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SOLVENT CHOICE, PHENOLIC QUANTIFICATION AND INVIVO POTENTIAL OF *phyllanthus amarus* WHOLE PLANT PHENOL RICH CONCENTRATE

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ABSTRACT

The traditional method of preparing herbal decoctions has been known for ages without certainty if the most efficacious phenolic bio-molecules of medicinal plants e.g. *Phyllanthus amarus* (*Schum and Thonn*) are being exploited. More so, high salt diet (HSD) intake has been established worldwide, to deleteriously induce hyperlipidemia-related hypertensive rubor, renal subjugation and adipocyte proliferation. The present study was to validate the best extraction method that would greatly tap the *Phyllanthus amarus* (*Schum and Thonn*) whole plant phenolic phytoconstituent and also evaluate its *in-vivo* role in the amelioration of high salt meal triggered hyperlipidemia, adiposity, and renal dysfunction in an animal model, within 8 weeks. Thirty-five male Sprague-Dawley rodents (170-180g) were grouped, and treated as follows: Group1: fed with normal rat chow; Group 2: HSD; Group3: HSD+75mg/kg/b.wt of PRE; Group 4: HSD+100mg/kg/b.wt of PRE; Group 5: HSD+150mg/kg/b.wt of PRE. Results showed that aqueous acetone concentrates exhibited the highest 2, 2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging activity (result not shown), and also with the highest content of total phenolics. The in-vivo results further revealed significant ($p < 0.05$) histopathological alterations in the renal architecture of group 2 fed high salt chow only, increased kidney weight, lipid profile, body fat deposit(54.5%) andconcentration of renal bio-products. It also established a significant dose-dependent recuperating potential in the groups co-treated with PRE when compared with rats on normal chow. The aqueous acetone solvent exhibited the best extraction medium for PRE and may thus be considered as an imminent therapeutic agent in managing high salt-driven Hyperlipidemia, Adiposity and Renal dysfunction.

Keywords: Hypernatremia, *Phyllanthus amarus,* Phenol-rich concentrate, Nephronecrosis, Acetone.

INTRODUCTION

Despite the health challenges scourging the world, the success accounts on the efficacy of natural remedies have been tremendous, further proving its integration into health care delivery management. More precisely, consumption of a high-salt diet (HSD) customized ready made meals has also been reported worldwide, resulting into hypernatremia-related pathologies, and renaldys function. The recommendation of dietary salt intake, more importantly in the regulation of related pathologies such as cardiovascular diseases, hypertension, adiposity, and tunica intima fibrosis had also received much attention in another study. It was established that high salt diet intake is the major cause of oxidative damage, hepatotoxicity and ultimately increasing the risk of vascular events of hyperlipidemia, atherosclerosis, myocardial infarction, obesity, stroke, and nephrotoxicity. Previous studies also established loss of leptin responsiveness in a high salt sensitive animals resulting into obesity. The influences of hypernatremia on the integrity of kidney architecture, functionality, infiltrating Urea, uric acid, creatinine and albuminbio-indicators have also been proclaimed, which was reported to be mediated through activation of renin-angiotensin's interaction in the kidney's subvention . In remedial inquisitiveness, supplementation with bio-active metabolites from crude plant origin as been established, perhaps through the suppression of angiotensinogen to centrally active peptide angiotensin Iactions inhypernatremia-drivenrenal morbidity. However, phytoconstituents, majorly

phenols, terpenes, and alkaloids, are not well exploited by traditional practitioners, though are documented agents for alternative medicine. Phenols, being the most beneficial secondary reductants from plants, a functional bearing scavenging hydroxyl group benzene subordinate, majorly acting as atherapeuticagent in disease control and protection in plants against pests, and even in animal consuming them as foods have not been extractively exploited. Normally, the leptin hormone regulating the potentiality of the kidney on renin-angiotensinactions, occurring within the human system could rejuvenate and prevent the body's homeostasis against a reasonable degree of health instability triggered by the high salt infringement. Nonetheless, the exposure of the body inertia to a high salt diet eventually submerged the anti-cascade call, triggering hypernatremia-related pathologies. *Phyllanthus amarus*, a medicinal plant belonging to *Euphorbiaceae* commonly used in alternative medicine as an anti-diarrhea, anti-diabetic, antioxidant, analgesic, anti-inflammatory, antihypertensive, antimicrobial, and hypolipidemic agent is hereby investigated. The phytochemical analysis revealed the presence of phenolic compounds (flavonoids), tannins, alkaloids, saponins, and terpenoids in aerial parts, with roots/seeds dominating with a reasonable amount of phenolic and terpenoid bio-constituents only. With this background, the study thus aimed to investigate the best extraction method for phenolic phytoconstituents in mg/g of gallic acid equivalent, provide an overview of the use of the *Phyllanthus amarus* whole plant phenolic rich bio-active constituents, its lethal dose, antiobesity, renal recuperating and antihyperlipidemic potential in high salt diet assaulted animal model.

MATERIALS AND METHODS

Plant Materials

The *Phyllanthus amarus* (*Schum & Thonn*) whole plant was sourced locally from alternative medicine Practitioners managing various diseases in Oja Igbo market, Ogbomosho and authenticated by Dr. Famuwagun I.B., Federal University of Technology, Akure (FUTA), Nigeria. The plant sample tagged 0255, was finally preserved at the FUTA herbarium for botanical referencing.

Plant Preparation

Phyllantusamarus (Schum & Thonn) whole plant was watched with clean water, air-dried at room temperature for 5weeks and eventually pulverized using an industrial fine grinding machine. The final powdered sample was kept in an air-tight amber bottle, and refrigerated for future extraction.

Plant Extraction

- **Preparation of Phenolic Rich-extract (PRF)**

The phenolic-rich extract was prepared using the methods of with little alteration.

Laboratory Animals

The experiment was performed using healthy adult male Sprague-Dawley rats (n=35), weighing between 170-180g, purchased from the animal unit, Department of Biochemistry, and housed in the animal colony of the Department. They were fed with commercial 8% high salt and normal rat chow from Funsab Enterprises, Agro and Livestock Raw Materials Merchant, Lagos, Nigeria.

Animal grouping

The animals (n=35) were divided into five groups of seven.
Group 1: Rats on a Normal chow diet
Group 2: Rats orally fed a high-salt diet
Group 3: HSD+75mg/kg/bodyweight of PRE.
Group 4: HSD+100mg/kg/bodyweight of PRE.
Group5: HSD+150mg/kg/bodyweight of PRE.

Acute Lethal toxicity evaluation of *Phyllanthus amarus* (Schum & Thonn) whole plantphenol-rich extracts

The acute lethal toxicity (LD50) of phenol-rich extracts (PRE) was determined using up-and-down techniques as described by .

Animal Sacrificing

After an overnight fasting, post-last administration, the animals were anesthetized with chloroform vapor for about 2minutes, sacrificed through an abdominal incision, renal organs harvested and blood collected using 5ml syringes into an Ethylene diamine tetraacetic acid (EDTA) and serum bottles. With the latter standing for 45minutes, centrifuged at 3000rpm for 15minutes, serum obtained was used for the biochemical analysis.

Biochemical evaluation

➢ **Qualitative test for Phenolics**

The phenolic affirmation was carried out as described by.

➢ **Total phenolic content**

Total phenolic concentration was determined spectrophotometrically against standard Gallic acid as quantified by.

➢ **Estimation of serum cholesterols' profile**

The following lipid cholesterols were estimated in the serum as proclaimed by analytical procedure using assay kits from Randox Laboratories Ltd, United Kingdom.

- **Assay for total cholesterol**

The total Cholesterol concentration was calculated as follows:

$$\frac{\text{Absorbance (A) of sample X concentration of standard (}\mathbf{\text{calibrator conc)}}}{\text{Absorbance of standard}}$$

Conversion factor, mg/dL x 0.0258 = mmol/L

•Estimation of triglycerides (TG)

The concentration of triglyceride was calculated as:

$$\frac{\text{Triglycerides (TG) (mg/dL) = A sample x 200 (calibrator conc)}}{A_{standard}}$$

Conversion factor, mg/dL x 0.0113 = mmol/L.

•Estimation of low and high-density lipoprotein

HDLc concentration was calculated as follows:

$$\frac{\text{A sample} \times \text{ Concentration of Standard} \times \text{Dilution Factor (200)}}{\text{A standard}}$$

While LDL-Cholesterol was quantified as:

LDL cholesterol = Total Cholesterol – HDLc - (TG/5)

➢Estimation of kidney biomarkers

•Serum creatinine determination

Serum creatinine concentration was quantified by the method described by.

•Serum urea determination

The concentration of urea was calculated as follows (mg/dl)

$$= \frac{\text{Absorbance for sample X Absorbance for standard}}{\text{Standard concentration}}$$

•Serum uric acid determination

Calculation of uric acid concentration was also estimated using the method described by .

•Serum total protein determination

Total protein was estimated as described by .

•Serum albumin determination

The concentration of albumin in the serum was estimated following the report of .

- **Serum globulins determination**

The total proteins of the plasma are divided into three fractions; albumin, globulins and fibrinogen. However, the measurement of protein is done on serum, which is the fluid that remains after the plasma has clotted, therefore fibrinogen, a plasma protein/clotting factor is already excluded. Consequently, the total globulin fraction was calculated by subtracting the albumin concentration from the total protein obtained from the serum .
Globulin level (g/dl) = Total protein concentration (g/dl) – albumin concentration(g/dl)

Computation of weight of the experimental animals and the harvested renal organs

The body weights and that of the harvested kidneys of all the rats were also measured.

Histological evaluation of the renal architecture

The renal architecture was evaluated after fixation in a phosphate-buffered solution (pH 7.4), stained with eosin, and preserved using 4% formaldehyde. Tissues were premounted on slides, viewed at X100 (H&E) and recorded .

Statistical Analysis

The data were cross-examined using a one-way analysis of variance (ANOVA) streamlined by the Newman-Keuls Multiple Comparison Test. The Statistical Analysis was performed using Graph Pad Prism (ver.5.0a). More so, all data were expressed as mean ± SD (n= 6) and considered statistically different at $p< 0.05$. Data with different superscripts were compared with the control group along the same column and are statistically different.

RESULTS

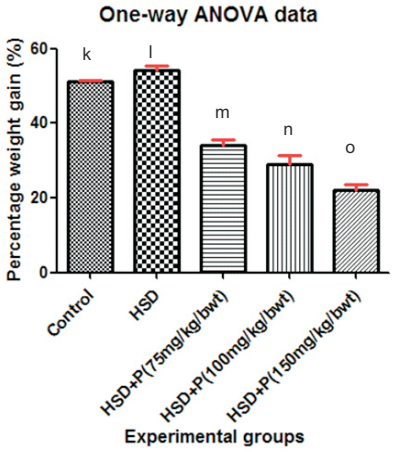

Figure 1: Outcome of phenolic-rich extract of *Phyllanthus* amarus (Schum & Thonn) whole plant on the bodyweight of rats fed with 8% high salt diet

Table 1: Outcome of Phenol rich extract (PRE) of *Phyllantusamarus* whole plant on lipid profile (mg/dl) of rats fed with 8% high salt diet.

Group	TC	HDL	TG	LDL	VLDL
Group1	91.21 ± 1.21^{e}	80.97 ± 1.14^{t}	70.11 ± 1.15^{a}	9.31 ± 1.00^{a}	14.62 ± 0.33^{a}
Group2	158.50 ± 1.26^{a}	48.38 ± 0.55^{u}	180.30 ± 1.30^{b}	58.05 ± 0.15^{b}	43.32 ± 0.25^{b}
Group3	130.30 ± 0.03^{b}	54.31 ± 0.33^{v}	161.20 ± 1.20^{c}	38.41 ± 0.08^{c}	29.21 ± 0.52^{c}
Group4	115.30 ± 1.35^{c}	72.51 ± 1.29^{w}	140.50 ± 0.15^{d}	21.52 ± 0.01^{d}	20.55 ± 0.16^{d}
Group5	99.21 ± 0.15^{d}	75.32 ± 1.19^{y}	100.30 ± 0.13^{e}	13.51 ± 0.03^{e}	15.59 ± 0.25^{ea}

Keys:

Group 1- Normal group

Group 2- High salt diet (HSD) fed group

Group 3- HSD+75mg/kg/bwt of PRE

Group 4- HSD+100mg/kg/bwt of PRE

Group 5- HSD+150mg/kg/bwt of PRE

Table 2: Outcome of Phenol-rich extract (PRE) of *Phyllantusamarus* whole plant on some markers of HSD-induced kidney toxicity.

Group1	31.80 ± 0.53^{e}	2.21 ± 0.02^{a}	0.62 ± 0.05^{a}	3.20 ± 0.01^{a}	6.05 ± 0.03^{a}	2.85±0.02e
Group2	58.21 ± 0.05^{a}	6.05 ± 0.05^{b}	1.41 ± 0.07^{b}	5.62 ± 0.02^{b}	7.05 ± 0.12^{b}	1.43 ± 0.10^{d}
Group3	50.15 ± 0.02^{b}	4.32 ± 0.02^{c}	1.10 ± 0.01^{c}	4.74 ± 0.10^{c}	6.05 ± 0.12^{ca}	1.31 ± 0.02^{c}
Group4	43.21 ± 0.02^{c}	3.96 ± 0.05^{d}	1.05 ± 0.03^{d}	3.83 ± 0.15^{d}	6.05 ± 0.55^{da}	2.22 ± 0.40^{b}
Group5	39.30 ± 0.08^{d}	3.01 ± 0.02^{e}	0.92 ± 0.04^{e}	2.98 ± 0.11^{ea}	6.05 ± 0.12^{ea}	3.07 ± 0.01^{ea}

Significant ($p < 0.05$) percentage increase in the bodyweight of the positive group (HSD assaulted) (54.5%) when compared with the negative rats on normal chow (51.2%) after 8weeks was established in this study, as depicted in Fig 1.The observed weight increase in the rats fed HSD agreed with the report of , who accounted for a correlation between leptin non-responsiveness, high salt diet and obesity in mice, amidst activation of endogenous fructose anabolism, and also resulting into fatty liver. However, there was a significant ($p < 0.05$) dose-dependent percentage decrease in the bodyweight of rats co-administered with phenolic-rich extract of the whole plant (34%, 29.3% & 22.9%) when compared with the negative control. The established ($p < 0.05$) dose-dependent and correlational decreases are consistent with the anti-obesity report of , on normoglycemic albino rats, administered aqueous extract of the same plant. The effect of *Phyllanthus amarus* extract on body weight as accomplished is thus attributed to the rich phenolic phytoconstituents that are present in this concentrate. More so, it's well accounted as shown in Table 1, a significant ($p< 0.05$) increase in the level of lipid profile, TC, TG, VLDL, and LDL with a corresponding($p< 0.05$) decrease in the level of HDL in the group fed high salt diet only (Group 2) when compared with rodents on normal rat chow after 8weeks.The result further revealed the ability of PRE, to significantly and dose-dependently recuperate the compromised lipid profile of the treated group to near normal. This was correlated with dose-dependent and metabolic improvement of HDL level. Worthy of note, is the more deleterious advancement of LDL than other lipid parameters of interest in the high salt assaulted group, which is also consistent with the report of .The significant dose-dependent increase ($p < 0.05$) in the serum level of urea, uric acid, creatinine and albumin of rats fed with HSD, was also established when compared with animals fed with normal chow (Table 2). However, groups 3, 4, and 5 co-administered with PRE at 75, 100, and 150mg/kg/bwt, respectively showed a dose-dependent decrease in the concentration of kidney bio-indicators (urea, uric acid, creatinine and albumin)to a near normal level with a corresponding increase in the concentrations of kidney marker, globulin. More importantly, PRE recuperated all compromised renal parameters to near normal levels after co-treatment when compared with the group fed with normal chow after 8weeks.

Histopathology of rats' kidneys fed HSD and co-treated with phenolic-rich concentrate

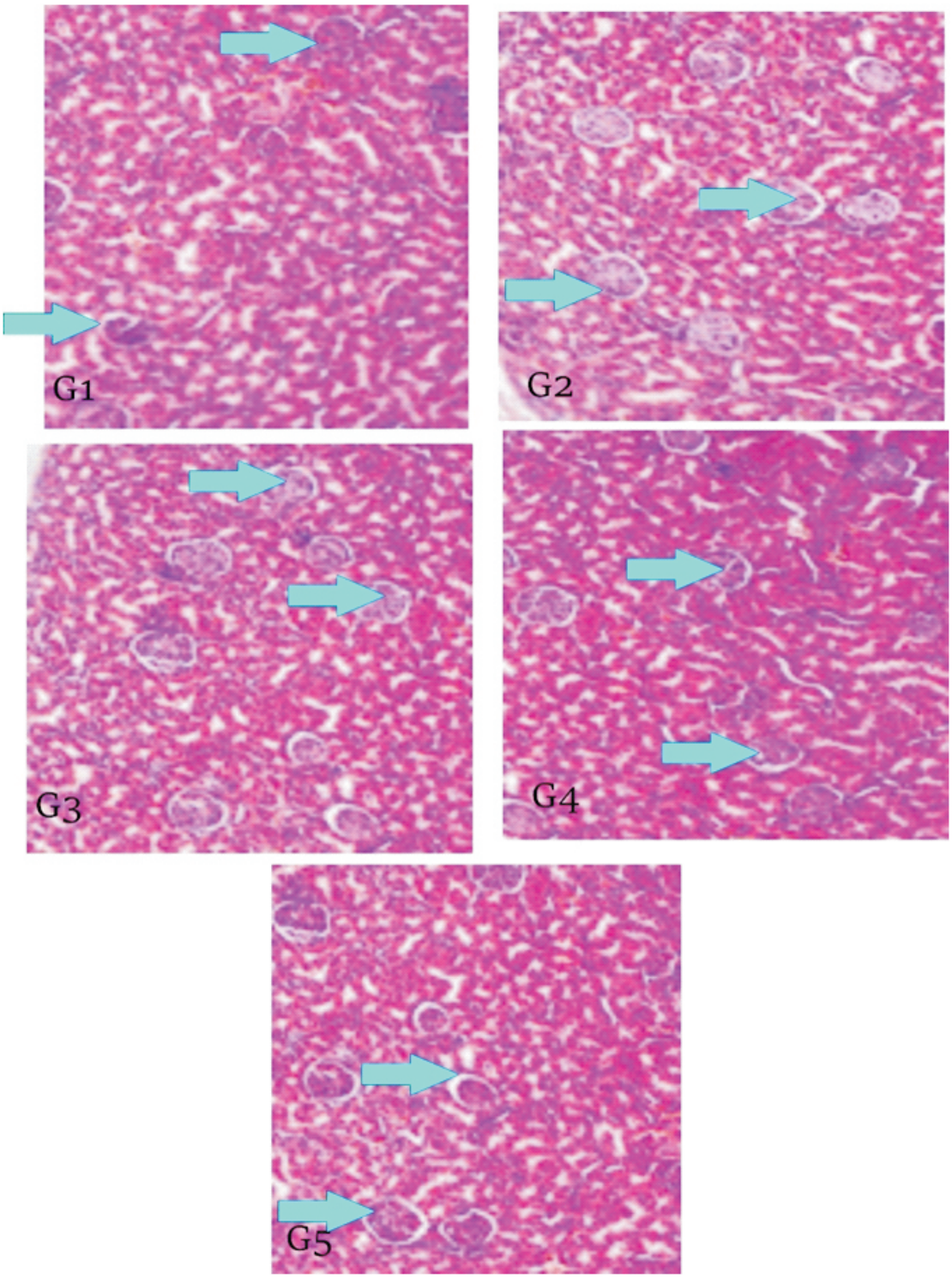

Fig 2: Sectional photomicrograph of kidney subjected to eosin staining procedure. G1- Group 1- Normal group. G2- High salt diet (HSD) fed group. G3- HSD+75mg/kg/bwt of PRE. G4- HSD+100mg/kg/bwt of PRE. G5- HSD+150mg/kg/bwt of PRE

Table 3: Weight of harvested kidneys of rats co-administered with PRE and HSD

Group	Weight of harvested kidney (g)
Group 1	0.60± 0.02[a]
Group 2	0.96± 0.02[b]
Group 3	0.85±0.01[c]
Group 4	0.70±0.02[d]
Group 5	0.61±0.01[ea]

Normal structural representations of the kidney were observed in group 1, fed with normal rat chow as presented in Figure 2. These were evidenced with normal kidney total weight, glomerulus and afferent arteriole when compared to group 2, fed HSD only (blue arrow). Assaulted intake of high salt diets, eventually distorted the kidney weight, corroded the renal topology, and the cortical histo-architecture leading to nephrosclerosis. However, groups 3, 4, and 5 co-administered with PRE at 75, 100, and 150mg/kg/bwt, respectively showed a significant and dose-dependent recuperation in the integrity of the kidney, with group 5 revealing near-normal kidney parenchyma, mesangial cells and renal weight.

Table 4: Phenolic content from solvent comparative study

Solvents	Phenolic content (mg/g) in Gallic acid equivalent (GAE)
Aqueous acetone(70%)	204.78±2.34[a]
Aqueous ethanol(70%)	165.50±1.78[b]
Aqueous Decoction	150.22±2.00[c]

The concentrate extracted from *Phyllantusamarus* whole plant using aqueous acetone, revealed a significant phenolic value of 204.78±2.34 mg/g of gallic acid extract (GAE) equivalent followed by aqueous ethanol with165.50±1.78 mg/g and lastly 150.22±2.00 mg/g by the aqueous vehicle. Interestingly, no such comparison could be found in the literature.

DISCUSSION

In consideration of the pertinent interest to exploit the full potential of *Phyllantusamarus* whole plant, it was accounted that the phenolic bio-constituents are more potent in plants extracted with acetone than other vehicles of interest and the metabolites there-in are responsible forintent pharmacological remediation reported in this study. The aqueous acetone concentrate also exhibited the greatest DPPH scavenging activities (data not shown) with the highest phenolic concentration of 204.78±2.34 mg/g of gallic acid extract (GAE) followed by 70% ethanol [165.50±1.78 mg/g of GAE] and aqueous vehicle [150.22±2.00mg/g of GAE]. This study further confirmed that, besides the commonly used traditional method of decoction, solvents such as aqueous acetone among others are of a better application for extracting beneficial phenolic phytoconstituents from medicinal plants. In addition, eight percent (8%) HSD (by weight) was established to induce repugnanthypernatremia-related adiposity by 54.5% when compared with rats on normal chow (51.2%), after the 8-week study. The observed weight increase with corresponding larger kidney hypertrophyper body weight observed in the rats fed with HSD, though agreed with the declaration of on high salt-sensitive populations, could have been triggered by the loss of leptinactions, adipocytokines and adipsinhormone stimulation amidst an increased appetite with concomitant endogenous fatty molecules anabolism and adipose tissue build-up. Moreover, the groups fed with HSD only were obviously noted to drink water more virulently than the group fed with normal rat chow throughout the study, consistently presumed to be triggered by the signal induction received from aldosterone, a renal hormone. Be as it may, the percentage dose-dependent decrease observed in the body weight of separate groups co-administered with PRE (75, 100 and 150 mg/kg body weight)of the *Phyllantusamarus* whole plant extract, though partly consistent with the account of on the aqueous extract of the same plant, could have been actuated by the efficacy of the rich metabolites in alleviating hypernatremia driven mitochondrial and endoplasmic reticulum (ER) stress, which ought to synthesize fatty adipose polymers. The rich plant concentrates also perhaps significantly potentiate the endothelial lipoprotein lipase decomposition of

fatty triglycerides to adenosine triphosphate (ATP) cycle, within the mitochondrion, tunica intima architecture and hence down regulating in tracellular fibrosis, adiposity, water retention and plasma-renalextracellular volume ratio. This eventually promotes the health countenance and weight reduction in treated groups after co-administration. The kidneys on the other hand are a pair of bean-shaped organs, with about a million tiny filters called nephrons specifically mandated to filter blood and eventually maintain homeostatic gradients, right levels of electrochemical and proteinous threshold (albumin and globulin), toxic urea, uric acid and nitrogenous creatinine in the blood. In this study, HSD severely corroded kidney architecture and its biomarkers leading to nephrosclerosis (figure 2)as revealed in the histology, which perhaps established the incidence of renal failure in some individuals consuming highly salty meals. This eventually resulted also into a significant increase in renal-related serum bio-molecules, albumin, nitrogenous urea, uric acid, creatinine and renal weight in rats fed with HSD when compared with the groups fed with normal chow after 8weeks. The aforementioned HSD agonist impairment triggered on the kidney integrity eventually resulted in dose-dependent related pathology; hyperlipidemia, and adiposity, associated with globulin depletion as previously established by . The report of further accounted for over-inductions of renin hormone, an angiotensinogenase, and aldosterone, from a compromised kidney's adrenal gland, in response to inflammatory high salt agonist, being the basic modalities behind the actuated nephrosclerosis, significant elevation in its markers, and volume gain of the kidney. However, co-administration of PRE (75, 100, and 150mg/kg/bwt) in other HSD-fed groups significantly and dose-dependent lyameliorated the pathology and perhaps hindered the renin response to near normal levels. This was also very apparent in the reduction coefficient of urea, uric acid, creatinine and albumin of high salt diet fed but co-treated rats to near normal levels as depicted in the renal photomicrograph. On a larger note, the PRE thus prevents HSD-induced renal hyper-activity of proteinous insurgence, kidney's histopathology and its renal sclerosis by counterbalancing the influx of electrochemical sodium/water gradient, being reabsorbed back to the blood lumen, which ought to inflame cardiac pressure. The attenuation effects potentiated by the phenolic-rich concentrates on the over-disposition of serum proteinous biomolecules, also resulted in an eventual increase in creatinine clearance credibility, a good venture for healthiness (table 2 refers). The resultant homeostasis accounted by PRE on HSD deranged kidney parenchyma, as recorded in Figure 2, its marker, globulin, and weight, also consolidated the remedial potency of the phytochemicalson albuminuria as reported by , and notably, recuperating over allagility in the co-treated groups. The dose-dependent efficacies established against the assaults from the groups co-administered with the rich concentrates, also consolidated a restoration report on renal parameters of rats treated with both Gallic and tannic acids extracts, surmountably reverting endoplasmic reticulum hyperinductivity and nephrotoxicity to near normal level. Furthermore, aldosterone from the kidneys' adrenal gland though peculiar with the regulation of sodium-potassium electro-chemical threshold in the kidney and plasma protein in the blood, yet, over elicitation of the hormone, consolidated with activation of Angiotensin converting enzymes (ACE) and Endothelin converting enzymes (ECE), have also been established in HSD agonist sensitive individual .Withal, the rich extracts potentiated a significant recuperation, which perhaps suppressed ACE, ECE and the conversion of angiotensinogen to centrally peptide angiotensin I, thence, to the extremely potent vasoconstrictor, angiotensin 11, by ACE is demobilized in co-treated groups. These potentialities of the phenol-rich plant concentrates maintain metabolic homeostasis, which favors kidney functionality, Endothelialnitric oxide synthase, hydrogen sulfide (H_2S) induction , cardiac inotropism, with concomitant reduction in ACE affiliated NADPH oxidase, other renal parameters and volume gain to near normal level, and eventually preventing high salt agonist-inducednephrosclerosis. Though, the total protein retains no significant concentration difference, in HSD fed group as accounted by throughout the study, however, urea, uric acid over elicitation, nephrotoxicity and albuminuria were dose-dependently attenuated by PRE, as also evidenced in the photomicrograph (figure 2) and could thus be recommended as therapeutic candidate in the treatment of HSD triggered renal injury. Most bio-

lipids synthesized by cholesterol synthase in the smooth and rough endoplasmic reticulum of the hepatocytes, are mostly trafficked through Golgi bodies into the bloodstream and are categorized into high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG) and very low-density lipoprotein (VLDL) cholesterols. Though VLDL is majorly transported in the form of TG along the blood lumen, however accumulation of the TG and LDL cholesterols are accounted to be atherogenic in animal models. The significant increase of TG and LDL in rats fed with HSD, could have been mediated or triggered by the denaturalization of tunica intima and hepatocyte, leading to hyperlipidemia in a counter-response, with corresponding actualization of adiposity and fatty liver. The established outrage ($p < 0.05$) in the lipid profile of various cholesterol (TC), TG, LDL, VLDL with significant ($p < 0.05$) decrease in the level of High-density lipoprotein (HDL)by the HSD agonist compared with rats on normal chow, could also have been actuated by the degradation inhepatic lipase functionality, vis-a-vis catabolism of fats and dietary triglycerides into extracellular nutrients, hence synthesis and proliferation of adipocytes. Nonetheless, the observed efficacies of PRE against HSD triggered hyperlipidemia in co-treated groups, attenuating hypernatremia-related impairment on tunica intima and hepatocyte by building hydrogen, covalent and/or ionic bonds, which is the common morphological manifestations of the intracellular ameliorative or radical scavenging mechanism of the hydroxyl functional group of most phenolics, was perhaps the Operandi of the extract metabolites, hence promoting the rich constituent anti-lipidemic efficacy and healthiness.

Aftermaths, the increase in HDL, a good cholesterol to a larger extent, initiated by the co-treatment, thus correspondingly actuated the extracellular trafficking of bad LDL from the peripheral tissues and endosomal lumen back to the hepatocytes for storage, through reverse cholesterol influence, thus limiting cellular toxicity and reducing the availability of LDL. The PRE amelioration potential might have also been mediated through inhibition of hepatic cholesterol synthase , increased lipid to bile acids conversion and excretion, enhanced endothelial lipoprotein lipase catabolism of TG to non-esterified fatty acid (NEFA), which consequently promote the availability of Adenosine triphosphate (ATP) in the tissues. These are instrumental and beneficial, essentially in high-density lipoprotein (HDL) efficiencies, and now in rats co-treated with the rich plant concentrates. Though, HSD assaulted animals revealed higher significant value in the level of LDL compared to other lipid parameters of interest, perhaps due to low reversed trafficking by HDL biomolecule, however, PRE potentiated elaborate lipid profile recuperation and recovery in a dose-dependent manner to near normal level. The basic mode of actions/remedial activity in the groups co-treated with the whole plant-richconcentrates could also have demobilized phospholipase A2 on the vascular intima, which ought to release fatty molecules and eventual formation of plaque indices. The other possible mechanisms by which high saltagonist induces hyperlipidemia and hepatorenal-related adiposity include; phospholipase enzyme hyperactivity , increased cardiac expression of the β-myosin heavy chain (MHC) ,calcium influx, over induction of sodium/potassium AT pasehomeostatic threshold , atherogenesis on vascular intima, profilerating peroxidation, endothelial on cogenesis and renal toxicityvia renin-angiotensin's modules of operandi, among others. Withal, PRE could have acted as a therapeutic agent, concisely alleviating all the aforementioned to near normal group.

CONCLUSION

It was established in this study that the general but common traditional method of preparing herbal decoction doesn't fit in to exploit the most efficacious phenolic phytoconstituents of medicinal plant, *P. amarus* to be precise. The aqueous acetone concentrate having revealed the highest phenolic concentration in mg/g of the standard Gallic acid equivalent (GAE) than other vehicles' extracts evaluated. This was also supported by showing greater DPPH scavenging ability among others (data not shown). The efficacy of the aqueous acetone concentrate (PRE) of the *Phyllantusamarus* whole plant, acting as an anti-obesity, anti-hyperlipidemia and renal recuperating agent in HSD assaulted rodents, was indisputably noted, and consolidated by the lethal dose above 5000mg/kg/body weight and safe doses of 75, 100, and 150mg/kg/bodyweight. These potencies accounted are very well related to the identified

phenolic compounds in *Phyllanthusamarus,* which include phyllanthin, hypophyllanthin, lignans , Hydroxybenzoic, hydroxycinnamic acid derivatives, gallic acid, flavonoids, ellagic acid derivatives, and protocatechuic acid, among others as accounted by. In all, the phenolic phytoconstituents from *P. amarus* are of research interest, which can be exploited using aqueous acetone and thus be therapeutically used as a novel complementary alternative candidate with very potent efficacy, in the prospect to manage HSD driven adiposity, hyperlipidemia and renal dysfunction.

List of Abbreviations

HSD- High salt diet
WHO- World Health Organization
PRE- Phenolic rich extract
SD- Standard deviation
TC- Total cholesterol
TG- Triglycerides
LDL- Low-density lipoprotein
HDLc- High-density lipoprotein cholesterols
ACE- Angiotensin converting enzymes
ECE- Endothelin converting enzymes.

Declarations

- **Ethics approval and consent to participate**

The protocol of the study was approved by the Institution's ethical committee on animal usage of the post-graduate school and gave an ethical approval number for the study (FBMS2019/012) in compliance with the world protocol of the National Institute of Health (NIH), (publication 85-23, 1985), for research animal ethics.

- **Consent for publication**

Not applicable.

- **Availability of data and materials**

All data generated and analyzed throughout the study are included in this published article.

- **Competing interests**

This is to declare that there is no existing conflict of interest in this study.

- **Funding**

The authors declare the study received no grant from any organization whatsoever.

- **Authors' Contributions**

OSO designed and supervised the study, in conjunction with AA. BSA provided logistic support, analyzed and interpreted the data, FBA, ATO and JPA evaluated the renal weight, solvent comparison and histology while TIF conducted the research, collected the data, and wrote the manuscript. All authors eventually read and approved the manuscript.

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- **Further study**

Though, this study validated the Nigeria folkloric use of ***Phyllanthus amarus (Schum & Thonn)*** *and* also established the composition to include phenolic agents, which could ameliorate the body against high salt diet-related impairments. However, there is a need to isolate, identify, characterize, and investigate the pharmacological bases of the phenol-rich concentrate, responsible for the potential accounted.

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Legends of Figures and Tables

1. **Figure 1:** Outcome of phenolic-rich extract of *Phyllanthus* amarus (Schum & Thonn) whole plant on the bodyweight of rats fed with 8% high salt diet.
2. **Figure 2:** Sectional photomicrograph of kidney subjected to eosin staining procedure
3. **Table 1:** Outcome of Phenol rich extract (PRE) of *Phyllantusamarus* whole plant on lipid profile (mg/dl) of rats fed with 8% high salt diet.
4. **Table 2:** Outcome of Phenol-rich extract (PRE) of *Phyllantusamarus* whole plant on some markers of HSD-induced kidney toxicity.
5. **Table 3:** Weight of harvested kidneys of rats co-administered with PRE and HSD.
6. **Table 4:** Phenolic content from solvent comparative study.

REFERENCES

Allender, P. Scott, Jeffrey A. Cutler, Dean Follmann, Francesco P. Cappuccio, Jane Pryer, and Paul Elliott. (1996). *Dietary Calcium and Blood Pressure: A Meta-Analysis of Randomized Clinical Trials*.

Amarini, Rebekah, Elise Landa, and Girish J. Kotwal. (2016). *Hypertension: Biochemical Basis and Current Management Strategies*.

Berger, Rebeca, Acaris Benetti, Adriana Girardi, Ludimila Forechi, Rafaella Oliveira, Paula Vassallo, and Mill Geraldo. (2018). "Influence of Long-Term Salt Diets on Cardiac Ca2+ Handling and Contractility Proteins in Hypertensive Rats." *American Journal of Hypertension* 31.

Boegehold, Matthew A. (2013). *The Effect of High Salt Intake on Endothelial Function: Reduced Vascular Nitric Oxide in the Absence of Hypertension*.

Busher, Janice T. (1990). "Serum Albumin and Globulin." in *Clinical Methods: The History, Physical, and Laboratory Examinations*, edited by H. K. Walker, W. D. Hall, and J. W. Hurst. Boston: Butterworths.

Chenevard et al. (2006). "Persistent Endothelial Dysfunction in Calcified Aortic Stenosis beyond Valve Replacement Surgery." Retrieved July 7, 2020

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1861314/).
Chinedu, Enegide, David Arome, and Fidelis Solomon Ameh. (2013). "A New Method for Determining Acute Toxicity in Animal Models." *Toxicology International*.

Ching Li, Paul Wai, C. S. Ho, and R. Swaminathan. (1994). "The Effect of High Salt Intake on Na+, K+-Atpase Activity of Tissues in the Rat." *Clinical and Experimental Hypertension* 16(3):327–40.

Drenjacnevic-Peric, I., B. Jelaković, J. H. Lombard, M. P. Kunert, A. Kibel, and M. Gros. (2011). *High-Salt Diet and Hypertension: Focus on the Renin-Angiotensin System*.

Ezeugwunne, et al, Chukwuma, Ogbodo, Okpogba, Analike, Okwara, Mbaeri, and Amah. (2018). "Effect of Phyllanthus Amarus Leaf Extract on the Serum Lipid Profile of Alloxan-Induced Diabetic Albino Wistar Rats in College of Health Sciences." *International Journal of Health Sciences & Research (Www.Ijhsr.Org)* 8(February):199–207.

Fujita, Toshiro. (2014). "Mechanism of Salt-Sensitive Hypertension: Focus on Adrenal and Sympathetic Nervous Systems." *Journal of the American Society of Nephrology : JASN* 25(6):1148–55.

Grillo, Andrea, Lucia Salvi, Paolo Coruzzi, Paolo Salvi, and Gianfranco Parati. (2019). "Sodium Intake and Hypertension." *Nutrients* 11(9).

Hurt-Camejo, Eva, Germán Camejo, Helena Peilot, Katarina Öörni, and Petri Kovanen. (2001). "Phospholipase A2 in Vascular Disease." *Circulation Research* 89(4):298–304.

James, D. B., O. A. Owolabi, N. Elebo, S. Hassan, and L. Odemene. (2009). "Glucose Tolerance Test and Some Biochemical Effect of Phyllanthus Amarus Aquoeus Extacts on Normaglycemic Albino Rats." *African Journal of Biotechnology*.

Khanna, A. K., F. Rizvi, and R. Chander. (2002). "Lipid Lowering Activity of Phyllanthus Niruri in Hyperlipemic Rats." *Journal of Ethnopharmacology*.

Komolafe, Kayode, M. T. Olaleye, T. I. Fasan, O. O. Elekofehinti, J. A. Saliu, and A. A. Akindahunsi. (2013). "Lowering Effect of Parkia Biglobosa Leaf Saponins in Triton-X 1339-Induced Hyperlipidemic Rats." *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4(1):576–85.

Konaté, Kiessoun, Adama Hilou, Jacques F. Mavoungou, Alexis N. Lepengué, Alain Souza, Nicolas Barro, Jacques Y. Datté, Bertrand M'Batchi, and Odile G. Nacoulma. (2012). "Antimicrobial Activity of Polyphenol-Rich Fractions from Sida Alba L. (Malvaceae) against Co-Trimoxazol-Resistant Bacteria Strains." *Annals of Clinical Microbiology and Antimicrobials*.

Kumar, Sunil, Preeti Chandra, Vikas Bajpai, Awantika Singh, Mukesh Srivastava, D. K. Mishra, and Brijesh Kumar. (2015). "Rapid Qualitative and Quantitative Analysis of Bioactive Compounds from Phyllanthus Amarus Using LC/MS/MS Techniques." *Industrial Crops and Products* 69:143–52.

Kusunoki, Masataka, Kazuhiko Tsutsumi, Daisuke Sato, and Takao Nakamura. (2012). "Lipoprotein Lipase and Obesity." 2012.

Kyohara, Mayu, Jun Shirakawa, Tomoko Okuyama, Yu Togashi, Ryota Inoue, Jinghe Li, Daisuke Miyashita, and Yasuo Terauchi. (2020). "Soluble EGFR, a Hepatokine, and Adipsin, an Adipokine, Are Biomarkers Correlated with Distinct Aspects of Insulin Resistance in Type 2 Diabetes Subjects." *Diabetology & Metabolic Syndrome* 12:83.

Lanaspa, Miguel A., Masanari Kuwabara, Ana Andres-Hernando, Nancy Li, Christina Cicerchi, Thomas Jensen, David J. Orlicky, Carlos A. Roncal-Jimenez, Takuji Ishimoto, Takahiko Nakagawa, Bernardo Rodriguez-Iturbe, Paul S. MacLean, and Richard J. Johnson. (2018). "High Salt Intake Causes Leptin Resistance and Obesity in Mice by Stimulating Endogenous Fructose Production and Metabolism." *Proceedings of the National Academy of Sciences of the United States of America*.

M., Prabhavathi R. and Prasad M. P. (2016). "Studies on Qualitative and Quantitative Phytochemical Analysis of Cissus Quadrangularis." *Pelagia Research Library Advances in Applied Science Research*.

Olorunnisola, Olubukola Sinbad, Peter Ifeoluwa Adegbola, Bamidele Stephen Ajilore, Ayodeji Zabdiel Abijo, Folorunsho Ayodeji Ajayi, and Olumide Samuel Fadahunsi. (2021). "Biochemical and Histological Investigation on the Protective Effect of Poly-Herbal Extract in High Salt Diet-Fed Male Wistar Rats." *Phytomedicine Plus* 1(4):100116.

Omage, Kingsley, Marshall A. Azeke, and Sylvia O. Omage. (2018). "Evaluation of the Efficacy of Acalypha Wilkesiana Leaves in Managing Cardiovascular Disease Risk Factors in Rabbits Exposed to Salt-Loaded Diets." *Clinical Phytoscience* 4(1):1.

Organization World Health. (2012). "WHO | Sodium Intake for Adults and Children." *World Health Organization*.

Philip, A. (2020). "High Sodium Intake - an Overview | ScienceDirect Topics." Retrieved February 15, 2021.

Rakhi Khabiya. (2019). "Spectrophotometric Determination of Total Phenolic Content for Standardization of Various Phyllanthus Species." *Asian Journal of Pharmaceutical and Clinical Research* 297–301.

Redón, Josep, Maria R. Oliva, Carmen Tormos, Vicente Giner, Javier Chaves, Antonio Iradi, and Guillermo T. Sáez. (2003). "Antioxidant Activities and Oxidative Stress Byproducts in Human Hypertension." *Hypertension*.

Rosón, María Inés, Silvana Lorena Della Penna, Gabriel Cao, Susana Gorzalczany, Marcela Pandolfo, Carolina Cerrudo, Belisario E. Fernández, and Jorge E. Toblli. (2011). "High-Sodium Diet Promotes a Profibrogenic Reaction in Normal Rat Kidneys: Effects of Tempol Administration." *Journal of Nephrology*.

Sedlak, Jozef, and Raymond H. Lindsay. (1968). "Estimation of Total, Protein-Bound, and Nonprotein Sulfhydryl Groups in Tissue with Ellman's Reagent." *Analytical Biochemistry*.

Tojo, Akihiro, and Satoshi Kinugasa. (2012). *Mechanisms of Glomerular Albumin Filtration and Tubular Reabsorption*.

Tripathi, Arvind K., Ram K. Verma, Anil K. Gupta, Madan M. Gupta, and Suman P. S. Khanuja. (2006). "Quantitative Determination of Phyllanthin and Hypophyllanthin in Phyllanthus Species by High-Performance Thin Layer Chromatography." *Phytochemical Analysis* 17(6):394–97.

Wong, Marty K. S. (2016). "Angiotensin Converting Enzymes." *Handbook of Hormones* 263-e29D-4.

Y, Hayakawa, Komaki H, Minatoguchi S, Yamada Y, Kanamori H, Nishigaki K, and Minatoguchi S. (2020). "High-Salt Intake Accelerates Functional and Histological Renal Damage Associated with Renal Tissue Overexpression of (pro)Renin Receptors and AT1 Receptors in Spontaneously Hypertensive Rats." *Clinical and Experimental Nephrology* 24(7).

Zuo, Yuegang, Chengjun Wang, Jiping Zhou, Amita Sachdeva, and Vanessa C. Ruelos. (2008). "Simultaneous Determination of Creatinine and Uric Acid in Human Urine by High-Performance Liquid Chromatography." *Analytical Sciences*