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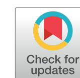
Research article

Phyllanthus amarus attenuated derangement in renal-cardiac function, redox status, lipid profile and reduced TNF-α, interleukins-2, 6 and 8 in high salt diet fed rats



Olubukola Sinbad Olorunnisola [a], Olumide Samuel Fadahunsi [a,*], Peter Ifeoluwa Adegbola [a], Bamidele Stephen Ajilore [b], Folorunsho Ayodeji Ajayi [c], Lamidi Waheed Babatunde Olaniyan [a]

[a] Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[b] Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[c] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University, Osogbo, Nigeria

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ABSTRACT

High salt diet (HSD) has been implicated in the etiopathogenesis of immune derangement, cardiovascular disorders and, metabolic syndromes. This study investigated the protective effect of ethanol extract of *Phyllanthus amarus* (EEPA) against high salt diet (HSD) induced biochemical and metabolic derangement in male Wistar rats. The rats were divided into 5 groups of 6 animals each as follows; control group fed with normal rat chow, negative control group, fed HSD only, animals on HSD treated orally with 75 mg/kg, 100 mg/kg, and, 150 mg/kg EEPA once daily. At the end of 8 weeks treatment, lipid profile (TG, TC, LDL, and VLDL), oxidative stress (catalase, reduced glutathione, and malondialdehyde), inflammatory (TNF-a, interleukins 2, 6, and 8), cardiac (lactate dehydrogenase, creatine kinase) and kidney function markers (urea, uric acid, creatinine) were assessed. Serum TG, TC, LDL, and VLDL content were significantly ($p < 0.05$) elevated in HSD-only fed rats, while HDL was significantly elevated in a concentration-dependent manner in EEPA treated animals. The extract produced a significant ($p < 0.05$) and dose-dependent increase in the antioxidant enzymes activities and a significant reduction in the malondialdehyde level. A significant ($p < 0.05$) dose-dependent reduction in serum TNF-alpha, IL-2, 6, and 8 of EEPA treated rats compared with HSD-fed rats was observed. More so, reduction in serum LDH, creatine kinase, creatinine, urea, and uric acid activity of extract-treated animals were noted. EEPA attenuated high salt diet-induced oxidative stress, inflammation, and dyslipidemia in rats.

1. Introduction

Since prehistoric times, salt has been an important part of human existence and nutrition. Due to its cheapness, sodium chloride is the most employed food preservative, seasoning, flavoring and, softening agent globally [1, 2]. The addition of salt to foods such as carbohydrates makes them palatable and brings out their natural flavor. There is overwhelming scientific literature on the critical and essential role of sodium in maintaining arrays of metabolic and physiological functions such as electrolytes balance, excitation of nerve, and muscle function [3, 4]. Although the amount of sodium needed to maintain homeostasis in adults is very low, dismayingly, there is an average global daily consumption of dietary salt, which is higher than the 2,000 mg per day recommended by the world health organization [5, 6, 7]. To protect public health, food regulatory agencies of most developed countries have introduced stringent laws to reduce sodium content in processed foods [8]. Consumption of high concentrations of salt can lead to the onset of severe pathological disorders and the development of metabolic syndromes [9, 10, 11]. Reports from research and epidemiological studies revealed that HSD is one of the chief risk factors in the prevalence and upward progression of hypertension which has afflicted about 30% of the world population [12, 13, 14]. More so, HSD has been implicated in increased cardiovascular morbidity, endothelial dysfunction, albuminuria, myocardial infarction, Alzheimer's disease, increased insulin resistance, impaired wound healing, cataract and, cancer [15, 16, 17]. The pathophysiology and physiological mechanisms involved in salt-induced metabolic disorders are not well deciphered, but alterations in renal function, fluid and sodium retention hormones, vascular relaxation,

* Corresponding author.
E-mail addresses: osfadahunsi83@lautech.edu.ng, osolorunnisola@lautech.edu.ng (O.S. Fadahunsi).

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decreased nitric oxide (NO) production and increased level of oxidative stress have been postulated to be associated [18,19]. *Phyllanthus amarus* is a popular medicinal plant belonging to the family Euphorbiaceae. It has gained relevance due to its varieties of usage in folkloric medicine. Furthermore, it has been documented to elicit a plethora of biological and pharmacological activities [20, 21, 22]. However, the potential of this plant to protect against HSD-induced metabolic and biochemical derangement is relatively understudied. In this context, the present study sought to investigate the effect of EEPA on some biochemical parameters in high salt diet-fed male Wistar rats.

2. Materials and methods

2.1. Plant identification, authentication, and preparation

Phyllanthus amarus was collected in Ogbomosho town (8°8′N°15′E) with the aid of a traditional herbalist. The plant was authenticated by a taxonomist at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The voucher number (LHB/4618) was deposited at the University Herbarium. The collected plant was rinsed with clean water to remove sandy particles and thereafter air-dried at room temperature for 2 weeks. The dried plant was pulverized into powder with a kitchen blender (EMEL: EM-242, Shanghai, China).

2.1.1. Preparation of ethanol extracts of Phyllanthus amarus (EEPA)

Five hundred (500 g) of the pulverized dried plant material was soaked in 1.5 L of analytical grade ethanol at room temperature for 72 h, after which the mixture was filtered with a muslin cloth and subsequently with filter paper. The filtrate was concentrated under pressure at 50 °C with a rotatory evaporator (Hexa Pharma Chem, India) and was freeze dried using a lyophilizer (De Novo Tech, India). The dried crude extract obtained was stored in airtight dark bottle and kept in the refrigerator (Haier Thermocool, Shandong, China) until further use.

2.2. Animal grouping and experimental design

All animal procedures in this study were performed following the guidelines of the research and ethics committee, Ladoke Akintola University of Technology (LAUTECH) for the use of laboratory animals. Ethical approval number LFBMSEC/016/2020 was collected from LAUTECH, Faculty of Basic Medical Science Ethical Committee on the 3rd of October, 2020. Thirty healthy Wistar rats with an average weight of 118 g were obtained from the animal house of the Department of Biochemistry, College of Basic of Medical Science, LAUTECH. The animals were housed in ventilated cages on a 12:12 h light-dark cycle and acclimatized for 2 weeks after which they were randomly separated into different groups of 6 animals each as depicted in Table 1.

2.3. Diet formulation and composition

The experimental diets (Table 2) were formulated as described by Mayyas *et al.* [71] at KESSMAT feeds, agro, and veterinary services (Ibadan, Nigeria).

2.4. Blood serum and tissue homogenate preparation

Animals were fasted overnight and sacrificed through cervical dislocation on the 56th day. Blood was collected through heart puncture and transferred into plain sample bottles. To obtain the serum, the blood samples were centrifuged at 4000 rpm for 10 min. The kidney was excised, washed in cold washing buffer thereafter homogenized in phosphate buffer saline (PBS) using Teflon homogenizer. The serum and the kidney homogenate were kept at -5 °C (Haier Thermocool, Shandong, China) for further biochemical analysis.

Table 1. Animals grouping and experimental design.

Groups	Treatment
1	Fed with normal rat chow (NRC)
2	Fed with high salt diet (4% NaCl) only
3	Fed with high salt diet (4% NaCl) + 75 mg/kg EEPA
4	Fed with high salt diet (4% NaCl) + 100 mg/kg EEPA
5	Fed with high salt diet (4% NaCl) + 150 mg/kg EEPA

Table 2. Composition of experimental diet.

Constituents	Composition	
	Normal diet	High salt diet (HSD)
Fat	5%	5%
Carbohydrate	62%	62%
Protein	20%	20%
Fibers	12.5%	12.5%
NaCl	0.5%	4%

2.5. Biochemical assay

2.5.1. Evaluation of oxidative stress markers

Kidney homogenates were used to evaluate the activity of antioxidant enzymes and the concentration of malondialdehyde.

2.5.1.1. Reduced glutathione (GSH). Reduced glutathione (GSH) activity was estimated in the kidney homogenate according to the procedure of Sedlak and Lindsay [23] and as earlier reported by Olorunnisola *et al.* [72].

2.5.1.2. Catalase (CAT). Catalase (CAT) activity was estimated in the kidney homogenate according to the method of Clairborrne [24] as previously reported by Olorunnisola *et al.* [72].

2.5.1.3. Malondialdehyde (MDA). MDA concentration, an index of lipid peroxidation was assayed according to the method of Ohkawa *et al.* [25] as previously reported by Olorunnisola *et al.* [72]. The concentration of MDA in nmol/g tissue was extrapolated from the standard curve.

2.5.1.4. Evaluation of serum renal function markers. Detection assay kits from Thermo Fisher Scientific, USA was used to evaluate the serum concentration of urea and creatinine. Uric acid was determined according to the protocol of the assay kit manufacturer (Abcam PLC, United Kingdom).

2.5.1.5. Evaluation serum cardiac function markers. Cardiac function markers vis-à-vis creatine kinase and lactate dehydrogenase were estimated spectrophotometrically in the serum using standard manufacturer procedure as provided in the assay kits (Sigma- Aldrich, Germany).

2.5.1.6. Evaluation of lipid profile. Serum lipid profile vis-à-vis high-density lipoprotein-cholesterol (HDL-C) and triglyceride (TG) was evaluated according to assay kit manufacturers (Elabscience, USA) and (Randox, England) respectively. Total cholesterol (TC) level was determined according to the method of Parakh and Jank [26]. Low density lipoprotein (LDL-C) and very low density lipoprotein – cholesterol (VLDL-C) concentrations were calculated according to Friedwald *et al.* [27].

2.6. Estimation of serum cytokines expression

Quantitative enzyme-linked immune-sorbent assay (ELISA) of tumor necrosis factor-alpha (TNF-a), interleukins-2, 6 and, 8 were carried out in

the serum on a 96-wells microplate using the rat ELISA kit according to the manufacturer's instructions (RayBiotech, USA).

2.7. Gas chromatography-mass spectrometric analysis of ethanol extract of P. amarus

The GC-MS analysis of ethanol extract of *P. amarus* was carried out as hitherto described by Adegbola *et al.* [73].

2.8. Statistical analysis

Data obtained in this study were expressed as mean ± standard error of the mean (SEM) and were subjected to normality test using Kolmogorov-Smirnov and Shapiro-Wilk's test. The data set followed a normal distribution and was subjected to Duncan's multiple post-hoc test. Significant difference was considered at P value <0.05 on the statistical package for social sciences (SPSS) 21.0.

3. Results

3.1. Effect of treatment on the weight of the experimental rats

The percentage (%) weight increase of rats in the experimental groups after 8 weeks is depicted in Figure 1. The normal (control) rats, demonstrated the highest percentage weight gain which was followed by 150, 100 and, 75 mg/kg extract-treated animals in a dose-dependent manner respectively. The lowest weight increase of 77.96% was noticed in the HSD-only fed rats.

3.2. Effect of EEPA on the serum lipid profile of HSD fed Wistar rats

Table 3 revealed the effects of *Phyllanthus amarus* extract on the serum lipid profile of rats fed with high HSD. The result showed a dose-dependent decrease in the serum LDL, TG, TC, and VLDL levels of rats treated with the plant extract. A significant and dose-dependent decrease ($p < 0.05$) was observed in the serum LDL, TG, TC and, VLDL levels of rats treated with the extract when compared with the group fed with the HSD without treatment. When the extract-treated groups were compared with the normal control, a significant increase ($p < 0.05$) was observed in the serum LDL, TG and, TC whereas, no significant difference ($p > 0.05$) was observed in the serum VLDL level of rats treated with 150 mg/kg of the extracts. Also, the serum HDL concentration of the normal control group showed no significant difference ($p > 0.05$) when compared with the group treated with 150 mg/kg of the plant extract. No significant difference ($p > 0.05$) were alsoobserved in the serum HDL levels of rats treated with 100 mg/kg and 150 mg/kg. More so, an in-significant difference ($p > 0.05$) in the serum HDL levels of 75 mg/kg extract treated and HSD diet only fed rats was observed.

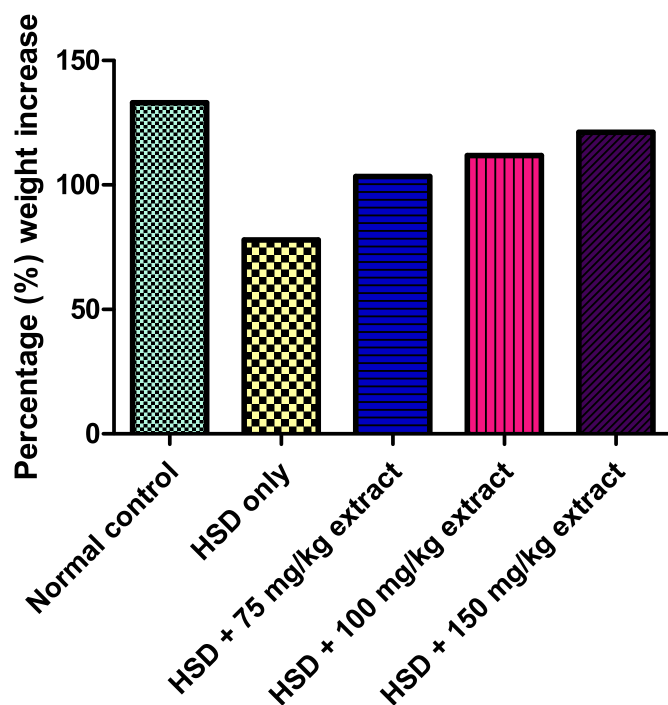

Figure 1. Percentage (%) increase in weight of experimental rats after 8 weeks.

Table 3. Serum lipid profile of rats fed with high salt diet and treated with extract of *Phyllanthus amarus*.

Groups	LDL (mmol/L)	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	VLDL (mmol/L)
Control (normal)	9.53 ± 0.11^a	71.07 ± 0.56^a	92.88 ± 1.05^a	80.97 ± 0.62^c	14.57 ± 0.61^a
HSD only	59.91 ± 1.29^e	183.65 ± 2.30^e	159.37 ± 0.49^e	48.49 ± 3.75^a	44.82 ± 1.38^d
HSD +75 mg/kg extract	38.84 ± 0.92^d	161.93 ± 1.60^d	130.10 ± 0.87^d	54.30 ± 2.25^a	31.78 ± 2.00^c
HSD +100 mg/kg extract	21.70 ± 0.53^c	143.27 ± 1.75^c	118.00 ± 1.42^c	73.50 ± 0.61^b	21.58 ± 0.66^b
HSD +150 mg/kg extract	13.50 ± 0.35^b	101.43 ± 2.04^b	100.70 ± 1.91^b	76.68 ± 0.91^{bc}	17.00 ± 0.59^a

Data were expressed as mean ± standard error of mean (SEM). Subscripts a, b, c, d and e indicates significant difference among all the groups based on Duncan's post-hoc test. Values with different superscript along the same column are significantly different ($p < 0.05$). *HSD: high salt diet; LDL: low density lipoprotein; TG: triacylglycerol; TC: total cholesterol: VLDL: very low density lipoprotein.

3.3. Effect of EEPA on the inflammatory markers of HSD fed Wistar rats

The effect of *Phyllanthus amarus* extract on the inflammatory markers of rats fed with a high salt diet is presented in Table 4. The result showed a dose dependent decrease in the levels of inflammatory markers of experimental rats. The serum level of Interleukin-6 and Interleukin-8 significantly decreased ($p < 0.05$) in a dose dependent manner when the extract-treated groups were compared with the group fed with a high salt diet alone, whereas, a significant increase ($p < 0.05$) was observed on comparison with the normal control group. Significant differences ($p < 0.05$) were also observed in the level of tumour necrotic factor (TNF-α) and Interleukin-2 of extract treated groups when compared with the

Table 4. Effect of *Phyllanthus amarus* extract on the inflammatory markers of rats fed with high salt diet.

Group	IL-8 (ng/mL)	TNF-α (ng/mL)	IL-2 (ng/mL)	IL-6 (ng/mL)
Control (normal)	7.93 ± 0.38^a	5.63 ± 0.33^a	1.46 ± 0.05^a	2.41 ± 0.07^a
HSD only	31.46 ± 0.50^e	8.44 ± 0.08^d	3.10 ± 0.08^d	5.32 ± 0.06^e
HSD +75 mg/kg extract	19.87 ± 0.22^d	7.77 ± 0.09^c	2.68 ± 0.04^c	4.39 ± 0.07^d
HSD+100 mg/kg extract	13.29 ± 0.70^c	6.38 ± 0.01^b	2.03 ± 0.01^b	3.63 ± 0.03^c
HSD+ 150 mg/kg extract	9.67 ± 0.38^b	6.02 ± 0.02^{ab}	1.93 ± 0.02^b	3.05 ± 0.01^b

Data expressed as mean ± standard error of mean (SEM). Subscripts a, b, c, d and e indicates significant difference among all the groups based on Duncan's post-hoc test. Values with different superscript along the same column are significantly different ($p < 0.05$). *HSD: high salt diet: IL: interleukin.

group fed with a high salt diet. No significant difference ($p > 0.05$) was observed between the normal control group and the group treated with 150 mg/kg of extracts. Between the groups treated with 100 and 150 mg/kg of extracts, no significant ($p > 0.05$) difference was observed in the serum level of TNF-α and IL-2.

3.4. Effect of EEPA on the oxidative stress indices of HSD fed Wistar rats

The results in Table 5 represent the oxidative stress parameters of rats fed with a high salt diet and treated with extract of *Phyllanthus amarus.*

Table 5. Oxidative stress parameters of rats fed with high salt diet and treated with extract of *Phyllanthus amarus*.

Groups	MDA (nmol)	CAT (nmol)	GSH (nmol)
Control (normal)	5.42 ± 0.05^{a}	56.50 ± 0.56^{e}	61.16 ± 0.11^{d}
HSD only	10.81 ± 0.05^{d}	22.13 ± 0.33^{a}	41.25 ± 0.41^{a}
HSD +75 mg/kg extract	8.21 ± 0.15^{c}	28.21 ± 0.05^{b}	45.35 ± 0.14^{ab}
HSD +100 mg/kg extract	6.54 ± 0.03^{b}	32.30 ± 1.12^{c}	49.42 ± 0.03^{b}
HSD+ 150 mg/kg extract	5.03 ± 0.07^{a}	47.31 ± 5.22^{d}	55.21 ± 0.01^{c}

Data expressed as mean ± standard error of mean (SEM). Subscripts a, b, c, d and e indicates significant difference among all the groups based on Duncan's post-hoc test. Values with different superscript along the same column are significantly different ($p < 0.05$). *HSD: high salt diet; MDA: Malondialdehyde; CAT: Catalase; GSH; Glutathione.

Dose-dependent changes were observed in the MDA, GSH concentration and catalase activity of the extract treated groups. The MDA concentration decreased significantly ($p < 0.05$) upon treatment of high salt diet fed rats with 75, 100 and 150 mg/kg of the plant extract when compared with the group fed with high salt diet alone. When the treated groups were compared with the control, only the group treated with 150 mg/kg of extract showed insignificant difference ($p > 0.05$). The catalase activity also increased significantly ($p < 0.05$) in a dose dependent manner when compared with the high salt diet fed group, whereas significant decrease ($p < 0.05$) was observed upon comparison with the normal control. The reduced glutathione (GSH) concentration was significantly increased ($p < 0.05$) in the group treated with 100 and 150 mg/kg of extract when compared with the group fed with high salt diet alone. No significant ($p > 0.05$) difference was observed in the group treated with 75 mg/kg of extract when compared with the high salt diet fed group. The GSH concentration in all the groups was significantly decreased ($p < 0.05$) when compared with the normal control group.

3.5. Effect of EEPA on the serum lactate dehydrogenase (LDH) and creatinine kinase (CK) of HSD fed Wistar rats

The cardio-protective effect of *Phyllanthus amarus* extract estimated by measuring the serum activity of LDH and CK is reported in Figures 2 and 3. The result revealed a dose-dependent decrease in the LDH activity of rats when treated with the plant extracts. The LDH activity

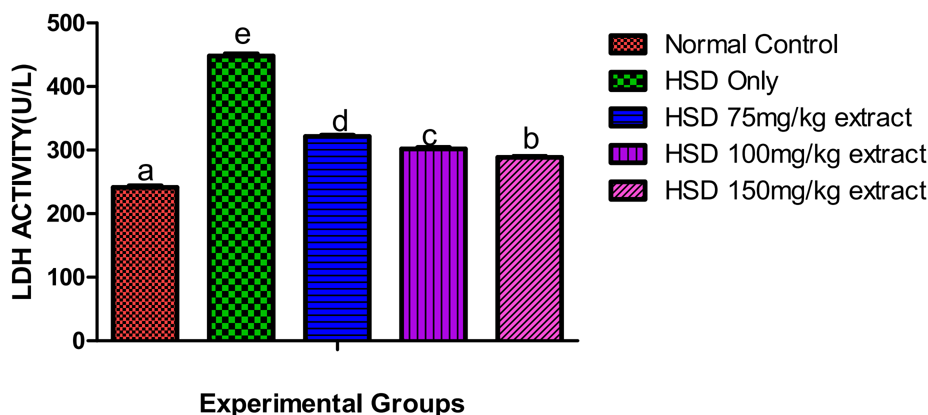

Figure 2. LDH activity of rats fed with high salt diet and treated with *Phyllanthus amarus* extracts. Data expressed as mean ± standard error of mean (SEM). Subscripts a, b, c, d, and e indicates significant difference among all the groups based on Duncan's post-hoc test. Bar chat with different superscripts is significantly different ($p < 0.05$). *HSD: High salt diet; LDH: Lactate dehydrogenase.

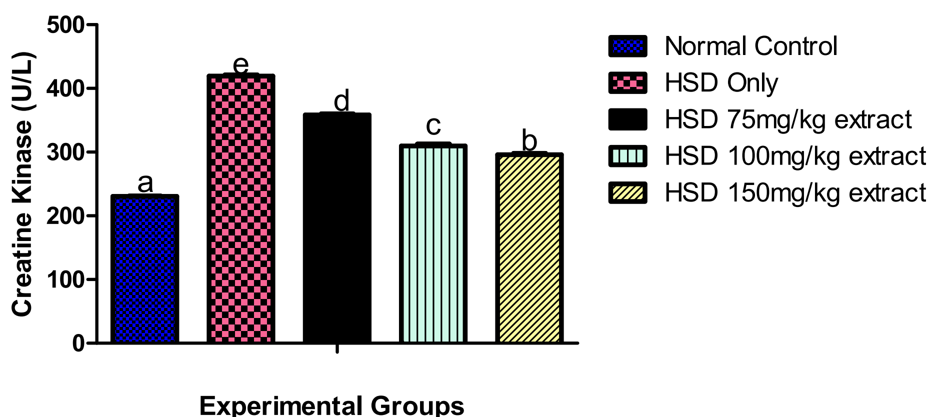

Figure 3. Creatine Kinase activity of rats fed with high salt diet and treated with *Phyllanthus amarus* extracts. Data expressed as mean ± standard error of mean (SEM). Subscripts a, b, c, d, and e indicate significant difference among all the groups based on Duncan's post-hoc test. Bar chat with different superscripts are significantly different ($p < 0.05$). *HSD: High salt diet.

significantly ($p < 0.05$) decreased in all the extract-treated groups when compared with the group fed with high salt diet alone. Similarly, the serum creatine kinase activity was significantly decreased ($p < 0.05$) upon treatment with *Phyllanthus amarus extract* when compared with the group fed with high salt diet alone and increased significantly ($p < 0.05$) when compared with the normal control group.

3.6. Effect of EEPA on kidney function markers of HSD fed Wistar rats

The renal function parameters assessed in this study included the serum urea, creatinine, and uric acid concentration. The result showed a dose-dependent change in the urea and creatinine activity of the extract-treated groups (Figures 4, 5, and 6). A significant decrease ($p < 0.05$) was

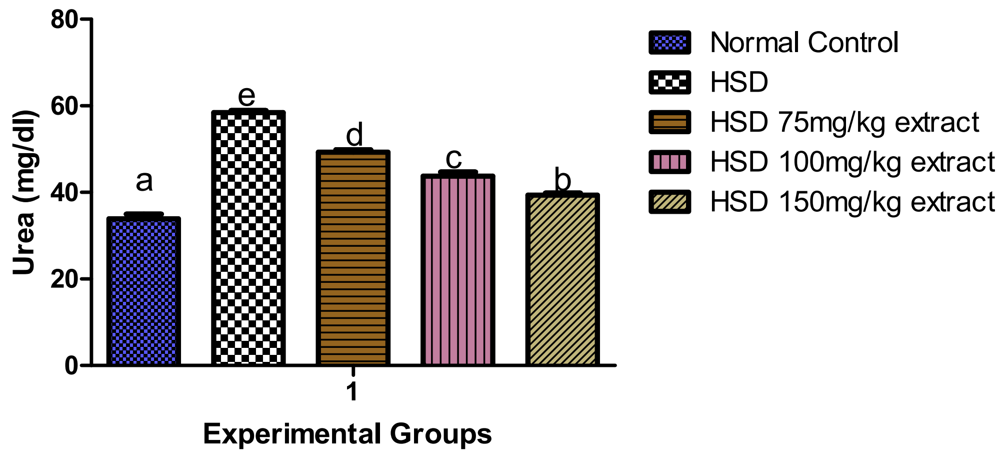

Figure 4. Serum urea concentration in rats fed with a high salt diet and treated with *Phyllanthus amarus* extracts. Data expressed as mean ± standard error of mean (SEM). Subscripts a, b, c, d, and e indicate significant differences among all the groups based on Duncan's post-hoc test. Bar chat with different superscripts are significantly different ($p < 0.05$). *HSD: High salt diet.

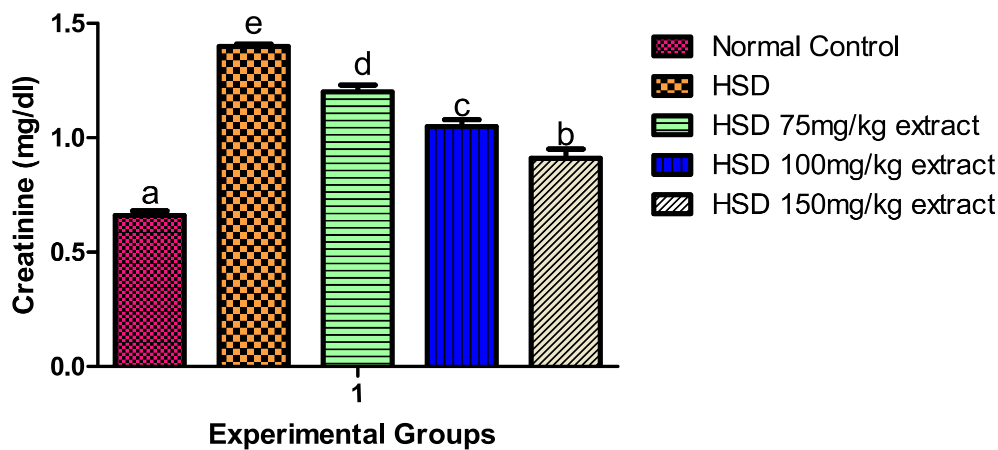

Figure 5. Serum creatinine concentration in rats fed with high salt diet and treated with EEPA. Data expressed as mean ± standard error of mean (SEM). Subscripts a, b, c, d, and e indicate significant differences among all the groups based on Duncan's post-hoc test. Bar chat with different superscripts are significantly different ($p < 0.05$). *HSD: High salt diet.

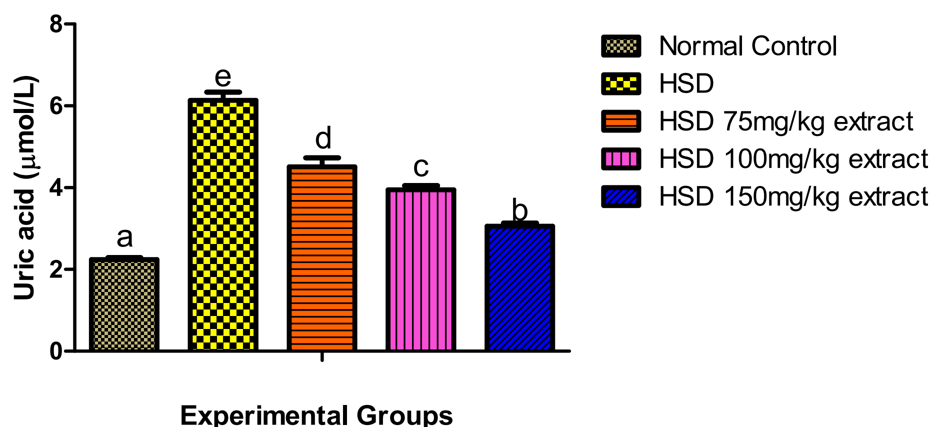

Figure 6. Serum Uric acid concentration in rats fed with high salt diet and treated with EEPA. Data expressed as mean ± standard error of the mean (SEM). Subscripts a, b, c, d and, e indicate significant differences among all the groups based on Duncan's post-hoc test. Bar chat with different superscripts are significantly different ($p < 0.05$). *HSD: High salt diet.

Table 6. GC-MS analysis and identified compounds from EEPA.

S/N	Retention Time	Identified Compounds	Peak Area (%)
1.	8.894	1H-Indole-3-acetic acid, 2-carboxy, methyl ester	2.48
2	10.264	3-Methyl-1-butanol, picolinyloxydimethyl silyl ether	3.55
3	11.939	2-Isopropylidenehydrazono-3-methyl 4-chloro-2,3-dihydrobenzothiazole	3.98
4	12.396	Pentanoic acid (octahydroquinolizin-1-yl)methyl ester,	3.38
5	18.436	4-(1H-[1,2,4]Triazole-3-carbonyl)-piperazine-1-carboxylic acid ethyl ester	23.53
6	19.553	1-(4′-Methoxymethylbiphenyl-4-yl)ethanol	17.14
7	22.751	1H-Carbazole-9-ethanol, 2,3,4,9-tetrahydro-.alpha.-[(2-pyridinylthio)methyl]-	3.47
8	29.908	Pyrido [2,3-b]indole,6-methyl-,1,2,4-Triazolo [4,3-b]pyridazine,	42.46

observed in both the urea, creatinine, and uric acid activity of the extract-treated groups when compared with the group fed with high salt diet, whereas the activity increased significantly ($p < 0.05$) when the extract-treated groups were compared with the normal control group.

3.7. GC-MS analysis of P. amarus

Table 6 depicts the compounds identified in the extract of *P. amarus* using gas chromatography-mass spectroscopy (GC-MS). The chromatogram showed eight prominent peaks (Figure 7). The identified compounds with their retention time and peak area are also showed in Table 6. The major compounds were Pyrido [2,3-b]indole, 6-methyl-, 1,2,4-Triazolo [4,3-b]pyridazine (42.46 %) corresponding to the most abundant, 4-(1H- [1,2,4]Triazole-3-carbonyl)-piperazine-1-carboxylic acid ethyl ester (23.53 %), and 1-(4′-Methoxymethylbiphenyl-4-yl) ethanol, 2-Propenoic acid, 2-cyano-3-[4-(1, 1-dimethylethyl)phenyl]-(17.14 %). Other less prominent compounds included 2-Isopropylidene-hydrazono-3-methyl 4-chloro-2,3-dihydrobenzothiazole (3.98 %), 3-Methyl-1-butanol, picolinyloxydimethyl silyl ether (3.55 %), 1H-Carbazole-9-ethanol (3.47 %), Pentanoic acid (octahydroquinolizin-1-yl)

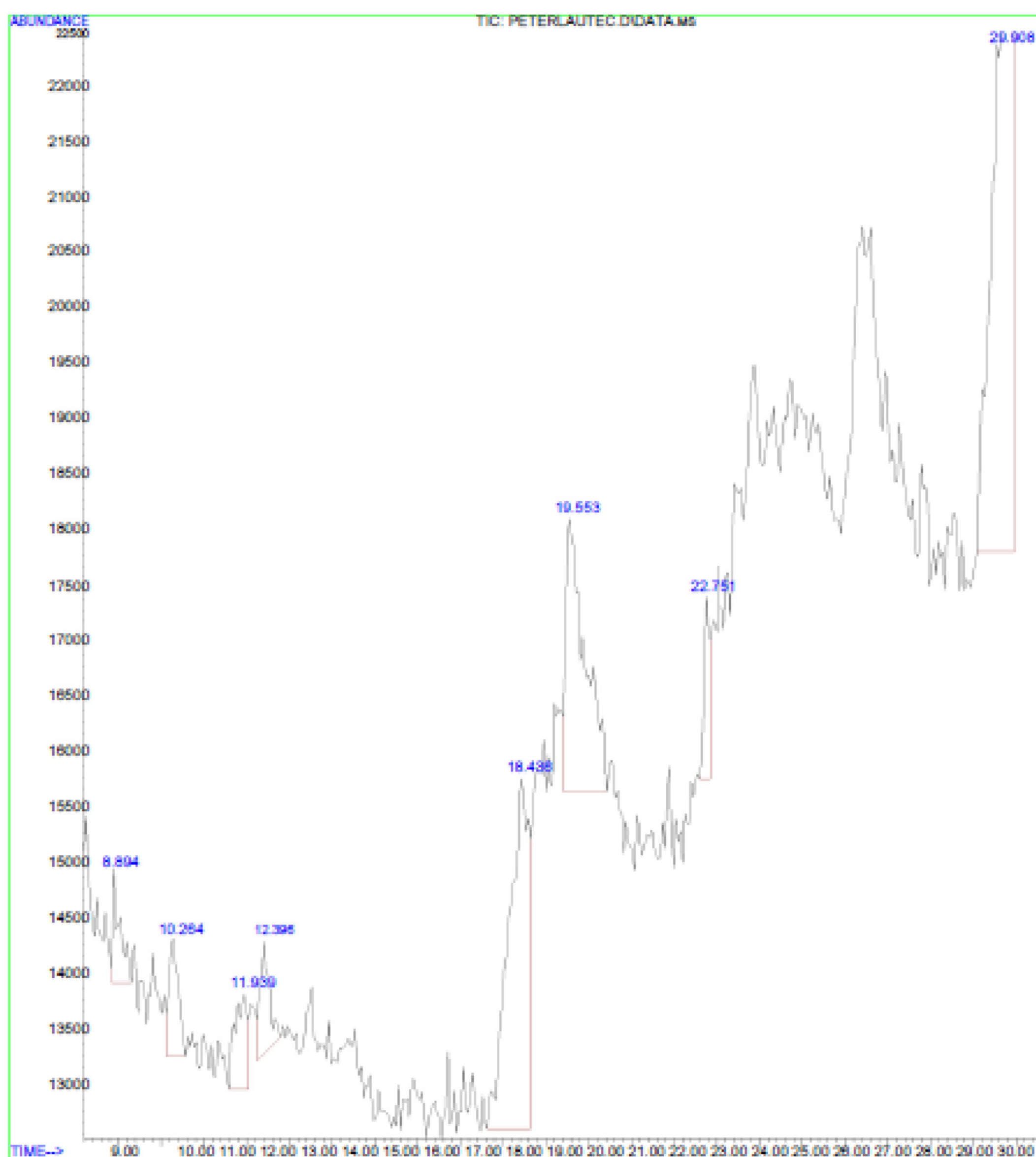

Figure 7. GC-MS spectrum of *Phyllanthus amarus* showing 8 prominent peaks.

methyl ester (3.38 %), 1H-Indole-3-acetic acid, 2-carboxy, methyl ester (2.48 %).

4. Discussion

High salt intake has been linked with an increase in renal blood flow, glomerular hyperfiltration with a decline, and impairment in kidney function. This impairment in kidney function predisposes to the development of hypertension and cardiovascular disorders [28]. Besides, through different pathways, high salt consumption has been implicated in the derangement of glucose metabolism, etiology of cognitive and immune dysfunction [15, 29, 30, 31]. A positive correlation between normal lipid profile and dietary salt restriction has been established and this can be linked to the reduced incidences of coronary heart disease, stroke, and myocardial infarction [32, 33]. The decreased serum HDL and a concomitant increased serum LDL, TG, TC, VLDL after treatment with high salt diet as noticed in this study is not unusual, as previous studies have demonstrated that a high salt intake can elicit derangement in lipid profile [34, 35, 36]. Serum LDL, TG, and cholesterol levels have been hitherto reported to be elevated, while HDL concentration was depleted in response to HSD [37, 38, 39, 40]. Although the mechanism of derangement in lipid metabolism in chronic and excess salt diet exposure is unclear, free radicals and inflammatory mediators have nevertheless, been hypothesized to be involved in the pathophysiology [39]. High salt diet causes an up-regulation in nitric oxide activity, which has been associated with the release of pro-inflammatory cytokines [41, 42, 43]. The reversal in deranged lipid profile in response to EEPA might be due to its constituent phytochemicals. As established by Schatz *et al.* [44], and Drenjancevic *et al.* [45], HSD caused severe renal damage, and neuropathology, resulting in increased activity of M1 macrophages and differentiation of Th17 cells, thus up-regulating IL-6, TNF-a, and IL-2. This phenomenon might be linked to the observed decrease in antioxidant enzymes activity vis-à-vis catalase and glutathione (GSH) in HSD-treated animals. Depleted level of GSH can be caused by increased generation of ROS, resulting in a buoyed level of malondialdehyde, TNF-α, interleukin-6 (IL-6), and deranged functioning of the immune system [46, 47, 48, 49]. HSD also up-regulates inflammatory molecules like vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule1 (ICAM-1), and nuclear factor-kappa B (NF-κB) [46]. Polyphenols are known for their different mechanisms of anti-inflammatory activity, ROS inhibition, and protection of antioxidant defenses [50]. *P. amarus* have been reported to demonstrate arrays of pharmacological and ssssbiological activities [51, 52]. Compounds detected in GCMS analysis in this study have been hypothesized to demonstrate antioxidant and anti-inflammatory activities among others [53, 54, 55]. Piperidine derivatives have been shown to exert antioxidant action by donating hydrogen atom(s) to free radicals [56, 57]. According to Jacob and Khan [58], Surankita *et al.* [59], elevated activity of LDH and CK with other biomarkers such as troponin are known to denote skeletal muscle injury and derangement in cardiac metabolism. In this study, these enzymes were elevated in response to HSD and are in tandem with submissions of Pisoni *et al.* [60] and Oloyo *et al.* [61]. The leakage of these enzymes from the tissues into the serum indicates disruption and loss of integrity of the cell membrane [62]. The kidneys play a pertinent and exclusive role in sodium regulation and reabsorption which is mediated by membrane transport proteins facilitating the movement of Na^{2+} across plasma membranes of renal epithelial cells [63, 64]. A substantial body of experimental and epidemiological reports have associated high sodium intake with glomerular hyper-filtration, kidney fibrosis, reduction in nephron number, impaired kidney function with elevated serum creatinine, urea, and uric acid activity [65]. In this study, it was discerned that administration of ethanol extract of *Phyllanthus amarus* reversed the elevated creatinine, urea, and uric acid concentration to near normal. Nephroprotective effects of *Phyllanthus amarus* phytoconstituents such as quercetin, astragalin, phyllanthin and epibubbialine have been documented [66, 67]. These compounds exert their action on molecular targets such as enzymes, receptors, and transcription factors [68]. More so, benzoic acid, pyridazine, carbozole and piperidine derivatives identified from the GC-MS analysis of EEPA have been reported to have arrays of pharmacological activities and can protect against cellular and tissue degeneration induced by toxic compounds [54, 55, 69, 70].

5. Conclusion

Our study indicates that *P. amarus* conferred considerable cardio and nephro protection against high salt-diet induced biochemical alterations and oxidative stress that may lead to cardiovascular-related diseases. Furthermore, some bioactive components belonging to benzothiazole, carbazole, benzoic acid, pyridazine, and piperidine derivatives present in *P. amarus* could be explored and useful for drug development.

Declarations

Author contribution statement

Olubukola Sinbad Olorunnisola: Conceived and designed the experiments.

Olumide Samuel Fadahunsi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Peter Ifeoluwa Adegbola: Performed the experiments; Analyzed and interpreted the data.

Bamidele Stephen Ajilore: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Folorunsho Ayodeji Ajayi, Lamidi Waheed Babatunde Olaniyan: Contributed reagents, materials, analysis tools or data.

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Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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