The role of poly-herbal extract in sodium chloride-induced oxidative stress and hyperlipidemia in male Wistar rats

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Abstract

Consistent consumption of high salt diet (HSD) has been associated with increased cellular generation of free radicals which has been implicated in the derangement of some vital organs and etiology of cardiovascular disorders. This study was designed to investigate the combined effect of some commonly employed medicinal plants on serum lipid profile and antioxidant status of aorta, kidney, and liver of high salt diet-fed animals. Thirty-five male Wistar rats were divided into 5 groups of 7 animals each. Group 1 and 2 animals were fed normal rat and 16 % high salt diet only respectively. Animals in groups 3, 4, and 5 were fed 16% high salt diet with 800, 400, and 200 mg/kg bw poly-herbal extract (PHE) respectively once for 28 consecutive days. Serum low-density lipoprotein (LDL), triacylglycerol (TG), total cholesterol (TC) and high-density lipoprotein (HDL), malondialdehyde, nitric oxide, catalase, superoxide dismutase, glutathione peroxidase, glutathione concentration, and activities were assessed in the aorta, kidney, and liver. PHE ($p < 0.05$) significantly reduced malondialdehyde and nitric oxide concentration and increased antioxidant enzymes and glutathione activity. Elevated serum TG, TC, LDL, and TC content in HSD-fed animals were significantly ($p < 0.05$) reduced to normal in PHE-treated rats while HDL was significantly elevated ($p < 0.05$) in a concentration-dependent manner in PHE treated animals. Feeding with PHE attenuated high salt diet imposed derangement in serum lipid profile and antioxidant status in the organs of the experimental rats.

Introduction

Nutrition is an important factor in maintaining the physiological and biochemical wellness of the biological system [1]. Constant consumption of diet deficient or excessive in micronutrients is associated with the development of degenerative and metabolic disorders [2]. Sodium chloride (NaCl) is probably the oldest spice in human history and has a multifunctional role in the modern-day food industry and biotechnology [3]. Recently, there is a considerable increase in the salt content of foods due to changes in human dietary habits vis-à-vis high consumption of industrialized, processed, and fast food [4,5]. Although, governmental and institutional awareness on the reduction of sodium consumption and negative health implications of high salt intake are well disseminated and publicized [6-9]. However, industrial suitability, gustatory delights, salt addiction, and consumer's acceptability are a few of the factors still influencing the continued demand, interest, and consumption of high salt diet (HSD) worldwide [3, 10]. Sodium is involved in several trans-membrane and physiological processes and is dominantly supplied via dietary salt [11, 12]. Unfortunately, uncontrolled and excessive consumption of salt has been linked to the development of cardiovascular disorders, endothelial dysfunction, lipid disorders, and Alzheimer disease [13, 14]. Increased activities of reactive oxidative species (ROS), infiltration of immune cells have been postulated as the likely mechanisms of high salt-induced renal damage and hypertension [15-17]. Natural products and plants with medicinal importance are highly coveted, and sought for throughout the world (Harvey et al., 2010; Newman and Cragg, 2012; Harvey 2015; Ruhsam and Hollingsworth, 2017). Pharmacological activities vis-à-vis cardio-protective, anti-inflammatory, antioxidant, anti-cancer prowess of these botanicals have been documented and attributed to their different phytochemicals [22]. Traditionally, many of these plants are employed as a concoction of poly-herbal mixture in the management of various diseases. It is a common practice and accepted belief in folkloric medicine that a combination of herbal plants would have a rapid and potentiated effect against the targeted ailments [23, 24]. With this background understanding, this study was designed at investigating the combined effect of aqueous leaf extract of *Annona muricata*, *Carica papaya*, *Moringa oleifera* and *Aloe barbadensis* on oxidative status and lipid profile of rats fed with high salt diet.

Materials And Methods

Plant source and extraction

Annona muricata, *Carica papaya*, *Moringa oleifera* and *Aloe barbadensis* were collected in Ogbomoso town (8°08'N4°15'E) and were authenticated by a taxonomist from the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The voucher numbers of the plants were deposited at the University Herbarium.

Preparation of Poly-herbal extracts (PHE)

Five hundred each of the fresh leaves of the four plants were pulverized using a kitchen blender and subsequently macerated and boiled in water at 100 degrees for 2 hours. It was left alone to cool under room temperature for 3 hours and was then filtered using a clean muslin cloth. The supernatant was freeze-dried and the lyophilized crude extract was stored in an airtight dark bottle and refrigerated until further use.

Acute toxicity and Determination of LD₅₀

Acute toxicity testing was carried out in two phases according to the method described by [25]. Firstly, nine animals were divided into three groups of three animals each. Each group of animals was administered 10, 100, and 1000 mg/kg of the poly-herbal extract, and no mortality was observed. In the second phase, 6 animals were distributed into 3 groups of two animals each and were administered higher 1600, 2900, and 5000 mg/kg of the PHE respectively. No mortality was observed after 24 to 48 hours among animals in all groups.

Animal grouping and experimental design

All animal procedures in this study were performed following the guidelines of the research and ethics committee, Ladok Akintola University of Technology (LAUTECH) for the use of laboratory animals. Thirty-five (35) healthy male Wistar rats weighing 140-150 g were obtained from the animal house of the Department of Biochemistry, College of Basic of Medical Sciences, LAUTECH. They were housed in ventilated cages on a 12:12 hour light-dark cycle and acclimatized for 2 weeks and were separated into 5 groups of 7 animals each as depicted in Table 1

Table 1: Animal grouping and experimental design

Groups	Treatment
1	Fed with normal rat chow only (positive control)
2	Fed with 16 % salt diet only (negative control)
3	Fed with 16% salt diet and 800 mg/kg of the poly-herbal extract once daily
4	Fed with 16% salt diet and 400 mg/kg of the poly-herbal extract once daily
5	Fed with 16% salt diet and 200 mg/kg of the poly-herbal extract once daily

Collection of blood serum and tissue preparation from treated rats

The animals were sacrificed through cervical dislocation on the 29th day after overnight fasting. Blood was collected through cardiac puncture using a 5 ml syringe and transferred into plain sample bottles. The blood samples were centrifuged at 4000 rpm for 10 minutes to obtain the serum. The kidney, liver, and aorta were excised, washed in cold washing buffer, and homogenized in phosphate buffer (10%w/v). The homogenates were centrifuged at 10,000 x gravitational force for 15 minutes at 4°C. The supernatants were collected and stored in the freezer at -18°C.

Antioxidant assays

Antioxidant enzyme activity and oxidative stress markers were estimated in the liver, aorta, and kidneys homogenates.

Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was evaluated according to the method of [26]. One and a half mL each of 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA, and 2 mM of pyrogallol were added to 70 µL of tissue homogenate. Change in absorbance was recorded at 420 nm for 3 min in a spectrophotometer.

Determination of glutathione concentration

Glutathione (GSH) activity was estimated according to the procedure of Ellman [27]. One hundred (100 μ L) of the tissue homogenate was diluted in 20 mL of phosphate buffer (0.1 M, pH 8). Fourty mL of 0.01 M 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added to 6mL of the mixture, and absorbance was read at 412 nm.

Determination of glutathione peroxidase activity

Glutathione peroxidase activity was determined according to the method of Reddy et al. [28]. To 3.0 ml of glutathione peroxidase substrate solution, 0.1 ml of the homogenate was added. To the test cuvette, 0.5ml of hydrogen peroxide was added and mixed. The change in absorbance was recorded every 30 seconds for 3 minutes in a spectrophotometer at 430nm.

Determination of catalase activity

Catalase activity was determined according to the method of Clairborne [29]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), 19 mM H₂O₂ and 20 uL tissue homogenate. The degradation of H₂O₂ was read spectrophotometrically at 240 nm for 1 min. and the catalase activity was calculated according to the formula: $K = 2.303/T \times \log (A1/A2)$

Where: K: Rate of reaction; T: Time interval (minutes); A1: Absorbance at time zero; A2: Absorbance at 60 seconds interval.

Determination of malondialdehydeconcentration

Estimation of malondialdehyde (MDA) concentration as an index of lipid peroxidation was assayed according to the method described by [30] . One mL of 20% trichloroacetic acid was added to 1 ml of the tissue homogenate thereafter 2 mL of 0.67% thiobarbituric acid was added. The mixture was incubated at 100°C for 15 min in a water bath and cooled. Six 8 mL of n-butanol was added and centrifuged at 3000 rpm for 15min. The absorbance of the clear pink supernatant was then read against a blank at 532 nm spectrophotometrically. The concentration of MDA is expressed in nmol / g of the tissue.

Determination of Nitric oxide concentration

The level of nitric oxide (NO) in the tissues was determined according to the method described by [31]. Succinctly, 200 μ L of the samples was incubated with 200 μ L of Griess reagent at 25 °C in the dark for 30 min. Absorbance was subsequently read at 548 nm.

Serum Lipid Assay

Collected serum samples were analyzed for lipid profile. High-density lipoprotein-cholesterol (HDL-C) was assayed using an assay kit (Elabscience, USA). Triglyceride (TG) content was evaluated by enzymatic method using an assay kit (Randox, England). Total cholesterol (TC) was determined according to the method of Parakh and Jank (1982). Low-density lipoprotein (LDL-C) and Very low-density lipoprotein – cholesterol (VLDL-C) was calculated according to Friedwald et al. (1972).

Statistical analysis

Data obtained in this study were expressed as mean $\pm$ SEM and subjected to one-way analysis of variance (ANOVA) using statistical package for social sciences 21.0. Duncan's multiple test was used to identify significance between means at $P < 0.05$

Results

Antioxidant status of the liver

The antioxidant enzyme activities and concentration of oxidative stress markers in the liver of rats in all experimental groups are depicted in Table 2. Treatment with poly-herbal extract caused a significant ($P<0.05$) and a dose-dependent reduction in MDA concentration in the liver of the treated rats when compared with the HSD group only. An insignificant elevation in GSH concentration ($3.25 \mu\text{mol/g}$) was observed in 800 mg/kg PHE treated animals when compared with NRC, HSD and 400 mg/kg PHE. However, it was significantly different when compared with $2.04 \mu\text{mol/g}$ noticed in the 200 mg/kg PHE treated animals. GPx activity was significantly lowered in HSD only fed rats ($0.55 \mu\text{mol/g}$), NRC ($0.63 \mu\text{mol/g}$) and 200 mg/kg PHE ($0.42 \mu\text{mol/g}$ when compared with 800 mg/kg PHE ($0.81 \mu\text{mol/g}$) and 400 mg/kg PHE ($0.73 \mu\text{mol/g}$) treated rats respectively. SOD, CAT, and GST activity was reduced in the liver of the high salt fed rats and were significantly ($P<0.05$) increased after treatment with 400 and 800 mg/kg PHE.

Antioxidant status of the kidney

Malondialdehyde, nitric oxide concentration, and antioxidant enzyme activities in the kidney of rats in all experimental groups are depicted in Table 3. Poly-herbal extract (400-800 mg/kg PHE) caused a dose-dependent and significant ($P<0.05$) reduction in MDA concentration in the kidney of the treated rats when compared with the HSD-fed-only group ($40.97 \mu\text{mol/g}$). The concentration of GSH in the kidney of HSD fed only ($0.91 \mu\text{mol/g}$) and 200 mg/kg PHE ($1.09 \mu\text{mol/g}$) fed animals were significantly ($P<0.05$) reduced when compared with NRC ($1.61 \mu\text{mol/g}$), 400 and 800 mg/kg PHE. Nitric oxide concentration was significantly ($p<0.05$) elevated in response to high salt loading. GPx activity of $0.31 \mu\text{mol/g}$ observed in HSD only fed was significantly ($P<0.05$) lower when compared with $0.55 \mu\text{mol/g}$, $1.09 \mu\text{mol/g}$, $0.76 \mu\text{mol/g}$, $0.76 \mu\text{mol/g}$ noticed in NRC, 800, 400 and 200 mg/kg PHE treated rats respectively. CAT ($10.78 \mu\text{mol/g}$ and $9.95 \mu\text{mol/g}$) and GST activity ($2.41 \mu\text{mol/g}$) noticed in the kidney of normal chow and 800 mg/kg PHE treated rats respectively were significant ($P<0.05$) when compared with other treatment groups. Although, the lowest activities was observed in the HSD only fed rats.

Table 2: Antioxidant enzymes activities and concentration of oxidative stress markers in the liver of rats

Treatment	MDA ($\mu\text{mol/g}$)	GSH ($\mu\text{mol/g}$)	NO ($\mu\text{mol/g}$)	GPX ($\mu\text{mol/g}$)	SOD ($\mu\text{mol/g}$)	CAT ($\mu\text{mol/g}$)	GST ($\mu\text{mol/g}$)
NRC	40.77 ± 0.43^b	2.77 ± 0.30^{ab}	7.59 ± 0.13^b	0.63 ± 0.04^{bc}	26.74 ± 2.38^c	7.42 ± 0.61^{bc}	5.90 ± 0.91^b
HSD only	75.64 ± 5.69^c	2.29 ± 0.44^{ab}	13.31 ± 0.59^e	0.55 ± 0.01^{ab}	18.58 ± 0.54^{ab}	1.27 ± 0.14^a	3.34 ± 0.54^a
HSD +800 mg/kg PHE	26.86 ± 0.86^a	3.25 ± 0.38^b	5.75 ± 0.12^a	0.81 ± 0.07^d	34.20 ± 3.60^d	8.44 ± 0.67^c	6.37 ± 0.81^b
HSD +400 mg/kg PHE	29.62 ± 1.69^a	2.67 ± 0.08^{ab}	9.70 ± 0.02^c	0.73 ± 0.01^{cd}	24.74 ± 0.35^{bc}	8.38 ± 1.20^c	5.66 ± 0.55^b
HSD +200 mg/kg PHE	41.60 ± 1.76^b	2.04 ± 0.23^a	11.56 ± 0.64^d	0.42 ± 0.05^a	16.17 ± 0.90^a	3.24 ± 0.97^a	4.34 ± 0.54^{ab}

Data were expressed as mean $\pm$ SEM. Values with different superscripts down the column are significantly different ($p<0.05$). NRC; normal rat chow; HSD; high salt diet; PHE: poly-herbal extract

Table 3: Antioxidant enzymes activities and concentration of oxidative stress markers in the kidney of rats

Treatment	MDA ($\mu\text{mol/g}$)	NO ($\mu\text{mol/g}$)	GSH ($\mu\text{mol/g}$)	GP _x ($\mu\text{mol/g}$)	CAT ($\mu\text{mol/g}$)	GST ($\mu\text{mol/g}$)
NRC only	24.40 $\pm$ 1.14 ^b	13.95 $\pm$ 0.89 ^{bc}	1.61 $\pm$ 0.25 ^{bc}	0.55 $\pm$ 0.02 ^b	10.78 $\pm$ 0.91 ^c	2.41 $\pm$ 0.39 ^b
HSD only	40.97 $\pm$ 0.79 ^c	22.47 $\pm$ 1.66 ^d	0.91 $\pm$ 0.04 ^a	0.31 $\pm$ 0.04 ^a	2.49 $\pm$ 0.00 ^a	0.50 $\pm$ 0.13 ^a
HSD + 800 mg/kg PHE	16.06 $\pm$ 0.22 ^a	8.97 $\pm$ 0.35 ^a	1.68 $\pm$ 0.15 ^{bc}	1.09 $\pm$ 0.03 ^d	9.95 $\pm$ 0.79 ^c	2.41 $\pm$ 0.39 ^b
HSD + 400 mg/kg PHE	19.07 $\pm$ 1.03 ^b	9.43 $\pm$ 0.44 ^a	1.50 $\pm$ 0.16 ^{bc}	0.76 $\pm$ 0.05 ^c	7.36 $\pm$ 0.79 ^b	1.39 $\pm$ 0.13 ^{ab}
HSD + 200 mg/kg PHE	38.90 $\pm$ 1.89 ^c	14.64 $\pm$ 1.33 ^c	1.09 $\pm$ 0.02 ^{ab}	0.76 $\pm$ 0.06 ^c	4.48 $\pm$ 0.50 ^a	0.50 $\pm$ 0.01 ^a

Data were expressed as mean $\pm$ SEM. Values with different superscripts down the column are significantly different ($p < 0.05$). NRC; normal rat chow; HSD; high salt diet; PHE: poly-herbal extract

Antioxidant status of the aorta

Antioxidant enzyme activities and concentration of oxidative stress markers in the aorta of rats in all experimental groups are depicted in Table 4. Malondialdehyde concentration of 61.54 $\mu\text{mol/g}$ observed in the aorta of HSD only fed rats was significantly higher when compared to all treatment groups. The lowest MDA level of 26.85 $\mu\text{mol/g}$ and 29.67 $\mu\text{mol/g}$ were observed in 800 and 400 mg/kg PHE treated rats only. The highest GSH and SOD activity of 3.25 $\mu\text{mol/g}$ and 25.27 $\mu\text{mol/g}$ was observed in the aorta of 800 mg/kg PHE administered rats respectively, while a significantly ($P < 0.05$) lowered activity was observed in the HSD only fed rats among all groups. The GP_x, CAT and GST activity of the aorta was observed to increase in a dose dependent manner in response to the PHE (200-800 mg/kg) treatment. Although, the significantly ($P < 0.05$) lowest activity of these enzymes was noticed in the HSD only fed rats. There was no significant difference in GP_x, CAT and GST concentration in the aorta of the NRC and PHE (200-800 mg/kg) fed rats.

Table 4: Antioxidant enzymes activities and concentration of oxidative stress markers in the aorta of rats

Treatment	MDA ($\mu\text{mol/g}$)	GSH ($\mu\text{mol/g}$ tissue)	NO ($\mu\text{mol/g}$ tissue)	SOD ($\mu\text{mol/g}$ tissue)	GP _x ($\mu\text{mol/g}$ tissue)	CAT ($\mu\text{mol/g}$ tissue)	GST ($\mu\text{mol/g}$ tissue)
NRC only	38.96 $\pm$ 1.86 ^c	2.77 $\pm$ 0.3 ^{ab}	6.65 $\pm$ 0.60 ^a	19.76 $\pm$ 2.18 ^{ab}	0.63 $\pm$ 0.04 ^b	7.42 $\pm$ 0.61 ^c	5.90 $\pm$ 0.91 ^b
HSD only	61.54 $\pm$ 4.08 ^d	1.93 $\pm$ 0.25 ^a	13.31 $\pm$ 0.59 ^c	17.17 $\pm$ 1.50 ^a	0.51 $\pm$ 0.03 ^a	1.24 $\pm$ 0.14 ^a	3.34 $\pm$ 0.54 ^a
HSD + 800 mg/kg PHE	26.85 $\pm$ 0.86 ^a	3.25 $\pm$ 0.38 ^b	6.70 $\pm$ 0.62 ^a	25.27 $\pm$ 2.29 ^b	0.71 $\pm$ 0.04 ^b	7.87 $\pm$ 0.50 ^c	6.37 $\pm$ 0.81 ^b
HSD + 400 mg/kg PHE	29.62 $\pm$ 1.69 ^{ab}	2.67 $\pm$ 0.08 ^{ab}	9.70 $\pm$ 0.02 ^b	21.46 $\pm$ 3.15 ^{ab}	0.66 $\pm$ 0.05 ^b	7.36 $\pm$ 0.91 ^c	5.61 $\pm$ 0.55 ^b
HSD + 200 mg/kg PHE	42.66 $\pm$ 2.93 ^c	2.03 $\pm$ 0.23 ^a	10.33 $\pm$ 1.06 ^b	18.56 $\pm$ 2.12 ^{ab}	0.68 $\pm$ 0.04 ^b	3.24 $\pm$ 0.9 ^b	4.34 $\pm$ 0.54 ^{ab}

Data were expressed as mean $\pm$ SEM. Values with different superscripts down the column are significantly different ($p < 0.05$). NRC; normal rat chow; HSD; high salt diet; PHE: poly-herbal extract

Serum lipid profile of treated rats

HDL and LDL levels in the serum of rats are depicted in Table 5. HDL concentration was significantly ($P<0.05$) elevated in the 800 and 400 mg/kg PHE treated rats, while LDL level was significantly ($P<0.05$) increased in HSD only treated animals. LDL level was however significantly reduced in a dose-dependent manner after treatment with the PHE. Triacylglycerol and cholesterol concentration in the serum of treated rats is shown in Figure 1. It was noted that triacylglycerol and cholesterol concentration in the serum of the HSD only fed rats were significantly ($P<0.05$) elevated when compared with other groups of experimental animals. There was a dose dependent decrease in serum TAG concentration after treatment with PHE. An appreciable and more pronounced reduction in cholesterol concentration was observed in 800 and 400 mg/kg PHE treated animals.

Table 5: Concentration of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) in the serum of rats

Treatment	HDL (mg/dl)	LDL (mg/dl)
NRC only	6.63±0.43 ^{ab}	35.06±1.97 ^c
HSD only	4.91±0.85 ^a	163.29±1.59 ^f
HSD +800 mg/kg PHE	22.83±0.72 ^d	9.67±1.67 ^a
HSD +400 mg/kg PHE	21.99±0.64 ^d	27.25±2.69 ^b
HSD +200 mg/kg PHE	12.09±1.20 ^c	89.30±3.76 ^e

NRC; normal rat chow; HSD; high salt diet; PHE: poly-herbal extract

Gas chromatography mass spectrophotometry (GC-MS) analysis

GC-MS spectrum of PHE with peaks and retention time is shown in Figure 2. The analysis of the poly-herbal extract revealed the presence of about 61 compounds (Table 6) with compounds such as Benzene-2-tert-butyl dimethylsilyloxy]-1-isopropyl-4-methyl- (8.83%), Deoxyqinghaosu (8.46%), Benzene, 1,1'-(1,2-cyclobutanediyl)-bis,trans- (5.15%), N-2-Acetylcyclopentylidene-cyclohexylamine (4.18%), 9,10-Anthraquinone-monohydrazone (3.83%), scopoletin (3.66%), 2,3-Diphenylcyclopropyl-methylphenylsulfoxide (3.59%), Bicyclo[3.3.1]nonan-2-one,1-methyl-9-(1-methylethylidene (2.45%) detected to be notably present.

Table 6: Compounds detected in the poly-herbal extract using GC–MS Analysis

Retention time		Identified Compounds	Peak area %
1	2.651	Arsenous acid, tris(trimethylsilyl)ester	0.09
2	2.905	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-1-Methyl-3-phenylindole	0.07
3	3.271	Cyclotrisiloxane, hexamethyl-	0.21
4	3.834	1,4-Bis(trimethylsilyl)benzene	1.18
5	4.341	Tris(tert-butyl dimethylsilyloxy)arsane	0.05
6	4.398	1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.06
7	4.764	4-Methyl-2-trimethylsilyloxy-acetophenone	0.13
8	4.905	1,3,5,7-Cyclooctatetraene	0.39
9	5.327	Cyclotetrasiloxane, octamethyl-	0.14
10	5.440	Trans-4-Dimethylamino-4'-methoxychalcone	0.39
11	5.834	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy) tetrasiloxan-1-ol	0.26
17	6.961	1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.05
18	7.158	Arsenous acid, tris(trimethylsilyl) ester	0.04
19	7.468	1,2-Bis(trimethylsilyl)benzene	0.11
20	7.722	1,1,1,3,5,5,5-Heptamethyltrisiloxane 1H-Indole, 1-methyl-2-phenyl	0.04
22	8.510	Cyclopentasiloxane, decamethyl-	1.22
23	8.736	5-Methyl-2-phenylindolizine	0.12
24	9.017	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane.	0.05
25	9.186	1,2,4-Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	0.04
26	9.327	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-	0.10
27	9.863	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	0.23
28	10.144	4-Bromo-3-chloroaniline	0.32
29	10.285	Cyclohexasiloxane, dodecamethyl-	1.47
30	10.651	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	0.54
31	10.961	Alpha.-D-Ribofuranoside ((2-pyridyl)-2,3-O-isopropylidene-1-thio-	1.28

32	11.384	Coumarin	1.01
33	11.609	Cycloheptasiloxane, tetradecamethyl-	1.85
34	11.947	Anthracene, 9,10-diethyl-9,10-dihydro-	0.38
35	12.313	3-Quinolinecarboxylic acid, 6,8-di fluoro-4-hydroxy-, ethyl ester	1.15
36	12.595	2-Ethylacridine	0.74
37	12.680	Cyclooctasiloxane, hexadecamethyl-	1.02
38	12.792	5,5'-Di(ethoxycarbonyl)-3,3'-dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethane	0.97
39	13.018	Trans-3-Ethoxy-b-methyl-b-nitrostyrene	1.58
40	13.187	Corydaldine	1.11
41	13.356	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-,trans-	5.15
42	13.778	Isopulegol	1.78
43	13.947	Bicyclo[4.1.0]hepta-2,4-diene, 2,3,4,5-tetraethyl-7,7-diphenyl-	2.93
44	14.144	2-Methyl-7-phenylindole	2.10
45	14.398	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	4.10
46	14.539	Scopoletin	3.66
47	14.680	Bicyclo[3.3.1]nonan-2-one,1-methyl-9-(1-methylethylidene)-	2.45
48	14.933	2,4,6-Trimethylphenyl isothiocyanate	1.79
49	15.074	Deoxyqinghaosu	8.46
50	15.271	N-(2-Acetylcyclopentylidene)cyclohexylamine	4.18
51	15.412	Fluorenoneoxime	2.58
52	15.750	6-Methoxy-2-hydroxyquinoxaline-4-oxide	2.80
53	15.947	Benzo[h]quinoline, 2,4-dimethyl-	1.51
54	16.088	Tris(tert-butyl dimethylsilyloxy)arsane Propiophenone, 2'-(trimethylsiloxy)-	1.87
55	16.257	9,10-Anthraquinone monohydrazone	3.83
56	16.426	1,2-Benzisothiazol-3-amine tms	2.02
57	17.215	Benzene, 2-[(tert-butyl dimethylsilyl)oxy]-1-isopropyl-4-methyl-	8.83
58	17.863	1,2-Bis(trimethylsilyl)benzene	2.10
59	18.229	Tetrasiloxane, decamethyl- 1,4-Bis(trimethylsilyl)benzene	1.49
60	18.595	2,3-Diphenylcyclopropyl)methylphenylsulfoxide, trans-	3.59
61	18.764	Trimethyl[4-(2-methyl-4-oxo-2-pent yl)phenoxy]silane	0.62

Discussion

Excessive consumption of dietary salt has been associated with increase production of free radicals which can overwhelm cellular anti-oxidant and defense mechanism [32, 33]. The deleterious effect and consequences of free radicals on vital organs have been clinically and experimentally established [34]. Reactive oxygen species when not appropriately regulated and/or quenched oxidizes important biological molecules in tissues [33, 35]. The kidney, liver, and heart are important organs that are central to the metabolic processes of the biological system. Hence, an oxidative insult to these organs will have a negative effect on overall cellular homeostasis. A considerable elevated MDA and NO level noted in the liver, kidney, and aorta homogenates of animals fed with 16% high salt diet without treatment indicate that there was a significant increase in lipid peroxidation and oxidation in these tissues relative to the NRC and 400 and 800 mg/kg PHE groups. Hence, suggesting a protective effect of the poly-herbal extract used in this study. Malondialdehyde is a product of membrane lipid peroxidation resulting from the harmful effect of superoxide anion ($\text{O}_2^\bullet$), hydroperoxyl radicals ($\text{HO}_2^\bullet$), lipid radicals ($\text{L}^\bullet$), peroxy-radical ($\text{LOO}^\bullet$), and peroxynitrite (ONOO^-) [36, 37]. The consequence of this is a considerable distortion in the conformation, physiological architecture, and integrity of the membrane as any major alteration and oxidation of the membrane lipids might have significant and negative aftermath on the signaling capacity and process of the cell [36, 38-39]. Nitric oxide is a free radical which is generated as an immunological response in many cell types [40, 41]. Although, there are contrasting reports on its clinical significance, however, increased concentration of different nitric oxide isoforms have been reported to manifest in cardiac and vascular diseases [42, 43]

High salt diet significantly reduced the SOD, CAT, and selenocysteine peroxidase in the liver, kidney, and aorta HSD only fed red rats. No major antioxidant improvement in these organs was noticed at the lowest dose of the extract but was more buoyed and significantly ($P < 0.05$) pronounced at higher dosages (400 and 800 mg/kg) of the PHE treatment.

Activities of enzymic antioxidants are useful indices and markers in the prognosis, progression, and prediction of some disease conditions [44]. Superoxide dismutase, catalase, and glutathione peroxidase are first-line defense antioxidants shielding the body against dangerous radicals vis-à-vis superoxide anion, peroxisomal, and mitochondrial hydrogen peroxide respectively [44].

Glutathione S-transferases catalyze the nucleophilic attack of glutathione (GSH) on electrophilic substrates, thereby decreasing their reactivity with cellular macromolecules [44]. Glutathione has many functions in the mammalian cell among which is the elimination and protection against reactive nitrogen and oxygen species [45]. In this present study, high salt diet reduced the concentration of GSH in the salt-treated animals, although not statistically different from the normal chow fed rats. GSH was only significantly elevated in 800 mg/kg extract-treated animals. The antioxidants depletion effect of high salt diet recorded in this study is in unison with previous scientific submission of [46]. As established by Bayorh et al. [47] Saidu et al. [48], the activity of antioxidant enzymes decreased, while ROS and MDA concentration increased in rats fed with 8% salt diet for 3 weeks. Batteries of experimental reports have documented that high sodium chloride can elicit derangement in lipid metabolism [49-51]. In this study, cholesterol, triglyceride, and low-density lipoprotein which are predictors of cardiovascular disorders [52] were elevated, while HDL level was reduced in the serum of salt-loaded rats. Nonetheless, the concentration of these markers was reversed to near normal after treatment with PHE. Oxidative stress has been reported to play a role in the derangement of lipid homeostasis through oxidation of accumulated low-density lipoprotein cholesterol in the plasma. This has been implicated in the development of atherosclerosis and heart attack [53]. The crude extracts and fractions of plants employed in this study have been discerned to contain different phenolics and important secondary metabolites such as caffeic acid, rutin, kaempferol, chlorogenic acid, procyanidins, catechin, and epicatechin with documented pharmacological and biological activities [54, 55]. Some empirical pieces of evidence have reported various extracts of *Annona muricata*, *C.papaya*, *Aloe barbdensis* and *M. oleifera* to demonstrate substantial anti-hyperlipidemic activities in experimentally induced pathological states [56-58]. Polyphenols such as flavonoids in plants and vegetables have been reported to modulate redox signaling pathways and induce transcription factors such as nuclear erythroid factor (Nrf2) which subsequently binds to antioxidant regulatory elements (ARE) in the DNA promoter region, thus

initiating the expression of cytoprotective and antioxidant genes with subsequently enhanced synthesis of enzymatic antioxidants [59-62]. Furthermore, active principles elicit anti-oxidant potential by donating and transferring hydrogen atom and single electron to free radicals thus disrupting their deleterious impact in the body [63]. It is worthy to note that compounds belonging to important classes of secondary metabolites vis-à-vis alkaloids, flavonoids, and terpenoids were recognized and detected in the chromatographic analysis. Active principles such as coumarin, scopoletin, and isooopulegol were detected in this study and have been documented to evoke antioxidant, inflammatory, anti-hyperlipidemic, and anti-bacteria activities [64-68]. Furthermore, isolated anthraquinones from different medicinal plants have exhibited in-vitro radical scavenging potentials [69, 70], while deoxyqinghaosu and corydaldine identified in this study have also been acclaimed to display arrays of biological activities [71].

Conclusion

High salt diet exposure elicited derangement in the antioxidant status in the assessed organs of the experimental rats. However, treatment with the different concentrations of the poly-herbal extracts caused a considerable increase in the concentration.

Declarations

Funding

Not Applicable

Conflicts of interest/Competing interests

The authors declares that no conflict of interest exists

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not Applicable

Authors' contributions

The research work was carried out in collaboration between all authors. OSO and OSF conceived and designed the study. Authors PIA, ODA and BSA managed experimental protocols and performed the experiments under the supervision of OSO. Author OSF managed the literature searches and wrote the first draft of the manuscript. Author PIA performed the statistical analysis. All authors read and approved the final draft of the manuscript.

Ethics approval

All animal procedures in this study were performed in accordance with the guidelines of the research and ethics committee, Ladoke Akintola University of Technology (LAUTECH) for the use of laboratory animals

Consent to participate

Not Applicable

Consent for publication

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Figures

Figure 1

Serum triacylglycerol and cholesterol concentration in the serum of rats. Data were expressed as mean $\pm$ SEM. Bar chart with different alphabets are significantly different ($p < 0.05$).

Figure 2

GC-MS chromatogram of the investigated poly-herbal extract