Abstract

Background: The home and traditional ways of preparing herbal decoctions have been known for ages through aqueous medium without much certainty or evidences if the most efficacious phenolic bio-molecules of herbal plant vis-a-vis *Phyllanthus amarus* (*Schum and Thonn*) whole plant is being exploited. More so, high salt diet (HSD) intake has been established worldwide, to deleteriously induce hyperlipidemia related hypertensive rubor, and mostly associated with the development of series of renal subjugation and adipocyte proliferation. The present study was to validate the best extraction method among others that would greatly tap the *Phyllanthus amarus* (*Schum and Thonn*) whole plant but its potent phenolic phytoconstituent and also evaluate the *in-vivo* role of its phenolic rich extract (PRE) in the amelioration of high salt meal triggered hyperlipidemia, adiposity, renal dysfunction in animal model, within 8weeks. Thirty-five male Sprague-Dawley rodents weighing between 170-180g were grouped, fed with the chow and treated as follows: Group1: fed with normal rat chow; Group2: HSD; Group3:HSD+75mg/kg/b.wt of PRE; Group4: HSD +100mg/kg/b.wt of PRE; Group5: HSD+150mg/kg/b.wt of PRE. Weights of the experimental rodents were measured, all rats sacrificed after an overnight fasting post the 8^{th} week, blood collected through abdominal incisions, for eventual biochemical, serum and renal histology, and weight of the harvested renal organs recorded.

Results: Results showed that aqueous acetone concentrates exhibited highest 2, 2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging activity more than that of aqueous ethanol and the vehicle (result not shown), and also with the highest content of total phenolics accomplished in the aqueous acetone extract. 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%) and concentration of renal bio-products: urea, uric acid, creatinine, and albumin. It

also established a significant dose dependent recuperating potential in the groups co-treated with PRE when compared with rats on normal chow.

Conclusion: The aqueous acetone solvent was established as the best extraction medium for phenolic concentrate more than that of the traditional ways of decoction and the ethanolic affiliate. More so, the PRE may be applied as imminent therapeutic agent in the management of high salt diet driven pathological derangement vis-à-vis Hyperlipidemia, Adiposity and Renal dysfunction.

Keywords: Hypernatremia; kidney, *Phyllanthus amarus* (*Schum and Thonn*)*;* Phenol rich concentrate; Nephronecrosis; Hyperlipidemia, Acetone.

Background

Despite all health challenges scourging the world, the testimonies and success accounts on the indisputable efficacy of natural remedies among the users has been tremendous, further proving its integration beyond reasonable doubt, into health care delivery management in Nigeria, and developed country like China. The nutritional benefit of balanced diets in the sustainability of homeostasis, and a healthy momentum cannot also be subjugated, most especially in this junks accustomed generation. More precisely, consumption of a high-salt diet (HSD) customised junks and readymade meals has also been reported worldwide, resulting into hypernatremia ("Hypernatremia," 2021) triggering obesity, hyperlipidemia (Oh et al., 2017), and renal dysfunction (Rosón et al., 2011), both in humans and animal models (Azinge et al., 2011). Recently, individuals have also been adapting a transition from a homemade diet to readymade meals available around, which are mostly preserved using high salt condiment. Several studies across the board have also shown that individual who depends on such, ready to eat meals now consume higher salt, perhaps used as flavor or seasonings in the former (Ahn et al., 2013), but deleteriously compromising the metabolic inertia (WHO, 2012). The recommendation of dietary salt (sodium chloride) intake, more importantly in the regulation of related pathologies such as

cardiovascular diseases (Oh et al., 2017), adiposity and tunica intima stiffness had also received much attention over the past years (Chenevard et al, 2006). It has also been established that high salt diet intake is the major cause of oxidative damages, and hepatotoxicity and ultimately increasing the risk of vascular events of hyperlipidemia, (Ezeugwunne et al., 2018), obesity, stroke, and nephrotoxicity (Rodriguez-Iturbe & Johnson, 2010). It has also been accounted to play a critical role in the pathogenesis of fibrosis (Wang et al., 2016), atherosclerosis, and myocardial infarction (Allender et al., 1996). Moreover, oxidative damages on lipidemic layers concomitantly induced by hypernatremia, triggering hyperlipidemia, organs' toxicity and glomerulus deteriorations have also been reported in salt sensitive individuals (Vaziri et al., 2000). Previous studies have also established loss of leptin responsiveness in high salt sensitive animals resulting into obesity, hyperlipidemia (Redón et al., 2003) and subsequent hypertension (Kusunoki et al., 2012). The influences of hypernatremia on the integrity of kidney architecture, functionality (Philip, 2020), infilterating Urea, uric acid, creatinine and albumin (Allender et al., 1996) bio-indicators have also been proclaimed in another research study (Al Disi et al., 2016), which was reported to be mediated through activation of renin-angiotensin's interraction in the kidney's subvention and greatly tantamount to kidney irregularities (Drenjacnevic-Peric et al., 2011).

In remedial inquisitiveness, towards the aforementioned high salt diet related metabolic infringement, supplementation with Nephroprotective bio-active metabolites from crude plant origin has been reported (Tienda-Vázquez et al., 2022), perhaps through the suppression of angiotensinogen to centrally peptide angiotensin I actions (Wong, 2016) and thus provide protection against hypernatremia driven Renal health issues (B. H. Lee et al., 2016). However, phytoconstituents, majorly phenols, terpenes, and alkaloids, are not well exploited by rural and traditional practitioners, but all are documented agents from medicinal plants, that are beneficial as protective therapeutics, against the endemic development of some health diseases (Azinge et al., 2011) e.g depletion on the levels of some renal and lipid related biomarkers such as globulin (Tojo & Kinugasa, 2012) and high density lipoprotein (HDL) respectively (Dullaart et

al., 2008), which cohabitedly resulting into albuminaria (Yadav et al., 2016) and adiposity clearance after therapeutic treatment (Komolafe et al., 2013). Plants in an entity, anabolised phenolic compounds in response to varied treats from other predactors such as pathogens, insects, human interventions and destructive wave radiation within the ecosystem (Hamuel, 2012). Phenols, being the most beneficial secondary metabolites and well established from plant, a functional bearing scavenging hydroxyl group benzene subordinate (Ali Ghasemzadeh, 2011), majorly acting as an reductant agent in disease control and protection in plant against pests, even in animal consuming them as foods has not been extractively exploited but noted to be of immense value than the other classes (Khoddami et al., 2013). The pursuit of these phenolic metabolite's potencies are thus very well germane and they are related to their scaffolding hydrogen donor abilities (Ghasemi et al., 2018). Unfortunately, none of the extraction outlines established so far in traditional/home made aqueous preparation decoction techniques in natural medicine, best suitable for phenolic extraction and such hidden treasures thus needs to be investigated. Hence, the best solvent choice was intensely tested for phenolic quantification in this study.

Normally, the leptin hormone regulating potentiality of the kidney on renin-angiotensin actions, occurring within the human system could rejuvenate and prevent the body homeostasis against a reasonable degree of health instability triggered by high salt infringement, if not overwhelmed. Nonetheless, the exposure of the body inertia to a high salt diet eventually induces hypernatremia ("Hypernatremia," 2021) related pathology (Rosón et al., 2011) with concomitant several metabolic derailments vis-a-vis hyperlipidemia, renalhepatotoxicity, intima peroxidation, and obesity with a high rate of mortality (Dobrian et al., 2003). With this background, the study thus aimed to compare the phenolic concentration in the decoction prepared in traditional way with that of 70% ethanol and 70% acetone in mg/g of gallic acid equivalent, provide an overview of the use of a whole medicinal plant named *Phyllanthus amarus* (Schum and Thonn), targeting the phenolic rich bio-active constituents, its lethal dose, antiobesity, renal recuperating and antihyperlipidemic potential in high salt diet assaulted

animal model. *Phyllanthus amarus* (Schum and Thonn), a medicinal plant belonging to *Euphorbiaceae* (Tang et al., 2014) commonly used in alternative medicine as anti-diarrhea, anti-diabetic (Adeneye et al., 2006), antioxidant, analgesic, anti-inflammatory, antihypertensive (Amaechina & Omogbai, 2007), antimicrobial (C. D. Lee et al., 1996), and hypolipidemic agents (Ezeugwunne et al., 2018) is hereby investigated. The phytochemical analysis was established to reveal 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 (Awasthi et al., 2015), while most of the High-performance liquid chromatography (HPLC) based retention time (RT) and ultraviolet (UV) data analyses of the phenolics in *Phyllanthus amarus,* revealed phyllanthin, hypophyllanthin, lignans (Tripathi et al., 2006). Hydroxybenzoic, hydroxycinnamic acid derivatives, gallic acid, flavonoids, ellagic acid derivatives, and protocatechuic acid, in which fifty two compounds were identified and characterized. (S. Kumar et al., 2015). In the previous study, it was further evident that the quantitative distribution of the phytoconstituents may vary due to geographical locations and hence its efficacy (Matou et al., 2021). Pharmacologically, all these compounds with five most intensified ones (S. Kumar et al., 2017) were quantified and recorded to exhibit metabolic activities such as anti-cancer, hepatoprotective, anti-inflammatory, diuretics, reductants, anti-viral, anti-bacterial, anti-hyperglycemic, and anti-hypercholesterolemic properties (K. B. H. Kumar & Kuttan, 2005).

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

Phyllantus amarus (Schum & Thonn) whole plant was watched with clean water, air-dried at room temperature for 5weeks (Handa et al., 2008) and eventually pulverized using an industrial fine grinding machine (Sulaiman et al., 2011). The final powdered sample kept in an air-tight amber bottle, refrigerated for future extraction.

Plant extraction

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

Phenolic rich extract was prepared by combining the methods of Konaté et al., (2012) and Złotek et al., (2016) with little alteration. The preserved powdered sample of the dried *Phyllantus amarus* (Schum & Thonn) whole plant (300g) was macerated with 1000ml of acetic acid /water/ acetone (2/28/70, v/v/v), for 72hours with subsequent shaking at intervals. The menstruum was later filtered using 150mm filter paper, subsequently rotary evaporated at 40°C under vacuum pressure, and freeze-dried to obtain the dried phenolic rich extract. The rich phenolic was afterward kept in amber bottles and preserved in the refrigerator for further investigation.

PHENOLIC RICH STEPWISE PROCEEDINGS

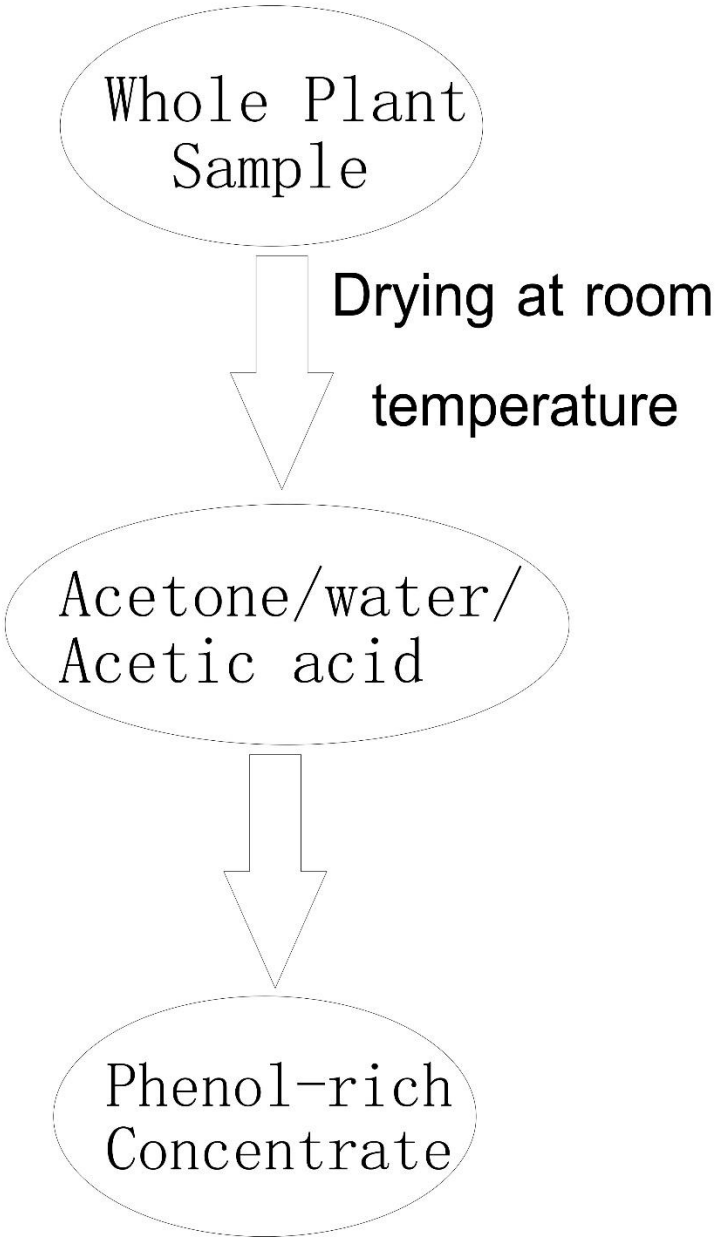

Figure 1: Stepwise diagram for phenolic-rich concentrate.

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 (Funsab Enterprises, Agro and Livestock Raw materials Merchant, Lagos, Nigeria), liberally supplied with water, and acclimatized for 2weeks before the commencement of the co-administration.

Animal grouping

The animals (n=35) were divided into five groups of seven.

Group 1: Rats on 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 plant phenol rich extracts

Standard analytical procedures were employed to evaluate the acute lethal toxicity (LD50) of phenol rich extracts (PRE), using up and down techniques as described by Bruce (1985) and Chinedu et al., (2013) in this study.

Animal Sacrificing

After an overnight fast, post-last administration, the animals were anesthetized with chloroform vapor for about 2minutes, sacrificed through abdominal incision, renal organs harvested and blood collected using 5ml syringes into an Ethylenediaminetetraacetic acid (EDTA) and serum bottles. With the latter

standing for 45minutes, centrifuged at 3000rpm for 15minutes, serum obtained was used for the biochemical analysis (Udinyewe et al, 2017).

Biochemical evaluation

- **Qualitative test for Phenolics**

The phenolic rich extract, 5g of the stock, was added to 2mls of 5% aqueous ferric chloride in a test-tube, shake slightly, the appearance of glittered blue color indicated a very rich phenolic phyto-constituent (M. & Prasad M. P., 2016).

- **Total phenolic content**

Total phenolic concentration in the extracts was determined spectrophotometrically against Gallic acid as standard using Folin-Denis and Folin-Ciocalteu reagents (Dietitian, 2013) and as quantified by (Mohd Zain & Wan Omar, 2018) and (Rakhi Khabiya, 2019) with little modification.

- **Estimation of serum cholesterols' profile**

The following lipid cholesterols were estimated in the serum as proclaimed by Komolafe et al., (2013) analytical procedure using assay kits from Randox Laboratories Ltd, United Kingdom.

i. Total cholesterol (TC)
ii. triglycerides concentrations (mg/dl)
iii. Low density lipoprotein (LDL)
iv. High density lipoprotein cholesterols (HDLc),

- **Assay for total cholesterol**

The total Cholesterol concentration was calculated as follows:

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

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

- **Estimation of triglycerides (TG)**

The concentration of triglyceride was calculated as:

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

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

- **Estimation of low and high density lipoprotein**

The HDL and LDL/VLDL cholesterol quantification kit provides a simple method for convenient separation of HDL and VLDL (very low density lipoprotein) in serum sample, and being easily quantified separately, using cholesterol oxidase mediated reactions.

The reaction was incubated for 60minutes at 37^0C and the absorbance was measured at 570nm in a micro-titre plate reader.

HDLc concentration was calculated as follow:

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

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 Zuo et al., (2008).

- **Serum urea determination**

Urea is enzymatically hydrolysed to ammonia in the presence of Urease and the ammonia then experienced a colour change to blue in the presence of phenol and hypochlorite solution. The absorbance of the blue solution was photometrically measured at 546nm which correspondingly quantified the concentration of urea. A commercial kit bought from LABKIT was used for the assay

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 Zuo et al., (2008).

- **Serum total protein determination**

Total protein was estimated as described by Sedlak and Lindsay, (1968).

- **Serum albumin determination**

The concentration of albumin in the serum was estimated in accordance with the report of Assink et al., (1984) and Busher, (1990).

- **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 plasma has clotted, therefore fibrinogen, a plasma protein, and a 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 (Busher, 1990).

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 kidney of all the rats were also measured.

Histological evaluation of the renal architecture

The renal architecture was evaluated after a fixation in 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 (Olorunnisola et al., 2021).

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 superscript were compared with the control group along the same column and are statistically different.

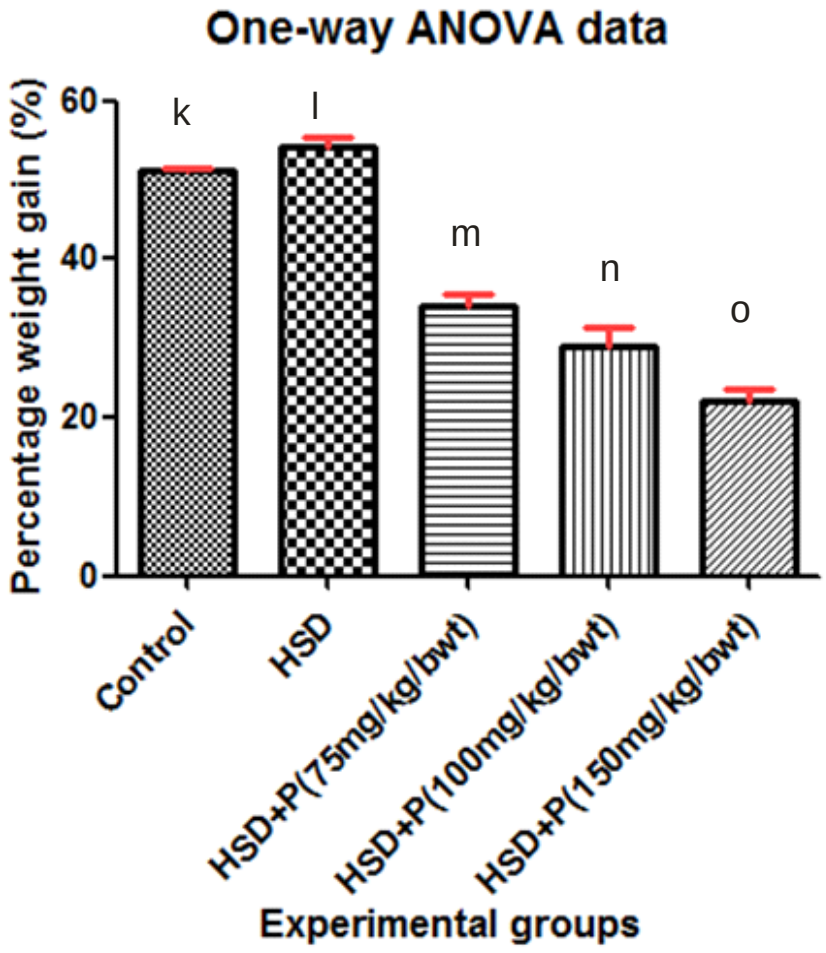

Figure 2: Outcome of phenolic-rich extract of *Phyllanthus* amarus (Schum & Thonn) whole plant on the bodyweight of rats fed with 8% high salt diet

Table 1: Outcome of Phenol rich extract (PRE) of *Phyllantus amarus* 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 *Phyllantus amarus* whole plant on some markers of HSD Induced kidney toxicity.

Group	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)	Total protein (g/dl)	Globulin (g/dl)
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 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 2. The observed weight increase in the rats fed HSD was in agreement with the report of Zhu et al., (2014), who postulated a closed relationship between adiposity and salt intake. Furthermore, the absurd obesity recorded is also in consistent with the report of Lanaspa et al., (2018), which accounted 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 James et al., (2009), 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 which 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, LDL with corresponding ($p< 0.05$) decrease in the level of HDL in the group administered high salt diet only (Group 2) when compared with rodents on normal rat chow after 8weeks (Lanaspa et al., 2018). The result further revealed the ability of PRE, to significantly and dose-dependently recuperated the compromised lipid profile of the treated group to near normal (Ara et al., 2008). This was correlated with dose-dependent and metabolic improvement of HDL level. Worthy of note, is the deleterious advancement of LDL more than other lipid parameters of interest in high salt assaulted group, which is also in consistent with the report of Komolafe et al., (2013).

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, 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 near normal level with corresponding increase in the concentrations of kidney marker, globulin. More importantly, PRE recuperated all compromised renal parameters to near normal level after co-treatment when compared with the group fed with normal chow after 8weeks.

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

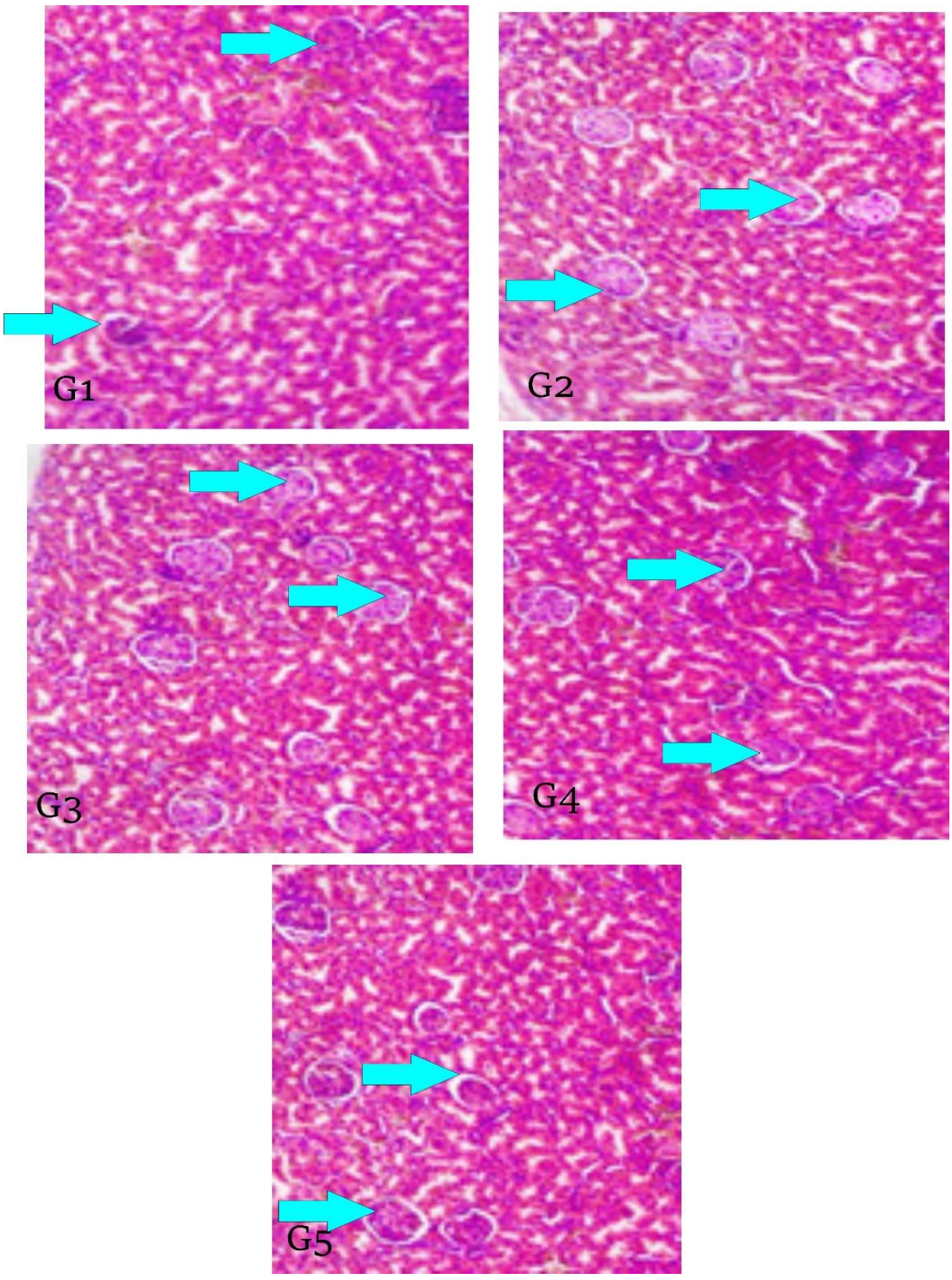

Fig 3: Sectional photomicrograph of kidney subjected to eosin staining procedure. G1- Group 1- Normal group. G2- High salt diet (HSD) fed group. G3- HSD+75mg/kg/bwt of PRE. G4- HSD+100mg/kg/bwt of PRE. G5- HSD+150mg/kg/bwt of PRE

Table 3: Weight of harvested kidney 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 3. 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, renal topology, and the cortical histo-architecture leading to nephrosclerosis. However, groups 3, 4, 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 (BHAT, 2022).

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 *Phyllantus amarus* whole plant using aqueous acetone, revealed a significant phenolic value of 204.78±2.34 mg/g of gallic acid extract (GAE) followed by aqueous ethanol with 165.50±1.78 mg/g and lastly 150.22±2.00 mg/g of gallic acid extract (GAE) by the aqueous vehicle. Interestingly, no such comparism could be found in the literature.

Discussion

In consideration of the pertinent interest to exploit the full potential of *Phyllantus amarus* whole plant, with the pharmacological efficacy of the aqueous extract to include, antihyperglycemic (Adeneye et al., 2006), antihypertensive (Amaechina & Omogbai, 2007), and diuretic (Yao et al., 2018). Previous studies had also reported the importance and efficacy of plant phenolic over all other secondary metabolites (S. Kumar et al., 2014). It was accounted that the phenolic bio-constituents are more potent in plants and also responsible for intent pharmacological remediation and repugnant perception reported in animals consuming such as food (Hamuel, 2012). Hence, a comparative evaluations/quantification of various phenolic concentrates prepared from mostly applied traditional method of plant decoction, aqueous ethanol and acetone were investigated using spectrophotometric method against Gallic acid as standard, and the rich extract's possible anti-hyperlipidemic, anti-adiposity and renal recuperating potentials in

HSD fed rats. Though, Awasthi et al., 2015 phytochemically qualitified the *Phyllantus amarus* plant bioactive metabolites to include phenolic phytoconstituents, alkaloids, saponins, flavonoids and terpenoids, while High-performance liquid chromatography (HPLC) based retention time (RT) and ultraviolet (UV) data analyses of the phenolics from *Phyllanthus amarus,* revealed phyllanthin, hypophyllanthin, lignans (Tripathi et al., 2006), Hydroxybenzoic, hydroxycinnamic acid derivatives, gallic acid, flavonoids, ellagic acid derivatives, and protocatechuic acid, in fifty two compounds identified (S. Kumar et al., 2015). While (Asiwe et al., 2021) and (Catharine, 2016) had also established the deleterious effects of high salt condimented meals on sensitive animals. However, it's very pertinent to verify the best extraction method in the quest to exploit the full potential of all the bio-active phenolic metabolites in *P. amarus* plant and its efficacy.

In this study, the aqueous acetone- concentrate exhibited 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 thus confirmed that, besides the commonly used traditional method of decoction, solvents such as aqueous acetone among others is of better application for extracting beneficial phenolic phytoconstituents (Khoddami et al., 2013) from medicinal plants for pharmacological application.

In addition, eight percent (8%) HSD (by weight) was accounted to induce hypernatremia ("Hypernatremia," 2021) 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 volume per body weight observed in the rats fed with HSD, though agreed with the declaration of (Organization World Health, 2012), on high salt sensitive population and the report of Zhu et al., (2014), could have been triggered by loss of leptin actions, an adipocytokines, (Kusunoki et al., 2012) and adipsin hormones stimulation (Kyohara et al., 2020) amidst an increased appetite (Horvath et al., 2010) with concomitant endogenous fatty molecules anabolism and buid-up (Dobrian et al., 2003), which eventually triggers

obesity (Lanaspa et al., 2018). 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 8week study, consistently presumed to be triggered by the signal induction received from aldosterone, a renal hormone (Fujita 2014). 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 *Phyllantus amarus* whole plant, though partly consistent with the account of James et al., (2009) 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 (Schulze *et al.* 2019). The rich plant concentrates also perhaps significantly actuate the endothelial lipoprotein lipase decomposition of fatty triglycerides to adenosine tri-phosphate (ATP) within the mitochondrion, tunica intima architecture and hence downregulating adiposity, water retention and plasma-renal extracellular volume ratio (Kusunoki *et al.* 2012). This eventually promotes the health countenance and weight reduction in treated groups after co-administsration (Kyohara *et al.* 2020).

The kidneys in 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 (Azinge *et al.* 2011). In this study, HSD severely deranged kidney architecture and its biomarkers leading to nephrosclerosis (figure 3) (Borrelli et al., 2020) as revealed in the histology. This eventually resulted into significant increase in renal-related serum bio-molecules, albumin (Omage *et al.* 2018), nitrogenous urea, uric acid, creatinine and renal weight in rats fed with HSD only when compared with the groups fed with normal chow after 8weeks. The aforementioned HSD assaulted impairment on the kidney integrity eventually resulted also into dose dependent related pathology; hyperlipidemia (Kang et al., 2016), and adiposity (Kang et al., 2017), associated with globulin depletion as previously established by (Tojo & Kinugasa, 2012). The reports of Amarini et al., (2016),

Men et al., (2013) and Fujita, (2014) further accounted 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 (Zhiwei Zhang, 2021), significant elevation in its markers (Drenjacnevic-Peric et al., 2011), and volume gain of the kidney (Duarte & Cooper-Dehoff, 2010) as accounted in high salt diet fed group. However, co-administration of PRE (75, 100 and 150mg/kg/bwt) in another HSD fed groups significantly and dose dependently ameliorated the pathology and perhaps hinder the renin response to near normal level (Drenjacnevic-Peric et al., 2011). 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 with PRE to near normal level as depicted in the renal photomicrograph (Oppelaar & Vogt, 2019). In a larger note, the PRE thus prevents HSD induced renal hyper-activity of proteinous insurgence (Oppelaar & Vogt, 2019), kidney's histopathology (F. J. He & MacGregor, 2004) and its renal sclerosis by counterbalancing the influx of electrochemical sodium/water gradient, being reabsorbed back to the blood lumen (Tojo & Kinugasa, 2012). The attenuation effects potentiated by the phenolic rich concentrates on over-disposition of serum proteinous biomolecules, also resulted into eventual increase in creatinine clearance credibility (Yadav et al., 2016), a good venture for healthiness. The resultant homeostasis accounted by PRE on HSD deranged kidney parenchyma, as recorded in figure 3, its marker, globulin, and weight, also consolidated the remedial potency of the phytochemicals on albuminuria as reported by (Omage et al., 2018) and (Tojo & Kinugasa, 2012), and notably, recuperating agility in the co-treated groups (AA Elagib & M. Nabiela, 2012). 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 (Schulze et al., 2019) and nephrotoxicity (Akomolafe et al., 2014) to near normal level. Furthermore, aldosterone from 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 (Fujita 2014). Withal, the rich extracts potentiated a significant recuperation, which perhaps suppressed ACE, ECE and the conversion of angiotensinogen to centrally peptide angiotensin I, thence, the conversion of inactive angiotensin I to the extremely potent vasoconstrictor, angiotensin 11, by ACE is demobilized (Wong, 2016) in co-treated groups. These potentialities of the phenol rich plant concentrates favor kidney functionality, Endothelial nitric oxide synthase (Philip, 2020), hydrogen sulphide (H_2S) induction (Boegehold, 2013), cardiac inotropism (Kass et al., 1987), with concomitant reduction in ACE affiliated NADPH oxidase (Boegehold 2013), other renal parameters and volume gain (Al Disi et al., 2016), to near normal level (Philip, 2020), and eventually preventing high salt agonist induced nephrosclerosis (Andonova et al., 2022). Though, total protein retains no significant concentration difference, in HSD fed group as accounted by Omage et al., (2018) throughout the 8week study, however, urea, uric acid over elicitation, nephrotoxicity, (Yadav et al., 2016) and albuminaria (Grillo et al., 2019) were dose-dependently attenuated by PRE, as also evidenced in the photomicrograph (figure 3) and could thus be recommended as therapeutic candidate in the treatment of HSD triggered renal injury.

Most bio-lipids synthesized by cholesterol synthase (Khanna et al., 2002) in the smooth and rough endoplasmic reticulum of the hepatocytes, are mostly trafficked through Golgi bodies into the blood stream and are categorized into high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG) and very low density lipoprotein (VLDL) cholesterols (Harsha et al., 2004). 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 HSD sensitive animals (Choi et al., 2020). The significant increase of TG and LDL (Redón et al., 2003) in rats fed with HSD, could have been mediated or triggered by denaturalization of tunica intima and hepatocyte (Grillo et al., 2019), leading

to hyperlipidemia in a counter response, with corresponding actualisation of adiposity and fatty liver (Prieur et al., 2008). The established outrage ($p < 0.05$) in the lipid profile of Total 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, though consistent with the report of Kang et al., (2016), could also have been actuated by the degradation in hepatic lipase functionality, vis-a-vis catabolism of fats (S. He et al., 2022) and dietary triglycerides into extracellular nutrients, hence synthesis and proliferation of adipocytes (*Lipase - an Overview | ScienceDirect Topics*, n.d.). Nonetheless, the observed efficacies of PRE against HSD triggered hyperlipidemia in co-treated groups. though supported by the report of Ezeugwunne et al., (2018), must have ameliorated the hypernatremia related impairment on tunica intima and hepatocyte (Grillo et al., 2019) by building hydrogen, covalent and/or ionic bonds, which is the common morphological manifestations of intracellular ameliorative or radical scavenging mechanism (Wink, 2015) of the hydroxyl functional group of most phenolics (Afanas'ev et al., 1989), hence promoting the rich constituent anti-lipidemic efficacy (Wang et al., 2016). 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 (Schulze et al., 2019), 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 (Khanna et al., 2002), increase lipid to bile acids conversion and excretion, enhance endothelial lipoprotein lipase catabolism of TG to non-esterified fatty acid (NEFA), which consequently promote the availability of Adenosine tri-phosphate (ATP) in the tissues (Kusunoki et al., 2012). These are instrumental and beneficial, essentially in high density lipoprotein (HDL) efficiencies (Dullaart et al., 2008), in rats co-treated with the rich plant concentrates. Though, HSD assaulted animals exhibited higher significant derangement in the level of LDL compared to other lipid parameters of interest, perhaps due to low

reversed trafficking by HDL biomolecule (Elizabeth Connor, n.d.), 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 rich concentrates could also have demobilised phospholipase A2 on the vascular intima, which ought to release fatty molecules and eventual formation of plagues indices (Hurt-Camejo et al., 2001). The other possible mechanisms by which high salt agonist induces hyperlipidemia and hepatorenal related adiposity include; phospholipase enzyme hyperactivity (Hurt-Camejo et al., 2001), increased cardiac expression of β-myosin heavy chain (MHC) (Berger et al., 2018), calcium influx (G. et al., 2015), overinducton of sodium/potassium ATpase homeostatic threshold (Ching Li et al., 1994), inotropism derailment (Kass et al., 1987), atherogenesis on vascular intima, profilerating peroxidation (Komolafe et al., 2013) and eventually endothelial modulations and renal toxicity via renin-angiotensin's modules of operandi, among others (Y et al., 2020). Withal, PRE could have act as therapeutic agent in alleviating all the aforementioned in a concise manner 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 highest phenolic concentration in mg/g of the standard Gallic acid equivalent (GAE) than aqueous ethanol and that of the vehicle extract 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 *Phyllantus amarus* (Schum. &Thonn) whole plant, acting as anti-obesity, anti-hyperlipidemia and renal recuperating agent in HSD assaulted rodents, as accomplished was well noted, and being 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 *Phyllanthus amarus*, which include phyllanthin, hypophyllanthin, lignans (Tripathi et al., 2006), Hydroxybenzoic, hydroxycinnamic acid derivatives, gallic acid, flavonoids, ellagic acid derivatives, and protocatechuic acid, among other fifty two compounds as accounted by (S. Kumar et al., 2015). While the established pharmacological activities by (Khoddami et al., 2013), (Mamza et al., 2012) and (Hamuel, 2012) propounded the phenolic culprits to be 3,5 di-t-butyl phenol, dihydroflavons, anthocyanidins, catechins, phenolic acid, benzoic, cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids in agreement with the former.

In light of all the aforementioned, phenolic phytoconstituents from *P. amarus* is of research interest, which can be exploited using aqueous acetone and thus be therapeutically used as a great complementary alternative agent 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 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 supports, analysed and interpreted the data, JPA evaluated the renal weight, solvent comparism 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: – Stepwise diagram for phenolic concentrate

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

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