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Serum Lipid Profile and Antioxidant Status of Salt- induced Hypertensive Rats Treated with an Antioxidants Rich Nutraceutical

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Abstract (Manuscript 74868)
There is increase evidence that hypertension is associated with increased levels of oxidative stress markers. The current work aimed to investigate the effect of an antioxidant rich nutraceutical on blood pressure, glucose, lipid profile, antioxidant status, nitric oxide, insulin, and malondialdehyde in salt loaded albino rats. The rats were placed on 8% NaCl for 3 weeks and then supplemented with 250 and 500mg/kg body weight of the nutraceutical with or without nifedipine for additional 3 weeks. The nutraceutical was prepared from onions, garlic, tomatoes, lemon, palm oil and crayfish in ratio of 5:5:2:4:2:2. Salt loading significantly increased the blood pressure. Supplementation showed significant ($P<0.05$) decreased in the blood pressure, glucose, insulin, total cholesterol, triglyceride, low density lipoprotein-cholesterol, atherogenic index, Malondialdehyde and increased in high density lipoprotein-cholesterol, antioxidant status and nitric oxide as compared with unsupplemented group. The percentage protection against atherogenesis indicated an average of 83.12 ± 2.53% for all the treated groups. The result also indicated significant positive correlation between the mean arterial blood pressure and total cholesterol, triglyceride, low density lipoprotein-cholesterol, atherogenic index and correlated negatively with high density lipoprotein-cholesterol and antioxidant activities. There was no significant difference in the parameters of supplemented group and the group treated with 10mg/kg of nifedipine. The results suggest that the nutraceutical may improve the antioxidant status and delayed the complications of hypertension in rats.
Key words: Cardiovascular disease, hypertension, nutraceutical, oxidative stress

INTRODUCTION

Hypertension and other cardiovascular diseases rank among the leading causes of mortality in industrialized nations [1], the leading and increasing contributor to the global disease burden and are responsible for one third of global deaths [2]. Hypertension is the most threatening risk factor for adverse cardiovascular outcomes, including stroke, myocardial infarction, renal failure and death [3]. Prevention of increased blood pressure therefore plays a critical role in reduction of those outcomes. Increased in the generation of reactive oxygen species and decreased antioxidant activities have been shown to be one of the mechanisms of the pathogenesis of hypertension [4]. Reactive oxygen species may act through several mechanisms to mediate vascular change in hypertension: direct actions on endothelial cells; increase quenching of nitric oxide, a vasodilator by O_2^-; production of peroxynitrite; and oxidative modification of low density lipoprotein [5]. Oxidized low-density lipoprotein has a number of potential pro-atherogenic activities contributing to important clinical manifestations of coronary artery disease such as endothelial dysfunction and plaque disruption [6]. Recent findings related to the renin angiotensin system, which is one of the most important mechanisms for blood pressure regulation, have provided an improved understanding of the pathophysiology of hypertension [7, 8]. However renin angiotensin system inhibition may provide end-organ protection independent of lowering of blood pressure.

Effect of antioxidant may result from either activation or mimicry of antioxidant defences. Alternatively, it may be due to interaction with factors involved in the activation of oxidative stress. Thus antioxidant treatment may prevent the hypertension and the associated organ alteration.

The use of nutraceuticals as an attempt to accomplish desirable therapeutic outcomes with reduced side effect as compared with other therapeutic agent [9] has been advocated in recent time. Therefore, this study aimed to investigate the effect of antioxidant rich nutraceutical on the possible complications that may arise from hypertension.

MATERIALS AND METHODS

Chemicals and Reagents

Analytical graded chemicals and reagents were used for this study. Nifedipine was sourced from Sunij Pharma, PVT Ltd, Ahmedabab, India.

Experimental Animals

Wistar rats of both sexes weighing between 120-150g were used for this study. The animals were purchased from Department of Biological sciences, Usmanu Danfodiyo University, Sokoto, Nigeria and were allowed to acclimatize for one week before the commencement of the experiment. They were fed pelletized growers' feed (Vital feed, Jos, Nigeria) and were allowed access to water *ad libitum* before and during the experimental period. The experimental protocol was approved by the Ethical committee of the Usmanu Danfodiyo University, Sokoto, Nigeria.

Preparation of Hypertensive Rats

The rats were placed on 8% NaCl diet [10] except the normal group. The animals were fed 8% salt diet for 3 weeks and treatment plus the challenging agent for additional 3 weeks.

Measurement of Blood Pressure

The baseline blood pressure was measured and recorded and subsequent measurement was done every two weeks. This was done by tail-cuff method using non-invasive Ugo Basile, series 58500 blood pressure recorder. Average of four readings was taken for each rat and the temperature of the rat was monitored throughout the measurement period. Mean arterial blood pressure was calculated according to the following equation: DP +1/3 (SP- DP) where SP and DP are systolic and diastolic pressure respectively.

Preparation of Antioxidant Micronutrients Supplements

Antioxidants nutraceutical was prepared locally from onions, garlic, tomatoes, lemon, palm oil and crayfish in ratio of 5:5:2:4:2:2. This was done by mixing 25g of onions; 25g of garlic and10g of tomatoes in 100ml distilled water and blended using electric blender. 10g of grinded crayfish was then added and blended once again. To this, 20g of lemon juice and 10g of palm oil were added, mixed and stored in refrigerator until required.

Grouping of Animals and Treatment

The animals were randomly divided into six groups of 5 rats each.

Group I Normal untreated (1ml of distilled water)
Group II Salt-loaded untreated (1ml of distilled water)
Group III Salt-loaded treated with 250mg/kg of nutraceutical
Group IV Salt-loaded treated with 500mg/kg of nutraceutical
Group V Salt-loaded treated with 500mg/kg of nutraceutical + 10mg/kg of nifedipine
Group VI Salt-loaded treated with 10mg/kg of nifedipine

The appropriate dosages of the nutraceutical and drug were administered to the animals orally once daily by intubation using intravenous cannula tube for 3 weeks. Twenty four hours after the last treatment, the animals were anaesthetized with chloroform vapour and blood samples were collected through cardiac puncture into labelled tubes for biochemical analyses. The animals were allowed to fast over night before the blood was collected. Weight changes of the rats were monitored throughout the experimental period.

Biochemical Analyses

The fasting serum glucose level was estimated by glucose oxidase method [11]. Serum total cholesterol [12], triglyceride [13] and high density lipoprotein-cholesterol [14] were determined by enzymatic method. Serum low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol was calculated by the formula of fredewald [15]. Atherogenic index was calculated as the ratio of LDL-cholesterol to HDL-cholesterol [16]. Colorimetric method was used for the estimation of serum total antioxidant status [17] and tissue malondialdehyde [18].

Cayman's Superoxide Dismutase Assay Kit was used for the estimation of SOD. The assay utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine at 450nm. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radicals.

The catalase activity was estimated using Cayman's Catalase Assay Kit. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured with 4- amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen at 540nm.

Glutathione Peroxidase was assayed using Cayman's Assay Kit. This assay measures glutathione peroxidase activity indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of hydroperoxide by glutathione peroxidase, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340nm.

Insulin was estimated by SPI bio rat insulin enzyme immunoassay kit**.** The assay is based on the competition between unlabelled rat insulin and acetylcholinesterase linked to rat insulin (tracer) for limited specific guinea-pig anti-rat insulin antiserum sites. The plate was then washed and Ellman's reagent added to the wells and the acetylcholinesterase tracer acts on the Ellman's reagent to form a yellow compound which was determined at 405nm.

Percentage Protection against Atherogenesis was calculated using the following equation:

$$\text{Protection} = \frac{AI\ of\ HC - AI\ of\ treated\ group}{AI\ of\ HC} x100$$

AI: atherogenic index; HC: Hypertensive control

Statistical Analysis

Values are expressed as mean ± standard deviation for 5 rats in each group. The biochemical parameters were analysed statistically using one way analysis of variance (ANOVA), followed by Turkey Kramer multiple comparison test using GraphPad Instat software. Differences were considered significant when $p<0.05$.

RESULTS

The result in Fig. 1 is the effect of treatment on % weight increase of the rats. The result indicated significant ($P<0.001$) weight increase in all the treated groups as compared to hypertensive control. There was no significant weight increase between the treated groups and normotensive control except the group that was treated with nifedipine ($P<0.001$). The group that received combined therapy gained the highest weight of 20.99±3.47% relatively similar to normotensive control (19.72±3.06), 250mg/kg of nutraceutical (19.02±2.87) and 500mg/kg of nutraceutical (19.10±2.82) respectively while hypertensive control gained the lowest weight of 3.28±0.46%.

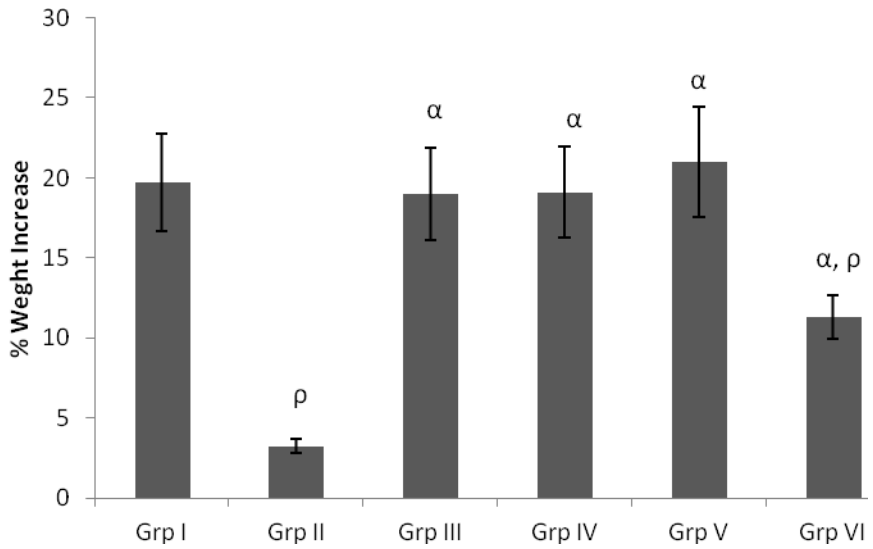

Figure 1: Effect of antioxidants rich nutraceutical on percentage body weight increase

Grp I- normal untreated, Grp II- salt-loaded untreated, Grp III- salt loaded treated with 250mg/kg of nutraceutical, Grp IV- salt loaded treated with 500mg/kg of nutraceutical, Grp V- salt loaded treated with 500mg/kg of nutraceutical and 10mg/kg of nifedipine, Grp VI- salt loaded treated with 10mg/kg of nifedipine. $^{\alpha}P<0.001$ when compared with grp II , $^{\rho}P<0.001$ when compared with grp I

Table I: Effect of antioxidant rich nutraceutical on the SBP, DBP and MABP

Group	SBP (mmHg)	DBP (mmHg)	MABP (mmHg)	PR (BPM)
I	117±2.4	75±1.64	88.41±1.87	258±12.40
II	141±1.94†	90±3.12†	106.66±2.08†	287±20.46
III	127±1.54*†	78±1.03*	94.39±0.54*†	253±27.08ᵝ
IV	126±0.54*†	79±0.83*†	94.77±0.64*†	251±11.03ᵝ
V	123±0.83*†	74±2.28*ᵅ	91.10±1.66*	253±19.77ᵝ
VI	125±1.83*†	78±1.87*	93.23±1.12*†	250±9.16ᵝ

SBP-systolic blood pressure, DBP-diastolic blood pressure, MABP-mean arterial blood pressure, PR- pulse rate, Grp I- normal untreated, Grp II- salt-loaded untreated, Grp III- salt loaded treated with 250mg/kg of nutraceutical, Grp IV- salt loaded treated with 500mg/kg of nutraceutical, Grp V- salt loaded treated with 500mg/kg of nutraceutical and 10mg/kg of nifedipine, Grp VI- salt loaded treated with 10mg/kg of nifedipine *P<0.001and ᵝP<0.05 when compared with group II, †P<0.001 when compared with group I, ᵅP<0.05 when compared with group VI

Table 2: Effect of antioxidant rich nutraceutical on lipid profile and atherogenic index

Group	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	AI
I	2.298±0.192	0.984±0.102	1.242±0.044	0.606±0.116	0.444±0.047	0.484±0.090
II	3.072±0.138†	1.268±0.069†	0.712±0.090†	0.712±0.090	0.572±0.033†	2.474±0.169†
III	2.128±0.227‡	1.004±0.108‡	1.114±0.160‡	0.554±0.064‡	0.452±0.049‡	0.500±0.073‡
IV	2.234±0.221‡	1.038±0.074ᵛ	1.232±0.062‡	0.524±0.141‡	0.468±0.034‡	0.420±0.107‡
V	2.110±0.067‡	0.956±0.066‡	1.216±0.075‡	0.466±0.069‡	0.430±0.029‡	0.378±0.047‡
VI	2.178±0.118‡	1.032±0.064ᵛ	1.198±0.027‡	0.506±0.071‡	0.464±0.029‡	0.418±0.05‡

TC- total cholesterol, TG- triglyceride, HDL-C- high density lipoprotein- cholesterol, LDL-C- low density lipoprotein- cholesterol, VLDL-C- very low density lipoprotein- cholesterol, AI- atherogenic index. Grp I- normal untreated, Grp II- salt-loaded untreated, Grp III- salt loaded treated with 250mg/kg of nutraceutical, Grp IV- salt loaded treated with 500mg/kg of nutraceutical, Grp V- salt loaded treated 500mg/kg of nutraceutical and 10mg/kg of nifedipine, Grp VI- salt loaded treated with 10mg/kg of nifedipine. ‡P<0.001, ᵛP<0.01and ᵝP<0.05 when compared with group II, †P<0.001, ᵖp<0.01and ᵃP<0.05 when compared with group I, ᵇP<0.001and ᵅP<0.05when compared with group VI

The effect of antioxidant rich nutraceutical on systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and pulse rate is presented in Table I. The result indicated significant decreased in the SBP (P<0.001), DBP (P<0.001), MABP (P<0.001) and PR (P<0.05) as compared with salt-loaded untreated group. There was no significant (P>0.05) difference in the SBP, DBP, MABP and pulse rate between the groups treated with nutraceutical and nifedipine. The effect of antioxidant rich nutraceutical on lipid profile is presented in Table 2. The result indicated significant decreased in the levels of TC, TG, LDL-C, VLDL-C and AI and increased in HDL-C of the treated groups as compared with the untreated group.

Effect of nutraceutical on % protection against atherogenesis (Fig 2) indicated no significant (P>0.05) variation in the % protection against atherogenesis of the group that received combined therapy (85.85±1.69%) and 500mg/kg of nutraceutical (83.07±2.26%) as compared with nifidipine (83.82±1.46) treated group but significant (P<0.05) difference was observed between the group that was treated with 250mg/kg of nutraceutical (79.75±2.51%) and nifedipine.

Table 3: Effect of antioxidants rich nutraceutical on glucose, insulin, MDA and TAS

Group	Glc (mmol/l)	Insulin (ng/ml)	MDA (µmol/l)	TAS (mmol/l)
I	5.62±0.36	1.48±0.32	0.290±0.034	1.33±0.106
II	5.77±0.44	2.10±0.31ᵖ	0.707±0.180†	0.72±0.175†
III	4.44±0.40‡,†	1.24±0.12‡	0.327±0.048‡	1.16±0.151ᵛ
IV	4.41±0.41‡,†	1.18±0.22‡	0.308±0.029‡	1.22±0.139†
V	4.19±0.22‡,†	1.12±0.20‡	0.274±0.042‡	1.28±0.175†
VI	4.71±0.38ᵛ,ᵖ	1.55±0.26ᵝ	0.314±0.024‡	1.18±0.189ᵛ

MDA- malondialdehyde, TAS- total antioxidant status; Grp I- normal untreated, Grp II- salt-loaded untreated, Grp III- salt loaded treated with 250mg/kg of nutraceutical, Grp IV- salt loaded treated with 500mg/kg of nutraceutical, Grp V- salt loaded treated with 500mg/kg of nutraceutical and 10mg/kg of nifedipine, Grp VI- salt loaded treated with 10mg/kg of nifedipine ‡P<0.001, ᵛP<0.01and ᵝP<0.05 when compared with group II, †P<0.001, ᵖp<0.01and ᵃP<0.05 when compared with group I, ᵇP<0.001, ᵅP<0.05 when compared with group VI.

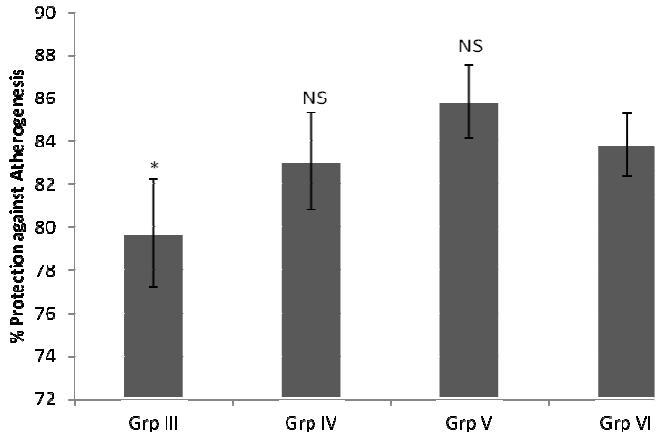

Figure 2: Effect of antioxidants rich nutraceutical on percentage protection against atherogenesis Grp III- salt loaded treated with 250mg/kg of nutraceutical, Grp IV- salt loaded treated with 500 mg/kg of nutraceutical, Grp V- salt loaded treated with 500mg/kg of nutraceutical and 10mg/kg of nifedipine, Grp VI- salt loaded treated with 10mg/kg of nifedipine.*P<0.05and NS- not significant when compared with grp VI,

Effect of antioxidant rich nutraceutical on glucose, insulin, MDA and total antioxidant status (Table 3) indicated significant decreased in the levels of glucose, insulin and MDA and increased in total antioxidant status of the treated groups as compared to the hypertensive control.

Effect of antioxidant rich nutraceutical on antioxidant enzymes and nitric oxide is presented in Table 4. The result showed significant increased in the activities of antioxidant enzymes and the level of nitric oxide between the treated groups and hypertensive control. No significant difference was observed between the nutraceutical and nifedipine treated group.

The correlation coefficient (r) of MABP against glucose, insulin, lipid profile and oxidative stress markers is presented in Fig 3. The result showed significant correlation between MABP and all the parameters with the exception of glucose, insulin and nitric oxide which showed insignificant correlation.

Table 4: Effect of antioxidants rich nutraceutical on antioxidant enzymes and nitric oxide

Group	Nitric oxide (µM)	Catalase (nmol/min/ml)	GPx (nmol/min/ml)	SOD (U/ml)
I	33.70±4.11	28.71±0.45	104.67±10.00	4.71±0.92
II	10.29±2.61†	13.62±1.80†	44.31±9.06†	2.51±1.15
III	15.11±3.91†	22.38±4.60ᵞ	81.50±9.13‡,†	5.12±0.83ᵝ
IV	20.33±3.82ᵞ,†	23.82±5.10‡	84.04±4.31‡,ᵖ	4.97±1.43ᵝ
V	40.00±3.24‡,ᵇ	27.12±2.97‡	91.68±4.31‡	5.14±1.19ᵝ
VI	20.74±3.23ᵞ,†	26.82±2.27‡	84.55±6.77‡,ᵖ	5.59±1.30ᵞ

GPx- glutathione peroxidase, SOD- superoxide dismutase; Grp I- normal untreated, Grp II- salt-loaded untreated, Grp III- salt loaded treated with 250mg/kg of nutraceutical, Grp IV- salt loaded treated with 500mg/kg of nutraceutical, Grp V- salt loaded treated 500mg/kg of nutraceutical and 10mg/kg of nifedipine, Grp VI- salt loaded treated with 10mg/kg of nifedipine. ‡P<0.001, ᵞP<0.01and ᵝP<0.05 when compared with group II, when compared with group II, †P<0.001, ᵖp<0.01and ᵃP<0.05 when compared with group I, ᵇP<0.001, ᵠP<0.05 when compared with group VI

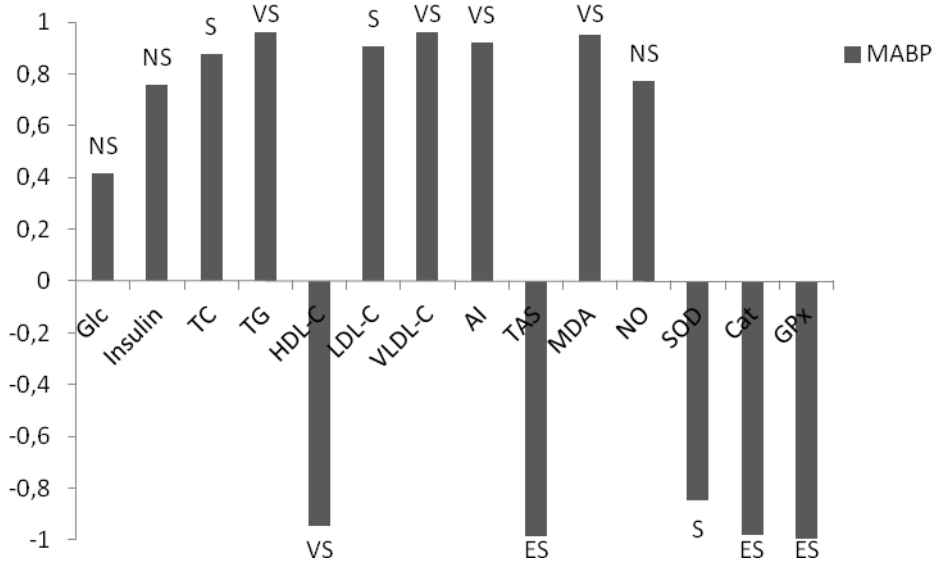

Fig 3: Correlation coefficient (r) of MABP against glucose, insulin, lipid profile, oxidative stress markers

MABP-mean arterial blood pressure, Glc- glucose, HOMA-IR- Homeostasis Model Assessment- Insulin Resistance GPx- glutathione peroxidase, SOD- superoxide dismutase, TC- total cholesterol, TG-triglyceride, HDL-C – high density lipoprotein cholesterol, LDL-C- low density lipoprotein cholesterol, VLDL–C - very low density lipoprotein cholesterol, AI- atherogenic index, TAS- total antioxidant status, MDA- malondialdehyde, NO- nitric oxide, ES- extremely significant, VS- very significant, S- significant

Discussion

Hypertension is a consequence of the interaction of genetic and environmental factors. Micronutrients plays critical role in the regulation of blood pressure and subsequent target organ damage. Endothelial and vascular smooth muscle dysfunction initiates and perpetuates essential hypertension. The optimal combination of nutrients may impacts significantly in the prevention and treatment of cardiovascular complications of hypertension [19]. The Short-term reduction in blood pressure using nutrition may have intermediate and long-term improvements in morbidity and mortality, including cardiovascular accidents, coronary heart disease, and myocardial

infarction [20, 21]. Hypertension was induced by placing the rats on 8% NaCl diet. Salt have been reported to cause hypertension in rats' models and human hypertension [10, 22, 23]. The result of this study showed that treatment attenuated the rising in the blood pressure of the rats as compared with the salt-loaded untreated control. Thus, the antihypertensive effect of nutraceutical in this model could be mediated through reduction in angiotensin II levels by inhibiting angiotensin converting enzyme, increased nitric oxide and decreased reactive oxygen species production or could be that the nutraceutical acts as calcium channel blockers that suppresses trap of nitric oxide by reactive oxygen species which allow vasodilation thereby stimulating NO synthase expression. The findings in our model corroborated several studies on the role of garlic [24, 25] and onion [26, 27] in the treatment of hypertension although in this study garlic and onion were used a long side with tomatoes, palm oil, cray fish and lemon juice. The insignificant difference between the nutraceutical treated groups and nifedipine control observed in this study further confirm that antioxidant rich nutraceutical could be used as an alternative therapy in the prevention and management of cardiovascular complications of hypertension.

It is important to note that; our findings supported the hypothesis for the use of nutraceutical as effective strategies in the treatment of oxidative stress related diseases like diabetes, atherosclerosis, myocardial infarction and renal diseases. The result of the study also indicated significant decreased in the levels of total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), ahterogenic index (AI), malondialdehyde (MDA), glucose and insulin and increased in the high density lipoprotein-cholesterol (HLDL-C), nitric oxide and antioxidant activities in the treated groups relative to the hypertensive control. Thus, the possible mechanism underlying the role of nutraceutical against lipid peroxidation, dyslipidemia, hyperinsulinemia and hyperglycemia could be attributed to the role of nutraceutical in scanvenging free radicals and increasing insulin sensitivity which boost glucose uptake by the cells. The significant positive correlations observed between MABP and TC, TG, LDL-C, VLDL-C, AI and MDA and significant negative correlation with HDL-C and antioxidant activities showed strong evidence that increased oxidative stress play significant role in the pathophysiology of hypertension and cardiovascular related diseases and underscores the effect of nutraceutical in preventing and treatment of the cardiovascular consequences of these diseases.

In conclusion, antioxidant rich nutraceutical lowered the blood pressure and glucose level and may have promising effect in the preventing and management of cardiovascular complications of hypertension and diabetes.

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