International Journal of Medical Science Research and Practice
Vol.9, Issue.2, pp.01-09, August (2022)

Research Paper

E-ISSN: 2347-7520

Terpenoid Rich Concentrate of *Phyllantus amarus (Schum Thonn)* Whole Plant's Potential in High Salt Steered Immunological Storm, Antioxidant and Blood Enzymes Derangement

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Available online at: www.isroset.org

Received: 18/Jun/2022, Accepted: 20/Jul/2022, Online: 31/Aug/2022

Abstract— High salt diet driven immunological storm and blood enzymes dissymmetry are established global health challenges, deleteriously inflicting intracellular organelles and related inflammatory cytokines out of homeostatic threshold. Statistic proclaimed by World Health Organization (2012) also reveals the preponderance of about a quarter of the world's global health linked oxidative damages to high salt-related influences. The aim of the study was to evaluate the serum enzymes' protective effect, antioxidant and immuno-boosting efficacy of qualitatively affirmed terpenoid rich-concentrate (TRC) of *Phyllanthus amarus* **(Schum and Thonn)** whole plant against Dawley rats fed with 8% salty chow (HSD) for 56days. Healthy male rats (n=40) were grouped as follows: Group 1: received only normal rat chow as control group; Group 2: Administered 8% high salt diet (HSD) only; Group 3: co-administered HSD+80mg/kg/bwt., of TRC; Group 4: HSD + 160mg/kg/bwt., of TRC; Group 5: HSD + 200mg/kg/bwt., of TRC. The results ($p<0.05$) indicated that TRC has a lethal dose (LD50) greater than 5000 mg/kg /b.wt, with a significant increase in the levels of modulating cytokines [interleukin 8 (IL-8), interleukin 6 (IL-6), interleukin 2 (IL-2), and Tumor necrosis factor-alpha (TNFα)], serum enzymes (AST, ALP, CK and LDH), consequent significant reduction in enzymatic antioxidant (GSH, Cat, SOD), but with measurable up-regulation of radical product, MDA, in the serum of rats fed HSD. Though, the deranged parameters of interest were dose-dependently remediated to near normal coefficient in the rodents co-administered with the rich concentrates. It's of interest that, the treated groups also expressed signs/physical improvement via; hair integrity, health status, and erection of fur to near normal. The results suggested that TRC contains bioactive compounds which could remediate HSD induced immunological storm, blood enzymes derangement and also complement orthodox medicine in the therapeutic search for oxidative related diseases triggered by high salt meals.

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Keywords— Blood antioxidant enzymes; *Phyllanthus amarus* (Schum and Thonn)*;* Terpenoid rich concentrate; Immunological Cytokines; Aspartate transaminase; and lethal dose.

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I. INTRODUCTION

The preponderance of deleterious complications associated with high salt diet as published by world health organization, is now calling for concern and instant appropriateness from the medics [1] [2]. Pharmacologically, the major chemotherapeutic-drugs used centrally to manage HSD affiliated pathologies, mostly acting as diuretics, vasodilators, endothelin converting enzymes inhibitors, and angiotensin II receptor blockers, are intensively expensive, rarely available to the rural dwellers and accounted to express immediate and/or future toxicological instincts [3], [4], [5]. Though, positive immunomodulating potential of bioactive xenobiotics on inflamed cytokines vis-à-vis; interleukin (IL)-2, IL-6, IL-8 and Tumor necrosis factor-alpha (TNF-α), have been timely established towards various inflammatory diseases. However, the use of beneficial chemotherapy/drugs has become a great concern to the re-occurrence of HSD infiltrated lesion [6] [7]. Consequently, natural phytochemicals from medicinal plants with lesser side effects, and in the pursuance to augment the availability of therapeutic agent to the rural dwellers on high salt diet triggered immunological and enzymatic damages, have also been given alternative and scientific considerations [8]. The biological roles of inflammatory cytokines as the basis to evaluate the immunological wholeness, cytotoxicity and as modulators of T-lymphocyte, neutrophil, and macrophages, are now pertinent in all recent immunological related diseases' therapy, management and study [9] [10]. In addition, non-pharmacological means of managing HSD affiliated inflammations also include lifestyle changes, intake of balanced diet, and/or nutraceuticals with potential efficacy.

Inflammatory cytokines are multiple immunomodulating agents, acting by binding to specific receptors on the cellular surface and thereby inducing changes in growth, development, irritability, and defense activities of the immune cells against pathogens. Incorrect regulation, concisely above threshold has been linked with a high concentration of immunological parameters/cytokines and mostly instigated by exogenous agonists. More so, a significant storming on the immune cell cytokines viz a viz; ; TNF-α, interleukin, and interferon-gamma (IFN-γ) in the interstitium have also been reported in both human and animal studies with high salt-sensitivity [11].

Amongst the most significant causes of the horrible diseases that have claimed the lives of millions of people globally, is the peroxidation of organs' macromolecules by free radicals. The body naturally releases free radicals as a byproduct of chemical reactions and defense during routine cellular functions amidst the conversion of food classes into metabolic nutrients. Simultaneously, antioxidants are potent radical scavengers also designated to remotely control and maintain the hyper-activity of the pro-oxidants [12]. Researchers are now very enthusiastic in determining the antioxidant status of intracellular metabolism and the relapse there-in, with the aim to evaluate salubrious status of the body inertia. [13]. It is evident that imbalances between the duo signify deleterious effects. Considerably, plants also undergo diverse bioactivities to release antioxidant, with enzymatic or non-enzymatic recuperative efficacies. More so, many isolates from the latter have been accounted to remediate oxidative relegation in various higher animals consuming them as foods. This is established by instinct ionic and/or hydrogen bonding by the various classes of well-known plant metabolites [14]. Related studies have also established the magnificient coefficient in the level of radical product Malondialdehyde (MDA) and corresponding downregulation in the activities of both enzymatic and non-enzymatic reductants in high salt sensitive individuals and animal models [15]. Despite this, bio-active plant metabolites have been documented as medicinal importance mitigating all these human pathologies and its co-morbidities triggered by excessive intracellular oxidation.

It's of worthy note that abnormal regulation of serum but hepatic related enzymes in response to exogenous agonists has also received more attention, especially in some very deadly metabolic rubor, compromising lactate dehydrogenase, creatine kinase, Aspartate transaminase, and Alkaline Phosphatase enzymatic threshold [16], [17], [18] [19] [20]. Withal, treating unhealthy cells with phytochemicals enriched meals appeared to counter the pathologies brought on by high salt agonist [21]. Consequently, *Phyllanthus amarus,*, a bitter medicinal plant, belonging to the *Euphorbiaceae* family was therefore investigated for possible efficacy against the deleterious effect of salty meal. It's commonly called 'Stonebreaker', by English speaking domain and has more than 600 species [22]. It is also accounted as a beneficial therapeutic plant commonly used by alternative medicine practitioners handling salt-related diseases in Ogbomosho and environ. The focus is thus to evaluate the In-vivo immunological recuperation of terpenoid rich concentrate of *P. amarus* whole plant in a salty diet triggered serum enzymatic derangement and oxidative damages. This aim will perhaps reduce the high prevalence of salty meal related pathologies as pronounced by [23].

II. RELATED WORK

Iranloye established the shortening window for implantation in a group of pregnant rodents and also recorded abortion in another pregnant group administered aqueous extract of *Phyllantus amarus* . Hence, traditional notion that Phyllanthus amarus can speedily use in sterility is not true [24].

The dose dependent toxicity of *Phyllantus amarus* extract in different rats administered 400 mg/kg, 800 mg/kg and 1,000 mg/kg was investigated. The extract actuated a reduction in the red blood cell (RBC) count, packed cell volume (PCV), haemoglobin concentration (Hb), but with an increase in the white blood cell (WBC). The result eventually revealed no significant toxicological values, though with a reduction in the weight of the experimental rodents, and significant immune boosting efficacy [25].

Aqueous leaves extract of Phyllantus amarus at doses 200, 400 and 600 mg/kg/body weight/day was noted to dose dependently suppress microbial parasites after treatment for four consecutive days in the suppressive tested infected mice more than standard antimalarial drug, Artesunate. [26].

Aqueous extract of *P. amarus* (AEPA) was accounted to ameliorate hypertension, and negative inotropism in salt-induced rat pathology. After 5 weeks of co-treatments, AEPA also significantly reduced Salt triggered high systolic blood pressure, normalised cardiac inotropism and recuperated significantly the deranged septum and tunica intima wall thickness in a dose dependent manner. Such beneficial effects thus seem to normalize the intra-vascular level of oxidative stress [27].

Phyto-chemical analysis of Ethanol extract of P. amarus (EPA, 80%) using a standardised ultra-high pressure liquid chromatography revealed the composition of niranthin, phyllanthin, ellagic acid, hypophyllanthin, phyltetralin, corilagin, isolintetralin, and gallic acid, while the extract also inhibited the anabolism of TNFα and Nitric oxide in lipopolysaccharide (LPS) -actuated microglial cells. Moreover, EPA also demonstrated neuroprotective abilities against LPS-induced microglial cells excitation through the inhibition of TNFα, and NF-κB via microglial activation bio-markers [28]

Administration of *P. amarus* extract (200 and 400 mg/kg/bodyweight) for 28 and 14 days significantly (*P<0.05) ameliorated the compromised memory integrity against induced spatial memory impairment in mice. The

observed protection was believed to be mediated through putative antineuroinflammatory mechanism. In conclusion, *P. amarus* extract exhibited neuroprotective properties against spatial memory impairment triggered by lipopolysaccharide [29].

Flavonoids (astragalin, kaempferol, quercetin, rutin), tannins (gallic acid, geraniin, corilagin, ellagic acid), lignans (phyllanthin, hypophyllanthin, niranthin), and triterpenes (lupeol, ursolic acid) extracted from *P. amarus*, were established to exert various anticancer and anti-inflammatory potencies via perturbation of NF-κB signaling cycles in animal model [30].

Ethanolic extract of *P. amarus* (PAEE) revealed antimicrobial efficacy against Gram-negative strains of bactaria, while Phyllanthin, an isolate, wasn't active against all strains tested. Addition of PAEE to the culture at an inhibitory concentrations was also accounted to inhibit fluoroquinolone-resistant Staphylococcus aureus. [31].

High performance liquid chromatography (HPLC) analysis of *P. amarus* extract revealed four compounds, which were corilagin, hypophyllanthin, phyllanthin, and niranthin, Using molecular docking, hypophyllanthin showed more acceptable histamine 1 receptor (H1R) binding action. Hence, *P. amarus* and its constituent, hypophyllanthin, could be used as antiallergic agent in negating the activation of H1R [32].

Hua established the terpenoid phytoconstituents of Phyllanthus to include Sesquiterpenes, Diterpenes and triterpenoids while further phytochemical analysis revealed the presence of Tannins, Lignans, Flavonoids, Alkaloids and sterols with their pharmacological activities to include cytotoxicity, antioxidant, anti-inflammatory, Hepatoprotective, antimicrobial, antimutagenic, anticancenogenic and antidiabetic [33].

P. amarus plants that is extremely rich in terpenoids e.g linalool, geraniol, citronellol, and monoterpene limonene (or citral) are accounted majorly in aromatherapeutics for the relief of anxiety and depression [34].

.III. Materials and Methods

Plant collection

The *Phyllanthus amarus* whole plant was sourced locally from the traditional healers selling herbs in Ogbomosho metropolis, and those passionate in managing HSD related pathologies. The medicinal plant was validated by Dr. Famuwagun, Department of crop, (FUTA) and Professor Ogunkunle A., the taxonomist, Department of Pure and Applied Biology, Faculty of Basic Medical Science, (LAUTECH), Oyo State, Nigeria. Voucher specimen of the plant tagged 0255 was eventually deposited at FUTA herbal laboratory store for a notable referencing.

Plant pulverisation

The *Phyllantus amarus* whole plant was cleaned and air-dried on a table at room temperature, pulverized using an industrial fine grader. The resulting grinded sample stored in an air-tight amber container and kept in the refrigerator for future use [35].

Fig 1: *Phyllanthus amarus (Schum & Thonn)* plant.

Rich concentrate extraction

- **Terpenoid Rich-concentrate (TRC)**

The fractionalization undergone a stardard procedure with little moderation as described by[36]; [37]; [38]; and [39]. The powdered *Phyllantus amarus* whole plant (700g) was extracted in methanol solvent (2000ml) using a soxhlet device, and the resulting methanolic menstruum dried using rotary apparatus. The eventual methanolic concentrate was again extracted with dichloromethanol organic solvent (800ml) in an automatic soxlet device integrated with suction pump, and immediately rotary evaporated [40]. The final terpenoid-rich concentrate, stored in amber bottle/s, preserved in the refrigerator until further dissolution, administration, and intensive research study.

- **Qualitative confirmation for Rich Terpenoids**

The rich concentrate was confirmed by adding 1.0ml of chloroform and 2ml of the concentrate to a few drops of sulphuric acid in a test tube, the appearance of deeply reddish-brown precipitate confirmed the rich terpenoid sample [41].

Laboratory animals and Ethical approval

Male Sprague-Dawley rats (170-190g) were gotten from the animal unit, LAUTECH, Nigeria. The rodents were fed with commercial salty (8%) and normal rat chow, liberally supplied with water, maintained on a normal 12-hour day/night cycle throughout the experiment. The animal care was guided by the rules, guidelines, and regulations of National Institute of Health (NIH), (1985), and scrutinized by the post graduate ethical committee of the college with approved number, FBMS2019/012.

- **Animal grouping**

The rodents (n=40) were divided equally into five groups, orally treated as follows for the 56day study [42].

Group-I served as normal group, treated with the vehicle (distilled water) and normal chow

Group-II administered a salty diet

Group-III administered salty diet+80mg/kg/bodyweight of terpenoid rich concentrate
G**roup-IV** administered salty diet +160mg/kg/ bodyweight of terpenoid rich concentrate
Group-V administered salty diet +200mg/kg/ bodyweight of terpenoid rich concentrate.

Haematological evaluation of experimental Animals
All rats were sacrificed immediately after midnight fasting post last administration, blood collected, and the serum separated for the immunological assay.

Acute Lethal toxicity evaluation
The acute lethal toxicity (LD50) was quantified, with little modification as described by [43] and [44].

Quantification of immunological signaling cytokines

- **Interleukin-8**

Interleukin-8, a cytokine of importance in inflammation, and triggered by endogenous factors, modulating monocytes/macrophages and many other cell types, such as endothelial cells was quantified as decribed by [45].

- **Interleukin-6 (IL-6)**

This was quantified as stated using automated kits [46].

- **Tumor Necrosis Factor-Alpha (TNF-α)**

The rat TNF-α ELISA procedure was used for the quantitative measurement of TNF-α in the serum according to the manufacturer's instruction.

- **Quantification of interleukin 2**.

The quantification was done using Interleukin 2 (IL-2) Immunoassay kit. Auto-assay protocols are also similar to existing sandwich ELISA guidelines [47].

Quantification of serum anti-oxidant status

- **Malondialdehyde (MDA)**

The serum Malondialdehyde level was estimated as described by [48].

- **Reduced glutathione**

The reduced glutathione (GSH) concentration was calculated using the method proclaimed by [49].

- **Super oxide dismutase (SOD) activity**

This was quantified as pronounced by [50].

- **Catalase (CAT) activity**

The catalase efficiency was accounted as proclaimed by [51].

Quantification of serum enzymes

- **Aspartate transaminase (AST) activity**

The activity of AST was quantified as accounted by [52].

- **Alkaline Phosphatase (ALP) activity**

This was calculated as described by [53].

- **Lactate dehydrogenase *(*LDH*)* activity**

The activity of LDH was estimated using the method of [54].

- **Creatine kinase (CK) activity**

The estimation of the CK activity was quantified as stipulated by Soroida.

Statistical Analysis

The data were litigated amidst one-way analysis of variance (ANOVA) with a significance level adopted at $p < 0.05$ (n=6) and the analysis structured, using Graph Pad Prism, subjected to Mean ± Standard deviation medium.

IV. RESULTS AND DISCUSSION

Table 1: Effect of terpenoid rich concentrate (TRC) of *Phyllantus amarus* whole plant on immunological parameters (ng/ml) of salty chow assaulted rats.

Group	TNF-α	IL8	IL6	IL2
Group1	5.72±1.50	7.50±1.30	2.32±0.20	1.42±0.10
Group2	8.32±3.20*	30.72±3.10**	5.21±2.60*	3.01±2.10*
Group3	8.05±0.04*	27.10±0.05*	4.97±0.03*	2.92±0.03*
Group4	7.45±0.01*	19.72±0.02*	4.02±0.01*	2.22±0.01*
Group5	6.55±0.04	13.94±1.03**	3.82±0.04*	1.99±0.04

The Data with astericks were scrutinized along the control group and are statistically different, using Graph pad prism

Keys
Group 1- Group administered normal chow diet.
Group 2- Salty diet (HSD) fed group
Group 3- Salty chow +80mg/kg/bwt of TRC
Group 4- Salty chow +160mg/kg/bwt of TRC
Group 5- Salty chow +200mg/kg/bwt of TRC
TNFα- Tumor necrosis, Factor-alpha, IL8- interleukin 8, IL2- interleukin 2, IL6- interleukin 6.

Table 2: Effect of terpenoid rich fraction (TRF) of *Phyllantus amarus* whole plant on markers of oxidative stress in salty chow assaulted rats.

Group	SOD U/mg/ protein)	Catalase (U/mg/ protein)	GSH (µmol/l)	MDA (nmol/ml)
Group1	0.05±0.03	56.50 ± 0.56	62.16± 0.11	5.42 ± 0.05
Group2	0.01±0.00	22.13±0.33*	41.25±0.41*	10.81±0.05*
Group3	0.01±0.03	25.22±0.01*	40.15±0.04*	9.38 ± 0.22*
Group4	0.02±0.03	30.31±0.05*	46.21±0.01*	7.92 ± 0.11*
Group5	0.04± 0.02	38.52±0.03*	51.22± 0.03*	6.03 ± 0.18

Table 3: Effect of terpenoid rich fraction (TRF) of *Phyllantus amarus* whole plant on serum enzymes (U/L) of salty chow assaulted rats.

Group	AST	ALP	LDH	CK
Group1	20.55±0.07	46.21±0.32	240.20±4.01	230.40±6.15
Group2	51.50±0.51*	84.32±0.10*	452.40±5.22*	420.20±8.03*
Group3	49.20±1.12*	74.32±0.02*	380.20±0.09*	349.60±0.04*
Group4	39.41±1.10*	70.22±0.12*	309.20±0.08*	320.40±0.02*
Group5	32.21±0.04*	63.42±0.02*	298.30±0.08*	301.30±0.04*

Administration of high salt agonist significantly increased the concentration of immunological cytokines, IL8, IL6, IL2 and TNF-α by 4.1, 2.3, 2.1 and 1.5; serum enzymatic activity, AST, ALP, LDH, and CK by 2.5, 1.8, 1.9 and 1.8;but decreased oxidative integrity, GSH, Catalase, and SOD by 1.5, 2.6 and 5.0 respectively, in rats fed salty diet when compared with the group on normal rat chow. However, the co-administration with TRF, dose dependently recuperated the bio-indicators of interest to near normal charge (Table 1, 2, 3). More so, the corresponding increase in the level of marker product MDA in assaulted group was also established with corresponding dose-dependent amelioration in co-treated rodents to near normal level.

Discussion

Phyllantus amarus whole plant has been used traditionally to treat some series of health challenges such as antimicrobial infections, inotropic derangement, tunica intima degradation, hypoglycemia, NF-κB, TNF-α proliferation, and tumor related pathology [55], [56], [57], [58]. Some beneficial phytochemicals to human have also been reported from the aqueous extract which include proteins, carbohydrates, alkaloids, glycosides, tannins, steroids and terpenoids. The increase in the prevalence of high salt related diseases has also become a worrisome consideration as published by WHO, which now proclaimed, 30% coefficient reduction in average intake of salt/sodium" [59]. The pandemic effect of high salt meal in the society thus constitutes the need and discovery of a therapeutic agent from bio-resources that could remediate high salt diet impaired metabolic derangement.

Precisely, there is no information on the metabolic potency of TRC from the *Phyllantus amarus* whole plant on health issues such as inflammatory cytokines, serum but hepatic related enzymatic and non-enzymatic antioxidants deregulation and eventual immunological deficiencies triggered by HSD. Nonetheless, it was established after 56days, that high salt diet significantly up-modulates the immuonological cytokines in rodents assaulted with salty chow only when compared with the group on normal diet. However, this was rather dose-dependently attenuated to near normal in another co-treated groups. The HSD driven immunological outrageousness might have been prestiged by corrosive tendencies and vascular rarefaction, triggered by high salt on endothelial cells of macrophages and leukocytes [60], [61]. However, the pharmacological potency of TRC to dose-dependently attenuate the endogenous agonist induced inflammations in another co-treated group was noted, perhaps by reduction tendencies via hydrogen bonding and radical coagulation. This was further correlated by [62]. While Ma et al., (2015) proclaimed significant interrelation of salty meal and eventual accumulation of Sodium in the interstitium, with consequent immunological inflammations and hypertension [63], [64], [65]. Hence, terpenoid could be applied as therapeutic agent in clinical variance and the management of compromised immunological modulators (IL8, IL6, IL2 and TNF-α) triggered by HSD. More importantly, IL8 having shown more notable variation than other modulatory cytokines is hereby suggested as the main marker in HSD driven clininal assessment.

HSD exacerbates considerable decrease in the reductant agility of high salt sensitive rodents in response to hypernatremia mediated electrochemical hypertonicity and is estabished to be a key influence in the etio-pathogenesis and assessment of excess radical cascades [66],. The levels of catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) reductants revealed a significant decrease in the group fed with HSD only when compared with the normal group in this study, though with a corresponding increase in the level of radical product malondialdehyde (MDA), signaling bio-macromolecule peroxidation. Despite all, the cascades concisely connotes metabolic unhealthiness, macromolecules denaturing and eventual DNA fracture, as inflicted by the salt agonist [67], [68]. This radical cascade was however dose dependently ameliorated in another groups co-treated with the rich concentrates to near normal levels, as collaborated by [69]. The HSD related infringement on redox activities of intracellular enzymatic and non-enzymatic antioxidants, the serum oxidative coefficients, also revealed free radicals generated by NADPH oxidase, though beneficial to a certain degree in pathogen arrest [70] . These reductants are synthesized from cytosols, cellular organs, and predominantly from hepatic ribosomes but mostly found along the tunica intima, and domiciled in the serum [71], [72], [73]. The agonist driven/perturbed hepatic antioxidants, are immediately released to the blood circulation, via rough endoplasmic reticulum, in tense activity to arrest oxidative stress, which the coefficient are usually applied to quantify antioxidant status [74]**.** Consequently, it is established to prescribe TRF as effective therapeutic agent in the management of HSD-triggered but haem based antioxidant derangement, perhaps inducing recuperative role on cytosols, cellular organs, and hepatic ribosomes, all which are designated to drive metabolic antioxidants, integrity.

In addition, previous studies had established a deleterious clinical variance of high salt on the integral coefficient of serum enzymes especially in salty meal sensitive population and animal model hepatotoxicity study [75]. Significant increase of Haem based but Hepatic related enzymes, AST, ALP, LDH, and CK are widely accounted in various deadly pathologies, sometimes triggered by hypernatremia influences [76], [77]. Hepatic stellate cells are magnanimous deteriorated in such liver fibrosis resulting into million deaths per year and primarily aetiologised by exogenous factors [78] [79]. These pathologies can be attenuated recuperatively in some rodents treated with secondary metabolites from plant origin as accounted by [80] [81]. In a worthy note, high salt diet increases significantly, the exposure of hepatic related enzymes to blood circulation in rats fed with HSD only in comparism with the group on normal rat chow, perhaps signalling Hepatic stellate cells cirrhosis in this study. However, the investigation further demonstrated the efficacy of TRC of the whole plant as therapeutic candidate in ameliorating the immediate pathological account, of notable increase in the activities of LDH, AST, ALP, and CK. Stellate cell deterioration actuated by salty meal agonist either directly or indirectly on damaged fibrotic tissue, epithelial cells, immunological and systemic metabolic dysfunctionality are all established ingredients of salty meal driven haem bases enzymatic derailment [82] . It's perhaps evident that TRF might have also attenuated the hepatic adverse effect and pathological intrusions on stellate and haem cells in a concise manner. This eventually revealed the efficacy of TFR in recuperating haem based enzymes to near normal levels.

Possible mode of actions

In salt-sensitive animals, the accumulation of disposed and hypertonic sodium ion (Na+) in the interstitial tissue and along the lumen, has been accounted to osmotically derail retention of water in isotonic body fluids. This interstitial hypertonic Na+ accumulation, amidst hypernatremia, increased the density and excitation of tonicity-responsive enhancer-binding proteinous molecule (TonEBP) which eventually triggers the upregulation of the gene encoding endothelial growth factor-C, activated by macrophages and hence inflammatory cytokines [83]. This thus evidenced by the significant increase of immunological modulators in this study. However, the induction on the enhancer binding protein (TonEBP) and gene encoding vascular endothelial growth triggered by high salt agonist is hereby inhibited via the TRF mediating potencies perhaps by radical – scavenger disposition [84].

While in another salt-sensitive (hypernatremia) induced angiotensin II (AngII) cardiovascular disorders, significant increase in serum enzymes and endogenous IL-10 level, co-morbidised with hepatic injury, increased arterial transaminases, blood pressure and oxidative stress [9] were continuously recuperated through enhanced nitric oxide (NO) mediated mechanism, and AngII-driven inhibiting responses after various phytochemical treatment [85]. This is also possible and in consistent with the efficacy of TRF, remediating deleterious damages on haem based enzymes, is very well established in co-treated groups. Moreover, sodium intrusion could also enter dendritic cells (DCs) via an amiloride-sensitive tunne, resulting into calcium influx, activating protein kinase, phosphorylation and hepatic demodulation [86]. It further triggers superoxide radicals, endothelial tumor and immunogenic isolevuglandin (IsoLG)-proteinous adducts [87]. Dendritic cells actuated by excess sodium agonist, also produce inflamed serum but hepatic related enzymes with exacerbation of experimental colitis in mice [88]. Nonetheless, TRC homeostatically perhaps coordinated all the aforementioned HSD induced infringements, recuperatively in a coordinated manner, and as accounted in the co-treated group.

LD50 Value

The acute toxicity study showed that the LD50 value of **terpenoid rich extract (TRC) of *Phyllanthus amarus*** whole plant is greater than 5000 mg/kg.

Morphological behavioral and physical assessment

Though, the HSD assaulted groups showed unhealthy signs via, yellowing of fur, stress, erection of fur, loss of hair, and appetite. However, co-administration with TRC significantly improved the morphological weaknesses, restfulness, and damp responses to near normal group.

V. CONCLUSION AND FUTURE SCOPE

Salt being the most important and essential electro-chemical element in the human body, where it utterly maintains and assists in homeostasis was nevertheless established, to be pathological, if gluttonized in various homes. The study further accounted for the presence of terpenoid phytoconstituent as part of the secondary metabolites domiciled in *Phyllantus amarus* plant, with the lethal dose above 5000mg/kg/b.wt. This study further re-established the folkloric rationale of the herbal plant in the management of salty diet-related immunological deficiencies, serum but hepatic related enzymatic derailment and oxidative stress. Hence, the terpenoid rich candidate can be pharmaceutically considered as a good agent in the management of the latter. In a worthy note, the results thus suggested that TRC contains therapeutic bioactive terpenoid metabolites which could remediate HSD induced immunological derangement, oxidative damage and serum but liver related enzymes outrageousness, and also, can be considered as a basis for further investigations on the characterization of the terpenoid bioactive compounds in the whole plant.

Authors' Contributions

OSO supervised all the study and drafting of the manuscript in agreement with AA, while BSA**,** CAO evaluated the data, and assay while TIF drafted the manuscript, harvested the plant sample, sacrificed, designated in rodent administration, and all necessary qualitative evaluations. Conclusively, all authors were intimated and approved the final manuscript before submission

Acknowledgements

The authors express appreciation to the laboratory technologist in charge of Biochemistry, LAUTECH, Ogbomoso, Oyo State, Nigeria, for their support. It is also pertinent to appreciate Dr. Famuwagun and Professor Ogunkunle A., the taxonomists that authenticated the plant and eventually preserved a sample with voucher number 0255 at the FUTA herbarium for future references.

Study Limitations

None.

Funding source

The authors declare no grant/funding from any country whatsoever was received.

Conflict of Interest

We wish to declare that there is no existing conflict of interest in this established claim.

List of Abbreviation

WHO- World Health Organization**;** TRC- Terpenoid rich concentrate; HSD- High salt diet; SD- Standard deviation; IL- Interleukins; TNF- Tumor necrosis factor.

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