertensive rats treated with *Annona muricata* leaves extract (100mg/kg, 200mg/kg or 400mg/kg), while Group H, I and J rats were hypertensive rats treated with *Curcuma longa* rhizomes extract (100mg/kg, 200mg/kg or 400mg/kg). The experimental hypertension was maintained for 42 days, alongside treatment with the antihypertensive drugs or plant extracts. Blood pressure was monitored by non-invasive method using an electro-sphygmomanometer (CODA, Kent Scientific, USA).

2.4 Sample collections and homogenate preparation

Blood sample was collected from the retro-orbital sinus into lithium heparinized bottles on day 43. After blood collection, the rats were humanely sacrificed by cervical dislocation. The heart, liver and kidney of each rat was carefully removed, immediately perfused with normal saline and blotted with filter paper. It was homogenized in cold potassium phosphate buffer (0.1 M, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 minutes with a cold centrifuge at 4 ^{0}C to obtain post-mitochondrial fraction. An estimation of serum total protein as well as determination of reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione transferase (GST), superoxide dismutase (SOD), malonaldehyde (MDA) and nitric oxide (NO) from the supernatant were carried out.

2.5 Data analysis

All values were expressed as mean±S.D. 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 was performed using Graph Pad Prism version 4.00.

3. RESULTS AND DISCUSSION

3.1 Result

3.1.1 Blood pressure

The systolic, diastolic and mean arterial blood pressures of hypertensive rats were significantly ($p<0.05$) higher than the normotensive rats. These were significantly ($p<0.05$) reversed in hypertensive rats treated with the methanol extracts of *A. muricata* leaves or *C. longa* rhizomes, or the standard antihypertensive drugs (lisinopril or Chlorothiazide) (Figure 1).

3.1.2 Antioxidant defense systems

The result shows that nitric oxide (NO) levels were significantly ($p<0.01$) decreased in untreated hypertensive rats, but was reversed in hypertensive rats treated with the extracts of *A. muricata* or *C. longa* or the antihypertensive drugs. NO levels in rats treated with the extracts were comparable to that in normotensive rats (Figure 2). Hypertension produced a significant ($p<0.01$) reduction in the heart, kidney and liver glutathione peroxidase (GPx) level in the untreated hypertensive group when compared to the normotensive rats. This was reversed in only hypertensive rats treated with *A. muricata* or *C. longa* (100mg/kg), while other hypertensive rats had reduced heart GPx levels. Depression of kidney GPx levels was reversed in all treated groups except in rats that received the higher doses of *C longa* (200mg/kg or 400mg/kg). A reversal of the depression in GPx was also observed in liver of treated rats, with significant ($p<0.05$) elevations of liver GPx in rats treated with *A. muricata* (200mg/kg and 400mg/kg) (Figure 3). Reduced glutathione (GSH) levels were also depressed in the heart, kidney and liver of untreated rats compared to normotensive rats, but a reversal was observed in all the treated hypertensive rats. Significant ($p<0.01$) elevations of heart and kidney GSH levels were also observed in hypertensive rats treated with methanol extract of *A. muricata* (400mg/kg) and *C. longa* (200mg/kg) (Figure 4).

Glutathione s-transferase (GST) levels significantly ($p<0.05$) declined in hearts, kidney and liver of untreated hypertensive rats compared to normotensive rats but was also reversed in all treated rats with significant elevations in the heart of hypertensive rats treated with *A. muricata* (400mg/kg) and the liver of rats treated with *C. longa* (100mg/kg and 200mg/kg) (Figure 5). The same pattern of decline in untreated hypertensive rats compared to normotensive rats and reversal in all treated rats was observed for superoxide dismutase (SOD) levels in the heart, kidney and liver of these rats. In addition, significant elevations in SOD levels were observed in kidneys of rats treated with *C. longa* (400mg/kg) and liver of rats treated with *A. muricata* (200mg/kg), while SOD were statistically unchanged in liver of rats treated with *A. muricata* (100mg/kg) and *C. longa* (100 and 400mg/kg) (Figure 6). Malondialdehyde (MDA) levels were significantly ($p<0.0001$) elevated in untreated hypertensive rats compared to normotensive rats, but were remarkably reversed to normal levels in treated rats with the extracts or antihypertensives (Figure 7).

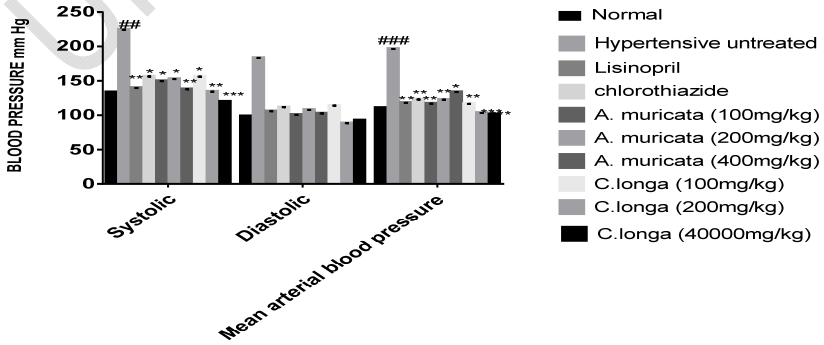

Figure 1: Blood pressure of uni-nephrectomized rats loaded daily with NaCl (1%) and treated with methanol extract of *Annona muricata* leaves or *Curcuma longa* rhizomes

Significantly (p<0.001) different from normotensive control; *Significantly different from hypertensive untreated (*p<0.05, ***p<0.0001)

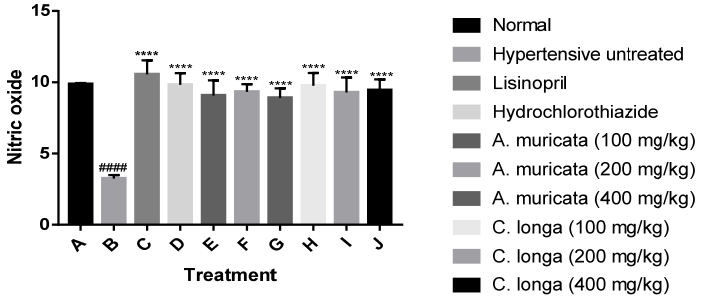

Figure 2: Effects of *Annona muricata* leaves, *Curcuma longa* rhizomes, lisinopril and chlorothiazide on serum NO.

Significantly different from the normotensive rats (####p<0.01); Significantly different from hypertensive untreated rats (*p<0.05, ****p<0.0001)

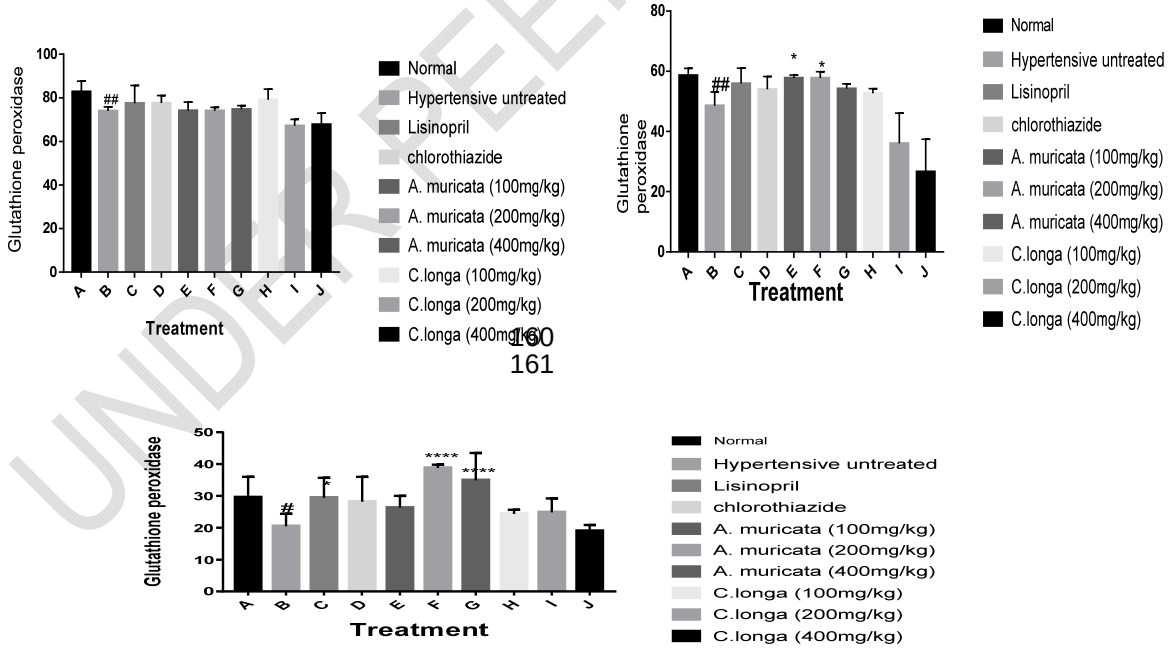

Figure 3: Effects of *Annona muricata* leaves, *Curcuma longa* rhizomes, lisinopril and chlorothiazide on heart, kidney and liver glutathione peroxidase.

Significantly different from the normal control group (p<0.01); *Significantly different from hypertensive untreated control (*p<0.05, ***p<0.001)

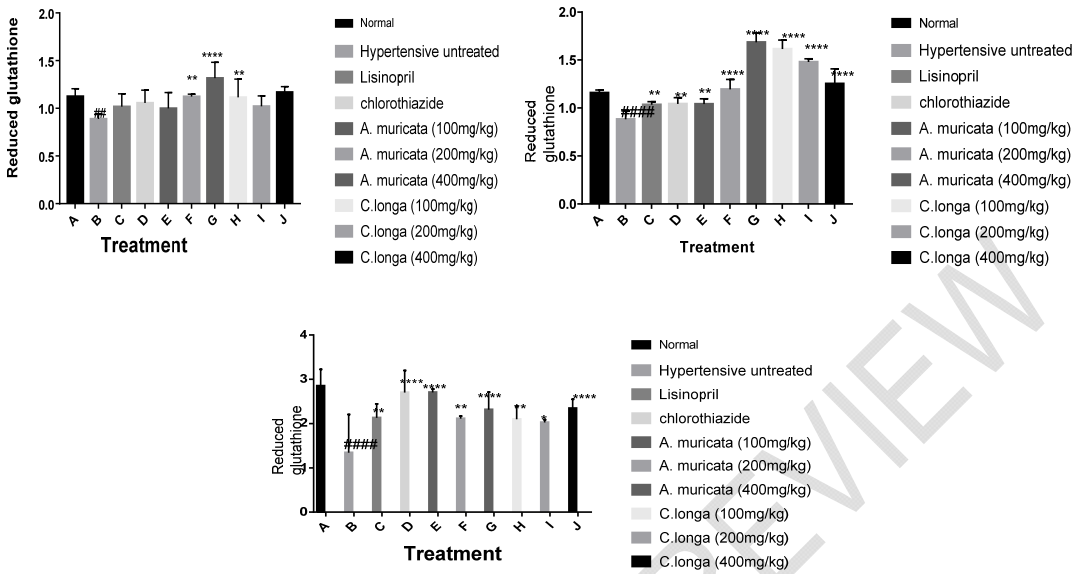

Figure 4: Effects of *Annona muricata* leaves, *Curcuma longa* rhizomes,lisinopril and chlorothiazide on heart, kidney and liver GSH.

Significantly different from the normal control group ($p<0.01$); *Significantly different from hypertensive untreated control (*$p<0.05$, ***$p<0.0001$)

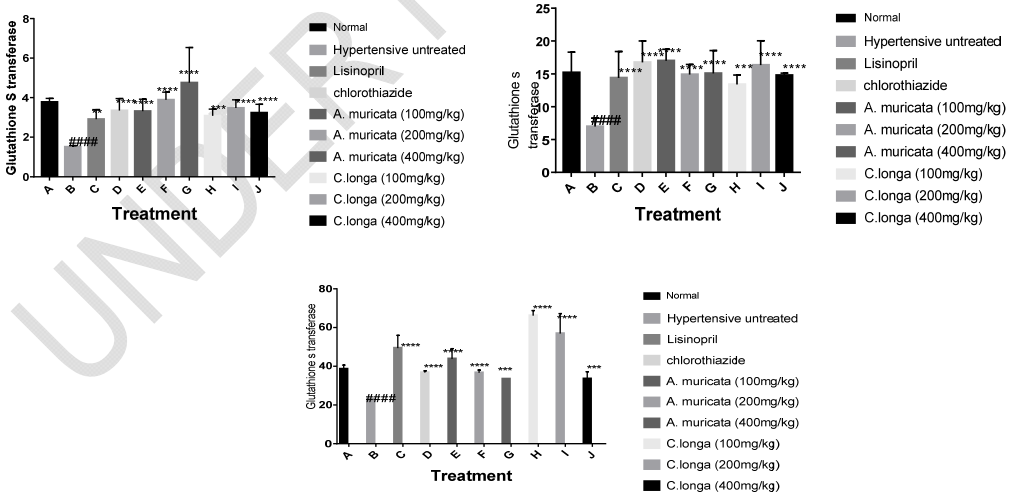

Figure 5: Effects of *Annona muricata* leaves, *Curcuma longa* rhizomes, lisinopril and chlorothiazide on heart, kidney and liver GST.

Significantly different from the normal control group ($p<0.01$); *Significantly different from hypertensive untreated control (****$p<0.0001$)

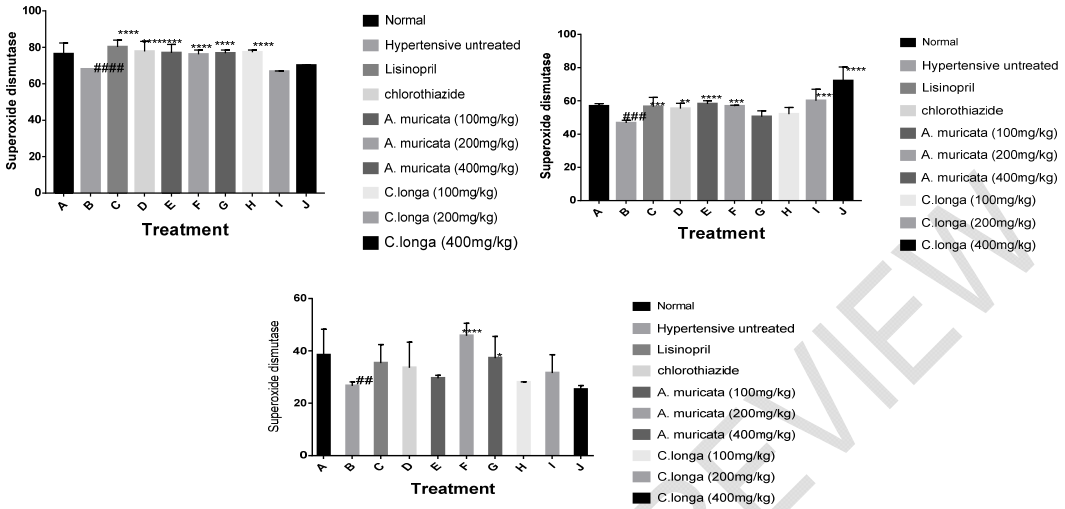

Figure 6: Effects of *Annona muricata* leaves, *Curcuma longa* rhizomes, lisinopril and chlorothiazide on heart, kidney and liver SOD.

Significantly different from the normal control group ($p<0.0001$); *Significantly different from hypertensive untreated control (****$p<0.0001$)

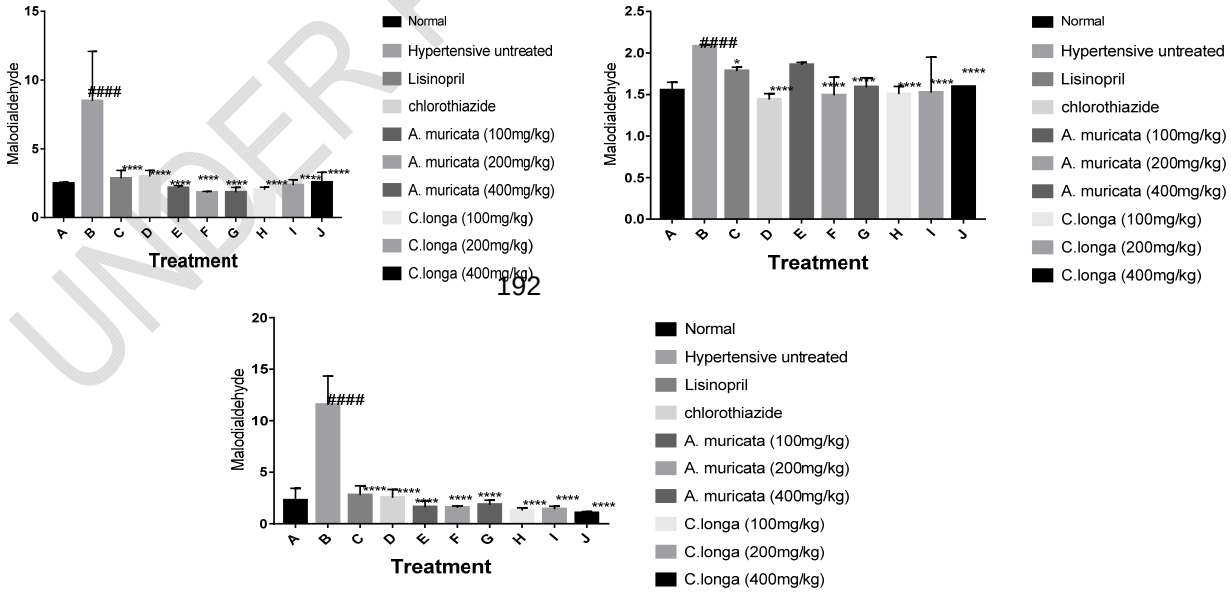

Figure 7: Effects of *Annona muricata* leaves, *Curcuma longa* rhizomes, lisinopril and chlorothiazide on heart, kidney and liver MDA.
Significantly different from the normal control group ($p<0.0001$); *Significantly different from hypertensive untreated control (****$p<0.0001$)

3.2 Discussion

Medicinal plants are now considered as the basis for health preservation and care worldwide [31]. Chronic degenerative diseases (hypertension, diabetes, cardiovascular and cancer) have reached epidemic proportions and are considered a serious health problem; therefore, the treatments of these diseases are of clinical importance [32]. In this study, hypertension was induced by unilateral nephrectomy and daily loading with sodium chloride (1%) which resulted in renal hypertension [33,34]. *Annona muricata* L. (Family: Annonaceae) and *Curcuma longa* (Family: Curcubitaceae) demonstrated potent antihypertensive properties evidenced by the reversal of the elevated blood pressure, restoration of antioxidants and reduction of oxidants generated in the induced hypertensive state.

The methanol extract of *A. muricata* leaves and *C. longa* rhizomes inhibited development of hypertension shown by normal systolic blood pressure, diastolic blood pressure and mean arterial pressure of these treated hypertensive rats. This is in agreement with an earlier report in which leaf extract of *A. muricata* caused a dose-dependent reduction in mean arterial pressure (MAP) in normotensive rats [27]. These researchers suggested that *A. muricata* lowered blood pressure through the blockage of calcium ion channel, and the Ca^{2+} antagonism was further demonstrated by its ability to relax high K^+ induced contractions [27]. *C. longa* has also been reported to have antioxidant and vascular protective effect [35] and exert antihypertensive effect by down-regulation of AT_1 receptor in vascular smooth muscle cells [36].

This study showed hypertension generated a remarkable oxidative stress which was significantly ($p<0.01$) reversed by the extracts of *A. muricata* and *C. longa*, alongside lowering of the blood pressure. The extracts showed significant ($p<0.05$) increase in antioxidant defense system and inhibition of generation of free radicals. Antioxidant defense systems of cells contain a variety of enzymatic and non-enzymatic scavengers. The enzymatic antioxidants of cells, including glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST) and superoxide dismutase (SOD) play a critical role in the attenuation of oxidative stress induced by reactive oxygen species [37]. Reduced glutathione substrate augments the activity of GPx and GST in catalyzing the hydrogen peroxide into oxygen and water. The reduced glutathione has the ability to reduce the oxidized glutathione, catalyzed by GR [38].

The first defensive mechanism against reactive oxygen species is provided by SOD, which attenuates oxidative stress through dismutation of O_2^-. Catalase enzyme has an important role in converting the endogenous H_2O_2 to water and oxygen [39]. The accumulation of H_2O_2 in cells results in the generation of highly reactive free hydroxyl radical (OH^-) through Fenton reaction, which has an important devastating

role in oxidative damages [40]. GPx degrades lipid peroxides to hydroxyl lipids and waters through conversion of glutathione to glutathione disulfide [41,42].

A major marker of lipid peroxidation is malondialdehyde (MDA) which increases during oxidative damage to cell membranes, inhibition of several important enzymes, reduced cellular function, and cell death [43,44]. The degree of lipid peroxidation can be determined by tissue MDA levels, which is a highly reliable marker of oxidative stress [45]. MDA is a highly reactive aldehyde which can cause toxic stress in cells and result in formation of covalent protein adducts known as advanced lipoxidation end-products, an analogy of advanced glycation end-products [46]. The result of this study shows that induction of hypertension produced a significant ($p<0.0001$) elevation of MDA in the heart, kidney and liver of untreated hypertensive group when compared to the normotensive rats. Treatment with methanol extract of *A. muricata* or *C. longa*, lisinopril and chlorothiazide produced a significant ($p<0.0001$) reduction in the heart, kidney and liver MDA. This indicates remarkable inhibition of lipid peroxidation which usually accompanies and further exacerbates oxidative stress and hypertension [47].

Depletion of nitric oxide (NO) was reversed in rats treated with methanol extract of *A. muricata* and *C. longa* in this study. Nitric oxide is generated from its precursor L-arginine by nitric oxide synthase (NOS). There are three isoforms of the enzyme; the two constitutive forms, endothelial and neuronal NOS (eNOS and nNOS) and the inducible isoform originally described in immune cells (iNOS). Nitric oxide effects its principle biological actions, including that of vascular smooth muscle relaxation, via soluble guanylate cyclase and production of the second messenger c-GMP [48]. Interestingly, *A. muricata* or *C. longa* treatment exhibited a good therapeutic profile with a marked increase of serum NO level thereby enhancing the vasodilatory effects of NO with resultant lowering of blood pressure.

4. CONCLUSION

In conclusion, methanol extract of *Annona muricata* and *Curcuma longa* ameliorated the oxidative stress which accompanies and exacerbates hypertension in uni-nephrectomized rats loaded with 1% sodium chloride. This study corroborated previous findings on the correlation between hypertension and oxidative stress [47,49], and improvement of renovascular hypertension following antioxidant treatment [50]. Further studies are warranted to establish the pharmacological principle responsible for the antihypertensive activity of these medicinal plants which can be progressed as antihypertensive drug candidates.

CONSENT (WHERE EVER APPLICABLE)

Not Applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as regulations set by the Animal Care and Use in Research Ethics Committee of the University of Ibadan with approval number UI-ACUREC/App/11/2017/054.

REFERENCES

1. Steinberg D, Witztum JL. Is the Oxidative Modification Hypothesis Relevant to Human Atherosclerosis? Circul. 2002; 105: 2107–2111.

2. Chilsom GM, Steimberg D. The Oxidative Modification Hypothesis of Atherogenesis. An Overview. Free Rad Biol Med. 2000; 28: 1815–1826.

3. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circul. Res. 2000; 87 (10): 840–44.

4. Wu L, Jourlink BH. Increased Methylglyoxal and Oxidative Stress in Hypertensive Rat Vascular Smooth Muscle Cells. Hypert. 2002; 39: 809–814.

5. Lerman LO, Nath KA, Rodriguez-Porcel M, Krier JD, Schwartz RS, Napoli C, Romero JC. Increased Oxidative Stress in Experimental Renovascular Hypertension. Hypert. 2001; 37: 541–546.

6. Rolliet MR, Rudd MA, Loscalzo J. Oxidative Stress and Renal Dysfunction in Salt-Sensitive Hypertension. Kidney Blood Press Res. 2001; 24: 116–123.

7. Dobrian AD, Davies MJ, Schriver SD, Lauterio TJ, Prewitt RL. Oxidative Stress in a Rat Model of Obesity-Induced Hypertension. Hypert. 2001; 37: 554–560.

8. Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M. Does Superoxide Underlie the Pathogenesis of Hypertension? Proc Natl Acad Sci USA. 1991; 88: 10045–10048.

9. Romero JC, Reckelhoff JF. Role of Angiotensin and Oxidative Stress in Essential Hypertension. Hypert. 1999; 34: 943–949.

10. Raij L. Nitric Oxide in Hypertension. Relationship with Renal Injury and Left Ventricular Hypertrophy. Hypert. 1998; 31: 189–193.

11. McIntyre M, Bohr DF, Dominiczak AF. Endothelial Function in Hypertension: The Role of Superoxide Anion. Hypert. 1999; 34: 539–545.

12. Yasunari K, Maeda K, Nakamura M, Yoshikawa J. Oxidative Stress in Leukocytes Is a Possible Link Between Blood Pressure, Blood Glucose and C-Reactive Protein. Hypert. 2002; 39: 777–780.

13. Orie NN, Zidek, W, Tepel M, Reactive Oxygen Species in Essential Hypertension and Non-Insulin-Dependent Diabetes Mellitus. Amer J Hypert. 1999; 12: 1169–1174.

14. Almeida MMB, de Lemos LG. Bioactive compound and antioxidant activity of fresh exotic fruits from Northern eastern Brazil. Food Res Inter. 2011; 44: 2155-2159.

15. Gutteridge JM, Halliwell B.Antioxidants: Molecules, medicines and myths. Biochem Biophys Res Comm. 2010; 393: 561-564

16. Mohamed DA, Hamed TE, AL-Okbi SY. Reduction in hypercholesterolemia and risk of cardiovascular diseases by mixtures of plant food. Grasas Aceites. 2010; 61 (4): 378-389. https://doi.org/10.3989/gya.021210

17. Akinloye DI, Osatuyi OA, Musibau OG, Yusuf AA, Adewuyi S. *In vitro* antioxidant activities, elemental analysis and some bioactive constituents of *Acalypha wilkesiana* Muellarg and *Acalypha wilkesiana* Java white leaf extracts. J Chem Soc Nigeria. 2016; 41(2), 150-157.

18. Saba AB, Oridupa OA. Search for a novel antioxidant, anti-inflammatory/analgesic or anti-proliferative drug: cucurbitacins hold the ace. J Med Plants Res. 2010; 4 (25): 2821 - 2826

19. George VC, Kumar DRN, Suresh PK, Kumar RA. Antioxidant, DNA protective efficacy and HPLC analysis of Annona muricata (soursop) extracts. J Food Sci Technol. 2014; 52(4); 2328–2335. https://doi.org/10.1007/s13197-014-1289-7

20. Muthu S, Durairaj B. Evaluation of antioxidant and free radical scavenging activity of Annona muricata. Eur J Exper Biol. 2015; 5(3): 39-45

21. Agu KC, Okolie PN. Proximate composition, phytochemical analysis, and in vitro antioxidant potentials of extracts of Annona muricata (Soursop). Food Sci Nutr. 2017; 5(5): 1029–1036. https://doi.org/10.1002/fsn3.498

22. Tanvir EM, Hossen MS, Hossain MF, Afroz R, Gan SH, Khalil MI, Karim N. Antioxidant Properties of Popular Turmeric (Curcuma longa) Varieties from Bangladesh. Journal of Food Quality Volume 2017, Article ID 8471785, 8 pages. https://doi.org/10.1155/2017/8471785.

23. Adewole SO, Caxton-Martins EA. Morphological changes and hypoglycemic effects of Annona muricata Linn. (Annonaceae) leaf aqueous extract on pancreatic B-cells of streptozotocin-treated diabetic rats. Afr J Biomed Res. 2006; 9: 173–187. https://doi.org/10.4314/ajbr.v9i3.48903

24. Adewole S, Ojewole J. Protective effects of Annona muricata linn.(annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. Afr J Tradit Complement Altern Med. 2009; 6: 30–41. https://doi.org/10.4314/ajtcam.v6i1.57071

25. Mishra S, Ahmad S, Kumar N, Sharma BK. Annona muricata (the cancer killer): A review. Glob. J Pharm Res. 2013; 2: 1613–1618

26. Priyadarsini KI. Chemical and structural features influencing the biological activity of curcumin. Curr Pharm Des. 2013; 19: 2093–2100

27. Nwokocha CR, Owu DU, Gordon A, Thaxter K, McCalla G, Ozolua RI, Young L. Possible mechanisms of action of the hypotensive effect of Annona muricata (soursop) in normotensive Sprague-Dawley rats. Pharm Biol. 2012; 50 (11): 1436–1441.

28. Adefegha SA, Oyeleye SI, Oboh G. Distribution of Phenolic Contents, Antidiabetic Potentials, Antihypertensive Properties, and Antioxidative Effects of Soursop (Annona muricata L.) Fruit Parts In Vitro. Biochem Res Int. 2015: 347673. doi: 10.1155/2015/347673.

29. Akinyemi AJ, Adedara IA, Thome GR, Morsch VM, Rovani MT, Mujica LKS, Duarte T, Duarte M, Oboh G, Schetinger MRC. Dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats. Toxicol Rep. 2015; 2: 1357-1366. doi: 10.1016/j.toxrep.2015.10.001.

30. Oyemitan IA, Elusiyan CA, Onifade AO, Akanmu MA, Oyedeji AO, McDonald AG. Neuropharmacological profile and chemical analysis of fresh rhizome essential oil of Curcuma longa (turmeric) cultivated in Southwest Nigeria. Toxicol Rep. 2017; 4: 391–398. doi: 10.1016/j.toxrep.2017.07.001

31. Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, Sun JN, Ma DL, Han YF, Fong WF, Ko KM. New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. Evid. Based Complement Alternat Med. 2013; 627375. https://doi.org/10.1155/2013/627375

32. World Health Organization (WHO). Preventing chronic diseases a vital investment. 2005; http://www.who.int/chp/chronic_disease_report/ full_report.pdf (accessed on 25.08.15).

33. Mozaffari MS, Wyss JM. Dietary NaCl-Induced Hypertension in Uninephrectomized Wistar-Kyoto Rats: Role of Kidney Function. J Cardiovasc Pharmacol. 2005; 33(5): 814-821

34. Lin HY, Lee YT, Chan YW, Tse G. Animal models for the study of primary and secondary hypertension in humans. Biomed Rep. 2016; 5(6): 653–659. https://doi.org/10.3892/br.2016.784

35. Nakmareong S, Kukongviriyapan U, Pakdeechote P, Donpunha W, Kukongviriyapan V, Kongyingyoes B, Sompamit K, Phisalaphong C. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. Naunyn Schmiedebergs Arch Pharmacol. 2011; 383: 519–529

36. Yao Y, Wang W, Li M, Ren H, Chen C, Wang J, Wang WE, Yang J, Zeng C. Curcumin exerts its antihypertensive effect by down regulating the AT1 receptor in vascular smooth muscle cells. Sci Reports. 2016; 6: 25579. https://doi.org/10.1038/srep25579

37. Singh S, Vrishni S, Singh B.K., Rahman, I., Kakkar, P., 2010. Nrf2-ARE stress response mechanism: a control point in oxidative stress-mediated dysfunctions and chronic inflammatory diseases. Free Rad Res. 2010; 44: 1267–1288

38. Bazzichi L, Ciompi ML, Betti L, Rossi A, Melchiorre D, Fiorini M, Giannaccini G, Lucacchini A. Impaired glutathione reductase activity and levels of collagenase and elastase in synovial fluid in rheumatoid arthritis. Clin Exp Rheumat. 2002; 20: 761-766

399 39. Fahmy HM, Mohammed FF, Abdelrahman RT, Abu Elfetoh MM, Mohammed YA. Effect
400 of Radiofrequency Waves Emitted from Conventional WIFI Devices on Some Oxidative
401 Stress Parameters in Rat Kidney. J. Drug Metab. Toxicol. 2015; 6: 195.
402 https://doi.org/10.4172/2157-7609.1000195

403 40. Park S, Imlay JA. High levels of intracellular cysteine promote oxidative DNA damage by
404 driving the fenton reaction. J Bacteriol. 2003; 185: 1942-1950.
405 https://doi.org/10.1128/JB.185.6.1942

406 41. Naziroglu M, Kokcam I. Antioxidants and lipid peroxidation status in the blood of patients
407 with alopecia. Cell Biochem Funct. 2008; 18: 169–173

408 42. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, Oxidative Damage and Oxygen
409 Deprivation Stress: a Review Ann Bot. 2003; 91(2): 179–194.
410 https://doi.org/10.1093/aob/mcf118

411 43. Abdel Fattah NS, Ebrahim AA, El Okda ES. Lipid peroxidation/antioxidant activity in
412 patients with alopecia areata. J Eur Acad Dermatol Venereol. 2011; 25(4): 403-8.
413 https://doi.org/10.1111/j.1468-3083.2010.03799.x.

414 44. Prie BE, Voiculescu VM, Ionescu-Bozdog OB, Petrutescu B, Iosif L, Gaman LE, Clatici
415 VG, Stoian I, Giurcaneanu C. Oxidative stress and alopecia areata. J Med Life. 2015;
416 8(Spec Issue): 43–46.

417 45. Davey MW, Stals E, Panis B, Keulemans J, Swennen RL. High-throughput determination
418 of malondialdehyde in plant tissues. Analyt Biochem. 2005; 347 (2): 201–207.
419 doi:10.1016/j.ab.2005.09.041

420 46. Farmer EE, Davoine C. Reactive electrophile species. Curr Opin Plant Biol. 2007; 10 (4):
421 380–6. https://doi.org/10.1016/j.pbi.2007.04.019

422 47. Touyz RM, Briones AM. Reactive oxygen species and vascular biology: implications in
423 human hypertension. Hypert Res. 2011; 34: 5–14

424 48. Francis SH, Busch JL, Corbin JD. cGMP-Dependent Protein Kinases and cGMP
425 Phosphodiesterases in Nitric Oxide and cGMP Action. Pharmacol Rev. 2010; 62(3): 525–
426 563. https://doi.org/10.1124/pr.110.002907

427 49. Wang H, Li H, Hou Z, Pan L, Shen X, Li G. Role of oxidative stress in elevated blood
428 pressure induced by high free fatty acids. Hypert Res. 2009; 32: 152–158

429 50. Nishi EE, Oliveira-Sales EB, Bergamaschi CT, Oliveira, TGC, Boim MA, Campos RR.
430 Chronic antioxidant treatment improves arterial renovascular hypertension and oxidative
431 stress markers in the kidney in Wistar rats. Am J Hypertens. 2010; 23: 473-480.
432 https://doi.org/10.1038/ajh.2010.1