GC/MS Analysis and a 30-day Toxicological Evaluation of a Nigerian Immunomodulatory Polyherbal Supplement (PHS)

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KEYWORDS
GC/MS, Herbal toxicity, Histology, Herbal supplement, Phytochemicals

ABSTRACT

Background and Purpose: A Nigerian-formulated polyherbal supplement (PHS) is consumed for its folkloric claims of boosting the immune system. The aim of this study was to toxicologically evaluate a 30-day administration of PHS.

Methods: Gas Chromatography/Mass Spectrometry (GC/MS) analysis was carried out on the ethanol extract of PHS. Thirty-two Wistar rats were randomly assigned to four groups. One group served as control while the other three groups were administered 250, 500, and 1000 mg/kg/day of PHS for 30 consecutive days. The animals were anesthetized, and cardiac puncture was used to obtain whole blood for hematology and serum biochemistry. In vivo, antioxidant assays were also carried out on the liver homogenates. The liver, kidneys, and heart were examined histologically.

Results: The most abundant compounds identified were eucalyptol, alpha-pinene, and phytol. There were no adverse hematological effects. Total cholesterol, LDL-C, and catalase were reduced significantly (P<0.05). At 1000 mg/kg/day, there was a significant increase in creatinine, sodium ion, and glutathione peroxidase concentration. The histological examination of the heart, kidneys, and liver revealed that PHS did not cause any major tissue lesions at 250 and 500 mg/kg/day. Tissue lesions and pathologies were obvious at 1000 mg/kg/day.

Conclusion: The polyherbal supplement seems to be relatively safe if used at doses up to 250 mg/kg/day. Higher doses may cause renal and hepatic lesions.

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INTRODUCTION

The status of the human immune system remains the bedrock for preventing and combating various infections, particularly viral infections, including COVID-19 (Jayawardena et al., 2020; Schultz and Aschenbrenner, 2021). In this instance, the COVID-19 pandemic, which the global health agencies still believe, is far from over (Lancet, 2023) still poses a huge public health challenge. This is largely due to limited access to FDA-approved therapeutic intervention in a resource-limited setting (Flip et al., 2022). Thus, a competent immune system is vital for the reduction of morbidity and mortality (Florindo et al., 2020; Sarkar et al., 2020). The host’s innate immune response to SARS-CoV-2 infection plays a major role in the reduction of morbidity and mortality. The clinical manifestations, disease pathogenesis, and clinical outcomes are also downstream consequences of immunocompetence or otherwise (Rovito et al., 2022; Shi et al., 2020). According to Hall et al. (2020), there are three basic phases of COVID-19 infections, namely: incubation period (generally asymptomatic), disease onset with respiratory symptoms, and severe disease phase. In the same vein, the host immune response in COVID-19 cases is classified into an early innate immune response (antiviral defense) phase localized in the lungs; an intermediate local/systemic transition immune response phase; followed by uncontrolled inflammatory responses and successive symptoms of cytokine storm (Calder, 2020; Mohammed et al., 2022). The overall functionality of the immune system is governed essentially by vitamins and micro-elements consumed in food plants (Thirumdas et al., 2021). Studies have shown that foodplant supplements have shown a positive impact on enhancing immunity against viral infections (Ekor, 2014; Mrityunjaya et al., 2020).

Phytotherapeutics, medicinal plants, or nutritional supplements make up an integral part of our existence (Pan et al., 2014; Kenny et al., 2022). Exploration of plants or their constituents for consumption either as medicines or food, dates to the very history of man. Scientific findings suggest that a higher percentage of Africans and Asians hinge on natural product medicines as the mainstay of therapy and not just as an alternative (Atanasov et al., 2015; Ahmad et al., 2021). This is attributed to their availability, affordability, and accessibility. Several frameworks for using nutraceuticals/phytotherapeutics as sources of therapeutic agents are being identified, profiled, and updated scientifically (Erb and Kliebenstein, 2020). Plants produce several secondary metabolites, forming complex compounds that may be harmful or beneficial to humans (Dwyer et al., 2018). To create a control system, most developed nations impose certain levels of regulations, which are reliable strategies for the monitoring of safety and standardization of these products, while providing quality assurance for any of such natural supplements (Dias et al., 2012; Hassen et al., 2022). To ensure safety, the scientific community has birthed three concepts. Firstly, there must be a study to show the safety profiles of any compound/product that is claimed to be beneficial to a living organism. The second is to assess the chemical constituents of the traditional medicinal agent and the third is to set the guidelines to investigate the proposed folkloric application which is a step toward drug development and discovery (Kiliś-Pstrusińska and Wiela-Hojeńska, 2021).

Over the last few decades, there has been an increased awareness of the safety profile of herb-based therapies such as phytotherapeutics, nutraceuticals, and polyherbal products (Kahraman et al., 2020). The untoward, erroneous, and unverifiable claim that plant-based herbs and nutraceuticals are always safe has led to several limitations to their use, especially in developed nations (Krzyszonoś and Piwowar-Sulej, 2022). One of the most difficult adverse events recently documented stems from intoxications associated with drug-herbal medicines interactions (Pan et al., 2014; Chang et al., 2021). There have been several reports of hepatotoxicity, nephrotoxicity, and cardiotoxicity consequent upon the use of herbal medications and formulas. In addition to the paucity of dosage information, misuse of herbal concoctions, and poor pharmacovigilance surveys for medicinal products, their consumption continues to pose a major public health challenge (Thakkar et al., 2020). In low- and medium-income countries, the use of herbal supplements therapeutically and prophylactically is still very trendy (Nyakudya, 2020). Drug-drug interactions can occur between herbal and conventional medicines via pharmacokinetic principles (Ziemman et al., 2019) e.g., drug absorption and metabolism; or by pharmacodynamics, through conflicting mechanisms of action, thus leading to a myriad of unwanted effects that could be life-threatening (Zhou et al., 2021).

This study explored the first and second scientific concepts required for the use of herbal supplements, which are the safety assessment of plant-based supplements, and the identification of bioactive metabolites present in the supplement.

MATERIALS AND METHODS

Chemicals and Solvents

Pentobarbital (anesthetic agent) and absolute ethanol were obtained from Sigma Alrich, USA; distilled water and Whatman filter paper were purchased from SUNAF Scientific Store (Ilorin, Nigeria).

Preparation of Polyherbal Supplement (PHS)

PHS, a natural product supplement containing: Garcinia kola (bitter kola) (5%w/w), and Zingiber officinale (ginger) (5%w/w); medicinal plants: leaves of Moringa oleifera (80%w/w), Ocimum gratissimum (5%w/w),
Vernonia amygdalina (5%/w/w), were compounded by Biofuel® and Natural Product Herbal Supplement; NAPHERBS, Ilorin, Nigeria. PHS (500 g) was macerated in 2.5 L of 50% ethanol for 72 h and then filtered using Whatman filter paper 1. The filtrate was concentrated using a rotary evaporator at 40°C (BUCHI Rotavapor® Model R-215, Switzerland) with the vacuum Model V-801 EasyVac® Switzerland. The concentrate was weighed and tagged PHS extract. A percentage yield of 14.01%/w/w was calculated using the formula below.

\[
\text{Percentage Yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of PHS powder (g)}} \times 100
\]

Experimental Animals
Wistar albino rats of both sexes (180-220 g) were obtained from McTenny Farms, a commercial private colony near Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, Nigeria, and housed under natural light and temperature conditions within the Laboratory Animal House facility of the College of Health Sciences, University of Ilorin, Nigeria. Rat pellets (Growers’ Mash, Ogo Oluwa, Ilorin, Kwara State, Nigeria) and water were made available ad libitum, and the animals were acclimatized for one week. Procedures involving animal handling followed the guidelines published by the National Institute of Health (NIH, 1996) and the University of Ilorin Ethical Review Committee (UIERC) with approval number: UERC/ASN/2020/2034.

Methods

Acute Toxicity Evaluation
Oral acute toxicity was evaluated according to a modified Lorke (1983) method. A single oral graded range of doses in phase 1 (n=3) with 10, 100, and 1000 mg/kg and phase 2 (n=1) with 1600, 2900, and 5400 mg/kg were administered to Wistar albino rats. During a 24-hour period, the animals were observed for signs of toxicity such as death, writhing, grooming, paw licking, diarrhea, and sedation.

A 30-day Toxicological Evaluation
Rats (32) of both sexes were randomly assigned to four groups, one as control and the others for doses of 250, 500, and 1000 mg/kg of PHS respectively. PHS extract and vehicle (distilled water, 600 μL for the control), were administered daily for 30 days using an orogastric tube.

Hematological Assays
At the end of 30 days of PHS administration, the rats were anaesthetized by an intraperitoneal administration of 30 mg/kg pentobarbital and blood was drawn by cardiac puncture. A portion of the blood sample was centrifuged for 20 min at 3000 g, and the serum was harvested and frozen for serum biochemical assay. The remaining portion of blood was put into a heparinized tube for hematological assay. A comprehensive hematological assessment was carried out to access the white blood cell count (WBC), red blood cell count (RBC), granulocyte (GRAN) and lymphocyte (LYM) percentages, etc., using an automated hematology analyzer from HORIBA Pentra™ XL 80, USA (Kale et al., 2019).

Serum Biochemical Assays
Serum electrolyte levels were evaluated using instruments from TECO Diagnostics (CA, USA). Renal function determinants such as serum creatinine and urea levels were evaluated using kits supplied by Spinreact™ autoanalyzer (Girona, Spain). Liver function enzymes including alanine transaminase (ALT), aspartate transferase (AST), and bilirubin levels were evaluated using the Beckman Coulter LH 780 analyzer. Lipid profile [total cholesterol (TC), triglycerides (Trig), high-density lipoprotein (HDLC-c), and low-density lipoprotein (LDL-c)] were analyzed using commercial kits obtained from Randox Laboratories Ltd (Crumlin, UK), with strict adherence to the manufacturer’s protocol.

Antioxidant Assays and MDA Levels
Superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) activities were carried out on the liver homogenate post-treatment protocol. Analytical kits obtained from MyBiosource Inc. (San Diego, USA) were used for the assay. Briefly, the kit contained the buffer, substrate (making up the substrate solution), and enzyme solution with enzyme diluent (the enzyme cocktail). The instrumentation control received 20 μL of double distilled water and 20 μL of enzyme working solution. Blank was composed of 20 μL of double distilled water and 20 μL of enzyme diluent while the sample was composed of 20 μL of sample and 20 μL of enzyme working solution. Substrate solution (200 μL) was added into each well with a multi-channel pipettor and mixed properly. The 96-well plate was incubated at 37°C for 20 min. The optical density (OD) of each well was measured at 450 nm with a microplate reader (Molecular Devices, CA, USA) (https://www.mybiosource.com/assay-kits/superoxide-dismutasesod/2540402; https://www.mybiosource.com/general-assay, kits/catalase/8243260; https://www.mybiosource.com/assay-kits/reduced-glutathione-gsh/2540433) Glutathione peroxidase and malonaldehyde (a marker of lipid peroxidation) levels were estimated using analytical kits obtained from MyBiosource Inc. (San Diego, USA) and Elab Sciences (Texas, USA) respectively, and following the manufacturer’s protocol. Briefly, tissue samples were rinsed in cold phosphate-buffered saline to remove residual blood and homogenized with 1 mL/0.1 g in assay buffer. The tissue and assay buffer mix were centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was used for the assay. Assay buffer, substrate, and glutathione reductase were premixed on ice according
to the manufacturer’s protocol. Twenty microliters of distilled water or supernatant were added to each well in experimental replicates. Then 160 µL of pre-warmed premixed reagent was added to each well, followed by 20 µL of hydrogen peroxide, and mixed quickly. The absorbance values were measured at 340 nm for 10 s and 190 s, representing A1 and A2 respectively (https://www.mybiosource.com/assay-kits/glutathione-peroxidase-gsh-px/9718985). For the MDA level, three major reagents containing clarificant, acid reagent (thiobarbituric acid) and a chromogenic agent were added successively to each experimental group in triplicate (including standard and experimental controls) according to the manufacturer’s protocol, and absorbance was read at 532 nm with a microplate reader (https://file.elabscience.com/Manual/biochemical_kits/E-BC-K025-M-Elabscience.pdf).

Histological Examination of the Liver, Kidneys, and Heart
Heart, liver, and kidney were fixed in 10% neutral buffered formalin as described earlier (Ojuade et al., 2021). Briefly, tissues were dehydrated by successively passing them through a gradient of mixtures of ethanol and water in an automatic tissue processor. They were dehydrated in ethanol at ascending concentrations of 70%, 80%, 90%, and 100%, and cleared in xylene before embedding in paraffin. Sections of 5 µm of tissues from different organs were dewaxed and stained with hematoxylin-eosin (H&E), mounted on charged microscope slides (Asala et al., 2021) for histopathological examination and under a low and high-powered field of Carl Zeiss binocular microscope (New York Microscope Company, NY, USA). IC-3 mounted camera was used for photographing the microscopic lesion as described by Akanbi et al. (2021).

Statistical Analysis
The statistical analysis used was the one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test (GraphPad prism IX software, Boston, USA). Significance was considered at \( P < 0.05 \). All results are expressed as the mean ± standard error of the mean.

RESULTS
Phytochemical Fingerprinting of Compounds in PHS
A total of about 109 compounds were obtained from the GC/MS analysis, consisting of various classes such as flavonoids, terpenoids and saponins. The most abundant compounds present include hexane-dioic acid, tetradecanoic acid, eucalyptol, alpha-pinene, phytol (Table 1) and the GC/MS spectra (Figure 1) shows the various peaks obtained by each of the constituents.

Acute Toxicity
No mortality was observed up to 5400 mg/kg dose of PHS. There were no observable signs of toxicity in phase 1 in both sexes of rat. There was no mortality in phase 2 of the test except for mild signs of toxicity such as transient writing, and piloerection.

Table 1: Phytochemicals identified in ethanol extract of PHS using GC-MS.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Peak No.</th>
<th>Area (%)</th>
<th>RT (minutes)</th>
<th>LIB ID</th>
<th>CAS</th>
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<tbody>
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<td>1</td>
<td>66</td>
<td>20.96</td>
<td>18.1</td>
<td>Hexanedioic acid</td>
<td>000103-23-1</td>
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<tr>
<td>2</td>
<td>48</td>
<td>5.71</td>
<td>14.0</td>
<td>Tetradecanoic acid</td>
<td>000544-63-8</td>
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<tr>
<td>3</td>
<td>9</td>
<td>5.38</td>
<td>5.9</td>
<td>Eucalyptol</td>
<td>000470-82-6</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>4.73</td>
<td>5.8</td>
<td>Benzene, 1,2,4,5-tetramethyl- o-Cymene</td>
<td>000095-93-2</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>4.54</td>
<td>4.4</td>
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<td>000080-56-8</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>4.45</td>
<td>6.2</td>
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<td>000099-85-4</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>4.37</td>
<td>18.5</td>
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<td>001330-86-5</td>
</tr>
<tr>
<td>8</td>
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<td>Phytol</td>
<td>000150-86-7</td>
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<tr>
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<td>3.28</td>
<td>14.4</td>
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<tr>
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<td>2.40</td>
<td>17.3</td>
<td>Octadecanoic acid</td>
<td>000057-11-4</td>
</tr>
</tbody>
</table>
Figure 1: GC/MS spectra obtained from ethanol extract of PHS.

**Effects of a 30-day PHS Administration**

**Hematological parameters**
After administering doses of PHS daily for 30 days, there were no statistical differences in the hematological indices such as WBC, LYM (%), GRAN (%), RBC, HCT and HGB (Figures 2A-D, 3A and 3B).

**Serum lipid profile**
At the dose of 500 mg/kg/day, there were significant ($P<0.05$) reductions in the level of total cholesterol and LDL-c (Figures 4A and 4C). High-density lipoprotein (HDL-c) and triglyceride levels were not significantly affected.

**Kidney function and serum electrolytes**
At the highest dose of 1000 mg/kg/day, the creatinine level was significantly ($P<0.05$) increased (Figure 5A), and this trend was also observed in the sodium ion level ($P<0.01$) (Figure 5D). The levels of urea and potassium ions were not altered across the groups (Figures 4B and 5C).

**Antioxidant parameters and MDA level**
There was a significant ($P<0.05$) reduction in the catalase enzyme concentration across the groups (Figure 6D). Glutathione peroxidase level was also significantly ($P<0.05$) reduced in the 1000 mg/kg/day treatment group (Figure 6C). Superoxide dismutase and reduced glutathione levels were not affected by PHS treatment (Figures 6A and 6B). The product of lipid peroxidation, malonaldehyde, was not affected by PHS treatment across the groups (Figure 6B).

**Liver function**
Across the groups, ALT, AST, and bilirubin levels were not altered by the 30-day PHS treatment.

**Histological findings**
The control group showed no visible lesion with hematoxylin and eosin stains for all the organs (Figures 9A, 10A and 11A). Also, the heart, liver and kidney from rats administered 250 mg/kg/day of PHS did not show any visible lesion (Figures 9B, 10B and 11B). At 500 mg/kg/day, the heart showed moderate cardiomyocyte degeneration (x400 magnification; Figure 9C). The liver in this treatment group (500 mg/kg/day) had multifocal areas of necrosis (x100 magnification), while the liver revealed hepatocyte degeneration and necrosis with accumulation of engorged Kupffer cells at the same magnification (Figure 10C). For the experimental group that received 500 mg/kg/day of PHS, the kidney showed diffused tubular degeneration and necrosis with interstitial cellular infiltration by macrophage and lymphoplasmacytoid cells (Figure 11C). At 1000 mg/kg/day, there was diffused coronary vascular congestion and cardiomyocyte necrosis observed in the heart (Figure 9D), while the liver showed diffuse coagulation necrosis (Figure 10D) and there was renal tubular degeneration in the kidneys (Figure 11D).
Figure 2: (A-D) Effect of PHS on white blood cells (WBC), percentage of lymphocytes (LYM) and granulocytes, red blood cell (RBC) count. Differences are not statistically significant when compared with the control; n=6.

Figure 3: (A, B) Effect of PHS on percentage of hematocrit (HCT) and hemoglobin concentration (HGB). Differences are not statistically significant when compared with the control; n=6.
Figure 4: Effect of a 30-day treatment with PHS on total cholesterol (T-Chol), low density lipoproteins cholesterol (LDL-C), high density lipoprotein (HDL-C) and triglycerides (Trig). *P<0.05 compared with control, n=6.

Figure 5: Effects of daily administration of PHS for 30 days on serum creatinine, urea, and electrolytes. *P<0.05, **P< 0.01 versus control, n=6.
Figure 6: Effect of the administration of PHS for 30 days on reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). *$P<0.05$; **$P<0.01$ versus control; $n=6$.

Figure 7: Effect of a 30-day treatment with PHS on malondialdehyde levels in rat sera. Values are not significantly different from control, $n=6$. 
Figure 8: Effect of a 30-day treatment with PHS on alanine transaminase (ALT), aspartate amino transaminase (AST), total and direct bilirubin concentrations in rat sera. Values are not significantly different from control, n=6.

Figure 9: Representative photomicrographs of hearts of rats treated with daily doses of PHS for 30 days. No visible lesions in hearts from groups A (control) and B (250 mg/kg) at x100. For group C (500 mg/kg) there were visible cardiomyocyte degenerations (stars) at x400. In group D (1000 mg/kg), there were diffuse coronary vascular congestion (arrows) and cardiomyocyte necrosis (stars) at x400. Staining was with H&E.
Figure 10: Representative photomicrographs of livers of rats treated with daily doses of PHS for 30 days. No visible lesions in livers from groups A (control) and B (250 mg/kg) at x100. For group C (500 mg/kg) there was hepatocyte degeneration and necrosis and accumulation of engorged Kupffer cells (arrows) x400. In group D (1000 mg/kg), there was diffuse coagulation necrosis (stars) x400. Staining was with H&E.

Figure 11: Representative photomicrographs of kidneys of rats treated with daily doses of PHS for 30 days. No visible lesions in kidneys from groups A (control) and B (250 mg/kg) at x100. For group C (500 mg/kg) there was severe diffuse tubular degeneration and necrosis (stars) with interstitial cellular infiltration by macrophage and lymphoplasmacytic cells (arrows) x400. In group D (1000 mg/kg), there was tubular degeneration and necrosis (stars). Staining was with H&E.
DISCUSSION

GC/MS profiling of the secondary metabolites present in PHS gives information of the phytomedicines responsible for its acclaimed immunomodulatory properties. Ranking high amongst the abundant phytochemicals were eucalyptol, alpha-pinene and gamma-terpinene. Eucalyptus oil which contains eucalyptol, has been reported to possess antiviral, antibacterial, and anti-inflammatory activities (Bravo et al., 2021). Eucalyptol has been used in combination with alpha-pinene for the common cold and other viral infections (Bravo et al., 2021). The abundance of these metabolites could be responsible for the purported folkloric immune-boosting claims of the supplement. Although, there are no scientific validations for these assertions. A molecular docking experiment has identified eucalyptol as an inhibitor of the main viral proteinase (Mpro/3CLpro) which is regarded as a suitable target for drug development against SARS infection due to its crucial role in polyproteins, which is essential for coronavirus replication (Strub et al., 2022). There has been a report on the anti-inflammatory benefits of terpenes, particularly, gamma-terpinene, which could reduce inflammatory symptoms by inhibiting the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-1 and nuclear transcription factor-kappa B (Prado-Audelo et al., 2021). As previously highlighted, knowing the constituents of a polyherbal formula could help delineate the mechanisms involved in its activity and provide a basis for its approval for human consumption.

In this study, the oral median lethal dose (LD₅₀) of PHS was greater than 5000 mg/kg. Judging from this acute toxicity test, the supplement could be considered safe and practically non-toxic (Lorke 1983; Erhirhie et al., 2018). In the 30-day toxicity evaluation, PHS did not alter the WBC counts, WBC differentials (lymphocytes, granulocytes), and RBC across the groups although there was a dose-dependent increase in WBC. Studies have shown that a reduction in WBC could predispose the animal to myriads of infections (Kanu et al., 2016; Femi-Oloye et al., 2020; Raeeszadeh et al., 2022). An increase in WBC and the differentials could mean an active response to infections or stress, while a decrease may suggest a chronic state of infection (Talargia et al., 2021).

Serum lipid profile is an indicator of cardiovascular wellbeing and particularly the risk of developing atherosclerosis and possibly other cardiac-related pathologies (Linton et al., 2019). Our findings revealed that PHS significantly reduced total cholesterol and LDL-c levels, particularly at the 500 mg/kg dose. Other parameters such as HDL-c and triglycerides were not significantly affected. High serum LDL-c and total cholesterol levels have been associated with increased cardiovascular mortality, but high HDL-c level is cardioprotective (Jung et al., 2022). This suggests that PHS could be beneficial in reducing the risk of atherosclerosis and other cardiovascular diseases.

At the highest dose of 1000 mg/kg, PHS significantly increased serum creatinine and Na⁺ levels. Increased creatinine level has been established to be an indicator of chronic kidney disease (CKD) (Mullens et al., 2020). Creatinine is a metabolic byproduct in the serum, generated by the metabolism of muscle cells. Usually, normal-functioning kidneys mop up creatinine out of the blood and excrete it from the system via the urine (Imo et al., 2019). When the kidneys are malfunctioning, the level of creatinine increases because of its build-up in serum. If the glomerular filtration rate (GFR) reduces, serum creatinine rises. In a clinical setting, serum creatinine level is an indirect marker of both GFR and overall kidney functions (Kellum et al., 2021). Sodium ion is an osmotically active cation and a main electrolyte in the extracellular fluid. It is responsible for the maintenance of the extracellular fluid volume and regulating the membrane potential of cells. The significant increase in the serum concentration of Na⁺ seen in rats administered the highest dose of PHS, could be the result of Na⁺ retention due to insufficient renal perfusion or intrinsic renal disease (Ellison, 2017). The present study suggests that chronic administration of high doses of PHS could be nephrotoxic.

Oxidative stress results from the inability of the body’s antioxidants to mop up free radicals produced by metabolic processes (Sharifi-Rad et al., 2022). It is an underlying mechanism for several disease conditions including cardiovascular, neurological, and metabolic disorders. Our findings showed a marked reduction in catalase (CAT) and glutathione peroxidase (GPx) concentration. Catalase is a crucial antioxidant enzyme that catalyzes the conversion of hydrogen peroxide to oxygen and water; thus, quenching the cytotoxicity of peroxyl radicals. GPx also plays a crucial role in conjunction with catalase in mopping up the reactive oxygen species (ROS) such as peroxyl radicals (Juan et al., 2022). This decrease in antioxidant protection by catalase and GPx is suggestive of a dysfunctional antioxidant system and could be a contributing factor in the development of diseases. Other antioxidant enzymes assayed such as, superoxide dismutase and reduced glutathione were not significantly affected by PHS treatment. Malondialdehyde (MDA), a product of lipid peroxidation was also not altered by PHS treatment.

The evaluation of liver and kidney functions is fundamental, as these vital organs have various mechanisms for removing toxic substances (Robinson et al., 2016). Although the liver and kidney are remarkable in removing toxins, the organs can become damaged during the detoxification process. Therefore, several biochemical parameters are used in the assessment of hepatic dysfunction and liver cell damage (Robinson et al., 2016).
Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are liver enzymes which can be used to access alterations in hepatic functions. Depending on the level of increase, their level can help in differential diagnosis. Increase in ALT, AST, ALP, and bilirubin denote hepatocellular disease. An elevation in ALP and bilirubin suggests cholestatic disease (Lala et al., 2023). Based on our findings, PHS did not have any significant effect on all the liver function indices assayed for.

The histological examination of the heart, liver and kidney is considered vital for showing organ/tissue damage consequent upon the use of toxic substances (Ibrahim et al., 2018). Our study revealed that there was no significant lesion at 250 mg/kg PHS treatment in the organs. However, the heart tissue showed some cardiomyocyte degeneration that worsened in the highest dose. There was also hepatic tissue degeneration and a more pronounced diffused coagulation necrosis at the highest dose. The kidney presented severe diffuse tubular degeneration and necrosis with interstitial cellular infiltration, culminating in tubular degeneration at the highest dose. This further corroborates the biochemical findings on the effect of PHS on kidney functions at the highest dose. Corroborative information from both histological and biochemical findings is crucial to understanding the safety profile of therapeutic and other herbal formulas (Pognan et al., 2023).

CONCLUSION

The findings show that PHS used at doses higher than 250 mg/kg for 30 days could impinge on kidney function and reduce antioxidant capacity. Therefore, caution must be employed in its use. Further research into more specific toxicities such as neurotoxicity, genotoxicity, and reproductive toxicity will establish a more detailed safety profile.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR’S CONTRIBUTION

All authors contributed to the development of the manuscript and approved the final draft. OO, SI, OBA, AN, MKB, MO, and SOA conceptualized and supervised the study. OO and SOA analyzed the data. SOA, OAA, JO, and AOA conducted the experiments. OBA conducted the histological examination and interpreted the micrographs.

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