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Phytochemical composition, acute and subacute toxicity profile of *Persea americana* seed oil in albino Wistar rats

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ABSTRACT

Objective: This study investigated the phytochemical composition and toxicity profile of Persea americana seed oil (PASO) in albino Wistar rats.
Methods: Chromatography-mass spectrometry (GC-MS) was used to analyse the chemical constituents of PASO. For the acute toxicity test, PASO was administered orally in a single dose of up to 3000 mg/kg body weight (bw). For the subacute toxicity test, the rats were divided into four (4) groups. Group I (normal control), while groups II, III and IV received 200, 300 and 400 mg/kg PASO daily, respectively, for 14 days.
Results: In the acute toxicity test, the lethal dose (LD_{50}) of PASO was estimated to be 1477.83 mg/kg. In the subacute toxicity test, PASO significantly increased ($p < 0.05$) aspartate aminotransferase, creatine phosphokinase, alanine aminotransferase, creatinine, alkaline phosphatase, urea, malondialdehyde, high density lipoprotein, interleukin 1-beta (IL-1β), tumour necrosis factor (TNF-α) and cardiac troponin and significantly decreased glutathione, red blood cells (RBC), packed cell volume (PCV), superoxide dismutase and catalase compared to the control group.
Conclusion: Our study showed that the LD_{50} of PASO is 1477.83 mg/kg body weight, which classifies it as a moderately toxic substance. In subacute toxicity, our results revealed that treatment with PASO resulted in an increase in liver enzymes, urea and creatinine, and inflammatory markers, and a decrease in antioxidant enzymes, suggesting that PASO impairs liver and kidney functions and may cause cardiac or muscle damage in albino Wistar rats.

1. Introduction

In the last two decades, research on functional foods, cosmetics and innovative pharmaceutical products derived from plants has increased significantly [1-3]. Currently, numerous plants and their derivatives have demonstrated their therapeutic efficacy in the control, prevention and treatment of various diseases [4-6]. They are also used in the production of functional foods and for the formulation of pharmaceutical and cosmetic products [7]. Recently, the search for oils extracted from indigenous, underutilized plants for use in the cosmetic, pharmaceutical and food industries has greatly increased [7], as the cost of importing edible oil is very high.

It is crucial to evaluate the toxicity profile of these plant-derived compounds before their use to avoid negative effects on humans and animals [8]. *Persea americana* (avocado pear) is a member of the Lauraceae family. Due to its therapeutic applications and nutritional benefits, it is one of the medicinal plants used extensively around the world [9]. In Nigeria, it is known as "Igba/apoka" in Yoruba and "ube-bekee" in Igbo. The fruit is green-yellow in colour and contains a single seed [9]. The pulp is sometimes referred to as a "superfood' due to its nutritional benefits and therapeutic importance [9].

In traditional medicine, different parts of *P. americana* are used to treat various diseases, such as anaemia, hypertension, diabetes, intestinal parasites, dysentery, inflammation and toothache [9]. Scientific studies have shown that different parts of *P. americana* have antiviral, anti-inflammatory, antidiabetic, trypanocidal, antifungal, antihypertensive, hypoglycemic and insecticidal effects [9,10]. However, most of these pharmacological effects have been associated primarily with the

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pulp and not the seeds, peel or leaves. The seeds are unused and inedible by-products that are usually discarded after consumption of the pulp. Investigating the potential dietary and therapeutic applications of underutilized agricultural food waste such as the seeds of *P. americana* could help reduce the significant environmental waste generated by this by-product [11].

Topical application of avocado seed paste is said to cure arthritis and dandruff, while the seed oil is included in various dermatological and cosmetic formulations [12,13]. In Nigeria, avocado seed powder is used for the prevention, control and treatment of hypertension and as a cardioprotective supplement [14-16]. The seeds are sliced, dried, ground and consumed either as tea or in combination with fermented cornmeal. In several countries, including Colombia, Mexico, Costa Rica, South Africa, Cuba and Ecuador, hot water extracts of dried seeds, leaves and fruits are used to promote sterility in women, as an abortifacient and as an emmenagogue [17].

A review of the literature revealed a lack of documented scientific studies on the phytochemical analysis and toxicity profile of *P. americana* seed oil. Therefore, this study aims to investigate the phytochemical constituents and toxicity profile of *P. americana* seed oil in albino Wistar rats.

2. Materials and methods

2.1. Plant collection and preparation

The seeds of *P. americana* were collected from a fully ripened avocado fruit grown in Arochukwu, Abia State, Nigeria. The authenticity was confirmed by Dr. Franklin Akanwa (Department of Plant Science and Biotechnology, Abia State University, Uturu). A total of 10 kg of the seeds were air-dried and then ground into powder using a mill. Exactly 50 g of the seed powder was placed in a thimble and then placed in the extraction chamber. For the extraction, 200 ml of n-hexane (BDH, Dorset, UK) was used in a Soxhlet extractor at a temperature of 60–80 °C for 1 h. After oil extraction from the seeds, the n-hexane was removed using a rotary evaporator (Henan, Model-RE20 0 0B, China) and the PASO was placed in a water bath at 40 °C for 24 h to remove all n-hexane residues.

2.2. Phytochemical analysis

The Agilent 7890A-5975C GC-MS system was used to analyze the chemical composition of PASO. The method described by Ukpai et al. [18] was used with minor modifications. The chemical compounds were identified using the National Institute of Standards and Technology (NIST) library.

2.3. Experimental animals

A total of eighty (80) healthy albino rats of the Wistar strain (male and female) (8 weeks old, weighing between 135 and 145 g) were purchased from Michael Okpara University of Agriculture Umudike, Nigeria. The rats were transported to the Department of Biochemistry, Abia State University, Uturu and allowed to acclimatize for 14 days before the experiment commenced. Throughout the experiment, the rats had free access to food and water and were kept at a temperature of 25–28 °C, humidity of 35–60% and a 12:12 h cycle. The 1998 World Health Organization ethical guidelines for good laboratory practice and the United States guidelines for the care and use of laboratory animals [19] were strictly followed. In addition, the study was approved for research by the Abia State University Ethics Committee (ABSU/REC/BMR/098).

2.4. Acute toxicity study

The acute toxicity assessment of PASO was carried out in accordance with OECD [20] 423 guidelines with minor modifications. Female albino rats of the Wistar strain (10 weeks old, weighing 150 ± 7 g) were divided into six groups of five (5) rats in each group. The rats were then administered oral doses of PASO at different concentrations (200, 500, 1000, 1500, 2000 and 3000 mg/kg). The rats were observed for 24 h after treatment and for 14 days. During this observation period, all behavioural changes and signs indicative of toxicity were recorded.

2.5. Subacute toxicity study

Healthy, unmated, disease-free (male and female albino of Wistar strain) rats (10 weeks old, weighing 150 ± 7 g) were divided into four separate groups (groups 1–4), each comprising six males and six females. To avoid mating, the male and female rats were housed in different cages. The treatment regimen was as follows: Group I (normal control), Group II (200 mg/kg PASO), Group III (300 mg/kg PASO) and Group IV (400 mg/kg PASO). After 14 days of treatment, the experimental rats were sacrificed on day 15 after an overnight fasting and following anesthesia by diethyl ether inhalation. Blood was collected by cardiac puncture and placed in bottles containing the anticoagulant EDTA for hematological analyses, while bottles without anticoagulant were used for biochemical analyses.

The relative organ weights were calculated using the equation below:

Relative organ weight = (Organ weight (g)/ Body weight of rat on sacrificed day × 100.

2.6. Biochemical analysis

Liver, kidney and lipid profile parameters were determined using a spectrophotometer and Randox test kits (Randox Laboratories Limited, Antrim, UK). The haematological parameters were determined in accordance with Bain et al. [21]. For the antioxidant biomarkers, the technique described by Kanu et al. [22] was used. ELISA was used for the measurement of tumour necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β), the colorimetric method for the determination of lactate dehydrogenase activity and the Abnova assay kit for the determination of creatine kinase. The troponin assay kit from Eagle Biosciences was used to measure cardiac troponin.

2.7. Histopathological analysis

The method described by Loha et al. [23] was used for histopathological analysis. The liver and kidney were fixed in 10% formalin. The sections of liver and kidney tissue were cut into 5 µm pieces. The 5 µm tissue sections were treated with paraffin wax and stained with hematoxylin-eosin dye and viewed under a light microscope by an experienced histopathologist. Histopathological lesions were scored using a semi-quantitative scoring system, with 0 representing normal tissue, 1 representing mild lesions (1–30%), 2 representing moderate lesions (31–70%), and 3 representing severe lesions (>70%).

2.8. Statistical analysis

The results were expressed as mean ± SD. The significance level at $p < 0.05$ was analyzed with a one-way analysis of variance (ANOVA) and a Tukey post hoc test using the R statistical package.

3. Results

3.1. Phytochemical analysis of the PASO

Table 1 shows the phytochemical constituents identified in PASO by GC—MS analysis. The GC—MS investigation of the chemical constituents of PASO provided information on the molecular weight, retention time and peak area associated with the different phytoconstituents. A

Table 1
GC-MS analysis of PASO.

Serial No.	Compound name	Molecular formula	Molecular weight (g/mol)	Retention time (min)	Quantity/Peak area (%)
1	9,12-Octadecadienal	$C_{18}H_{32}O$	264.40	5.24	9.10
2	Hexadecane	$C_{16}H_{34}$	226.41	6.88	1.23
3	Benzene	C_6H_6	78.11	7.43	0.45
4	Carbonic acid	H_2CO_3	62.03	8.52	1.45
5	17-pentatriacontene	$C_{35}H_{70}$	490.90	8.88	3.58
6	Undecane	$C_{11}H_{24}$	156.31	8.99	3.35
7	Tetracosane	$C_{24}H_{50}$	338.65	9.14	1.18
8	Heptane	C_7H_{16}	100.21	9.62	3.22
9	Hydroxylamine	H_3NO	330.30	10.58	2.09
10	Cyclohexadecane	$C_{16}H_{32}$	224.42	18.28	1.78
11	Palmitic acid	$C_{16}H_{32}O_2$	256.40	18.48	1.94
12	1-Decanol	$C_{10}H_{22}O$	158.28	21.11	0.93
13	2,4-Di-tert- butylphenol	$C_{14}H_{22}O$	206.32	22.13	9.67
14	Erucic	$C_{22}H_{42}O_2$	338.57	23.44	2.24
15	1-Nonadecene	$C_{19}H_{38}$	266.50	28.09	2.97
16	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	30.53	1.60
17	3-Eicosene	$C_{20}H_{40}$	280.50	30.70	2.54
18	1-Ethanone	$C_4H_6O_2$	42.04	30.89	0.64
19	Oleic acid	$C_{18}H_{34}O_2$	282.46	32.04	0.57
20	1-Docosene	$C_{22}H_{44}$	308.60	32.18	1.27
21	9-Octadecenal	$C_{18}H_{34}O$	266.50	32.59	2.05
22	9-Octadecenoic acid	$C_{18}H_{34}O$	282.50	33.88	0.97
23	13-Octadecenal	$C_{18}H_{34}O$	266.50	37.07	0.25

total of 23 different chemical constituents were detected in PASO. The analysis revealed that 2,4-di-tert-butylphenol is the predominant constituent with a retention time of 22.130 and a percentage composition of 9.67, while 13-octadecenal is the least abundant constituent with a retention time of 22.130 but a percentage composition of only 0.25 (Table 1; Fig. 1).

3.2. Toxicity studies of PASO

In acute toxicity evaluations, rats treated with 1000, 1500, 2000 and 3000 mg/kg PASO showed behavioural changes and signs of toxicity such as restlessness, general weakness, clustering and death. Administration of PASO at 200 and 500 mg/kg body weight and the control group did not result in behavioural changes, signs of toxicity or death (Table 2). However, administration of PASO at doses of 1000, 1500, 2000 and 3000 mg/kg body weight resulted in mortality of 20, 60, 60

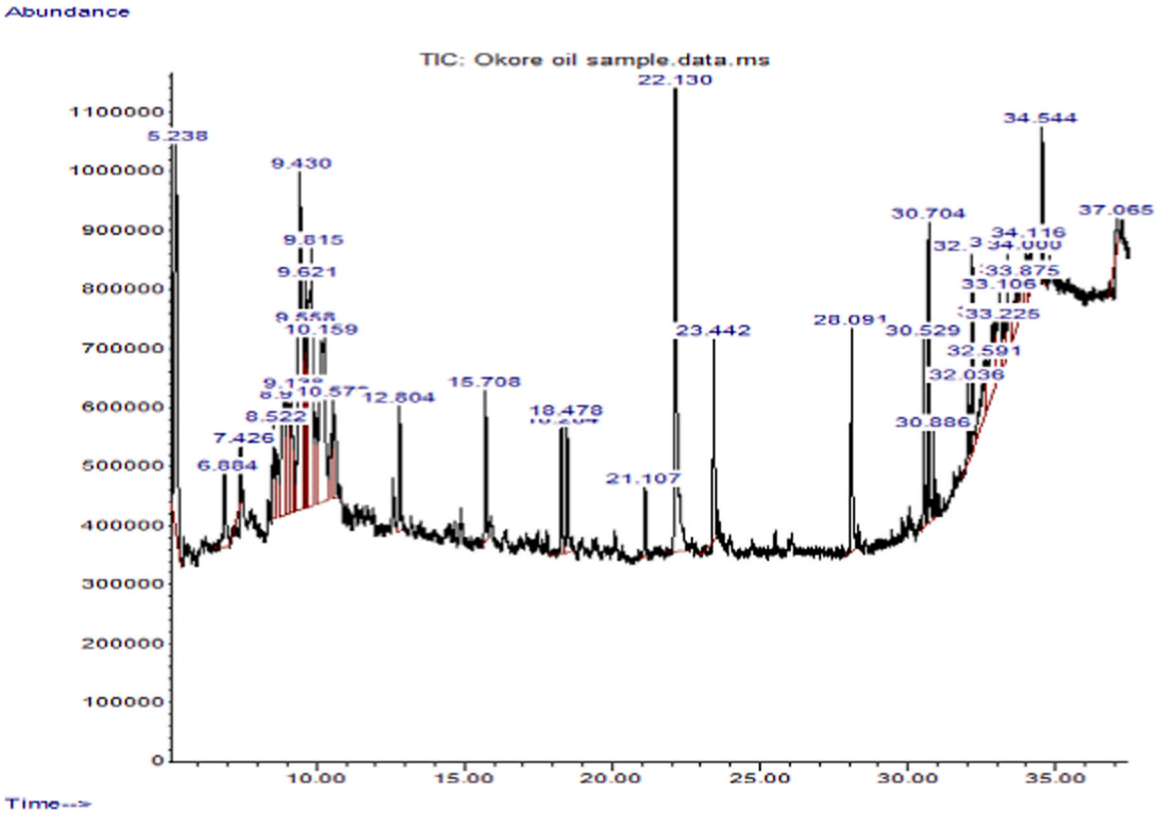

Fig. 1. Chromatogram reflecting the peaks of the distinct compounds of the GC-MS spectra analysis of PASO.

Table 2
Acute (oral) toxicity study of albino Wistar rats after 24 h administration of PASO.

Group	Dose (mg/kg)	Behavioural change/signs of toxicity	Death/number of animals	Mortality (%)
Control	0	No behavioural changes or toxic effects	0/5	0
PASO	200	No behavioural changes or toxic effects	0/5	0
	500	No behavioural changes or toxic effects	0/5	0
	1000	Restlessness, weakness, clustering and death	1/5	20
	1500	Restlessness, weakness, clustering and death	3/5	60
	2000	Restlessness, weakness, clustering and death	4/5	80
	3000	Restlessness, weakness, clustering and death	5/5	100

and 100%, respectively. At the maximum dose of 3000 mg/kg body weight, all experimental rats were dead (Table 2). The LD_{50} of PASO was estimated to be 1477.83 mg/kg body weight.

In the subacute toxicity study, a significant ($p < 0.05$) percentage reduction in body weight gain of PASO-treated groups compared to control groups (Table 3). However, there were no significant changes ($p > 0.05$) difference in organ weights was observed between the PASO-treated and control groups and the PASO groups in both male and female rats (Table 4). In liver function parameters, a significant increase ($p < 0.05$) in AST, ALP and ALT, was recorded in PASO-treated rats compared to the control groups. However, no significant changes ($p > 0.05$) in albumin, globulin, total protein and bilirubin were observed when the PASO-treated groups were compared with the control groups across all doses in both sexes (Table 5). Fig. 2 shows the histological examination of the liver of albino rats treated with different doses of PASO. The control group exhibited normal architecture of the hepatocytes, which were organized in interconnected cords around the central veins (V) in the males, while the portal areas (including the hepatic artery, hepatic veins and bile ducts) appeared structurally intact in the females. Administration of 200 mg/kg PASO resulted in mild hepatic inflammation characterized by mild (1–30%) infiltration of mononuclear inflammatory leukocytes in the portal areas (P). In contrast, treatment with 300 and 400 mg/kg PASO resulted in mild (1–30%) cellular swelling of hepatocytes near the portal areas (P), with affected hepatocytes appearing pale and swollen (arrow), thereby occluding adjacent hepatic sinusoids in rats.

The effects of PASO on renal function parameters in rats are shown in Table 6. When comparing the control group with the PASO-treated group, a significant increase ($p < 0.05$) in urea and creatinine levels was observed in rats treated with 400 mg/kg PASO. However, no significant changes were observed in uric acid, Na^+, Cl^-, K^+ and HCO_3^- in all PASO-treated groups compared to the control group. The histopathological effects of PASO on the kidneys of male and female albino rats are shown in Fig. 3. The normal control group exhibited typical renal histoarchitecture with normal glomeruli (G) located within their respective Bowman's capsules and surrounded by a network of healthy renal tubules (indicated by an arrow). The groups receiving 200 and 300 mg/kg PASO showed no significant changes in renal histopathology in either sex. In contrast, the group receiving 400 mg/kg PASO exhibited mild

Table 3
Effect of 14 days treatment PASO on body weights of albino Wistar rats.

		PASO (mg/kg)		
Parameter	Control	200	300	400
Male				
Day 1	150.92 ± 6.44	153.87 ± 5.76	152.84 ± 7.21	150.87 ± 6.89
Day 14	164.77 ± 7.50	160.54 ± 9.44	157.22 ± 6.35	154.90 ± 8.48
WC	13.85	6.67	4.38	4.03
% WC	9.18[b]	4.34[a]	2.87[a]	2.67[a]
Female				
Day 1	151.90 ± 7.20	155.90 ± 6.33	154.22 ± 7.37	153.63 ± 4.98
Day 14	166.45 ± 8.10	163.64 ± 5.84	160.77 ± 8.56	159.27 ± 6.75
WC	14.55	7.74	6.55	5.64
% WC	9.58[b]	4.97[a]	4.25[a]	3.67[a]

Mean ± SD, n = 6; WC, Weight Change. Within each row, values bearing the same letter show no significant difference (P > 0.05). Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively.

Table 4
Effect of 14 days treatment PASO on organ weights of albino Wistar rats.

		PASO (mg/kg)		
Organ weight (g)	Control	200	300	400
Male				
Liver	3.93 ± 0.15	4.21 ± 0.41	4.68 ± 0.18	4.53 ± 0.34
Heart	0.47 ± 0.03	0.41 ± 0.10	0.47 ± 0.08	0.43 ± 0.08
Kidney	0.88 ± 0.11	0.80 ± 0.21	0.86 ± 0.20	1.03 ± 0.05
Lungs	0.80 ± 0.08	0.83 ± 0.12	0.68 ± 0.08	0.72 ± 0.21
Testes	1.65 ± 0.30	1.49 ± 0.17	2.05 ± 0.30	2.05 ± 0.20
Spleen	0.40 ± 0.07	0.46 ± 0.03	0.50 ± 0.15	0.52 ± 0.12
Female				
Liver	3.61 ± 0.42	3.58 ± 0.11	3.79 ± 0.39	4.11 ± 0.50
Kidney	0.79 ± 0.12	0.75 ± 0.17	0.83 ± 0.12	0.87 ± 0.15
Heart	0.47 ± 0.09	0.32 ± 0.04	0.30 ± 0.04	0.38 ± 0.09
Lungs	0.57 ± 0.03	0.73 ± 0.10	0.78 ± 0.11	0.69 ± 0.10
Spleen	0.45 ± 0.06	0.48 ± 0.07	0.49 ± 0.05	0.52 ± 0.10

Mean ± SD, n = 6. Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively.

Table 5
Effect of PASO on liver function parameters of male and female albino Wistar rats.

		PASO (mg/kg)		
Parameters	Control	200	300	400
Male				
Total protein (TP) (g/dL)	5.73 ± 0.16[a]	5.61 ± 0.07[a]	5.50 ± 0.07[a]	5.18 ± 0.23[a]
Albumin (g/dL)	3.51 ± 0.10[a]	3.59 ± 0.17[a]	3.31 ± 0.12[a]	3.00 ± 0.21[a]
Globulin (g/dL)	2.22 ± 0.17[a]	2.03 ± 0.18[a]	2.19 ± 0.12[a]	2.18 ± 0.16[a]
ALT (U/L)	24.00 ± 2.92[a]	36.00 ± 2.12[b]	42.00 ± 3.39[c]	47.40 ± 2.88[d]
AST (U/L)	38.20 ± 2.86[a]	45.40 ± 2.30[b]	56.80 ± 3.27[c]	57.80 ± 3.83[c]
ALP (U/L)	67.40 ± 3.05[a]	71.40 ± 2.07[b]	72.20 ± 1.30[b]	74.00 ± 4.30[b]
Bilirubin (mg/dL)	0.55 ± 0.04[a]	0.60 ± 0.09[a]	0.68 ± 0.07[a]	0.70 ± 0.07[a]
Female				
Total protein (TP) (g/dL)	5.44 ± 0.15[a]	5.24 ± 0.14[a]	5.07 ± 0.13[a]	4.80 ± 0.20[a]
Albumin (g/dL)	3.30 ± 0.07[a]	3.18 ± 0.06[a]	2.92 ± 0.03[a]	2.63 ± 0.15[a]
Globulin (g/dL)	2.14 ± 0.14[a]	2.06 ± 0.10[a]	2.15 ± 0.12[a]	2.17 ± 0.23[a]
ALT (U/L)	24.00 ± 1.58[a]	27.60 ± 1.34[a]	34.00 ± 2.92[b]	43.40 ± 4.39[c]
AST (U/L)	42.80 ± 1.92[a]	45.00 ± 3.46[b]	46.40 ± 2.70[b]	54.60 ± 3.13[c]
ALP (u/L)	67.80 ± 1.92[a]	69.40 ± 1.14[b]	70.00 ± 1.58[b]	71.40 ± 4.16[b]
Bilirubin (mg/dL)	0.57 ± 0.03[a]	0.52 ± 0.05[a]	0.73 ± 0.05[a]	0.74 ± 0.04[a]

Mean ± SD, n = 6. Within each row, values bearing the same letter show no significant difference (P > 0.05). Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively. AST= aspartate aminotransferase, ALT= alanine transaminase, ALP= alkaline phosphatase

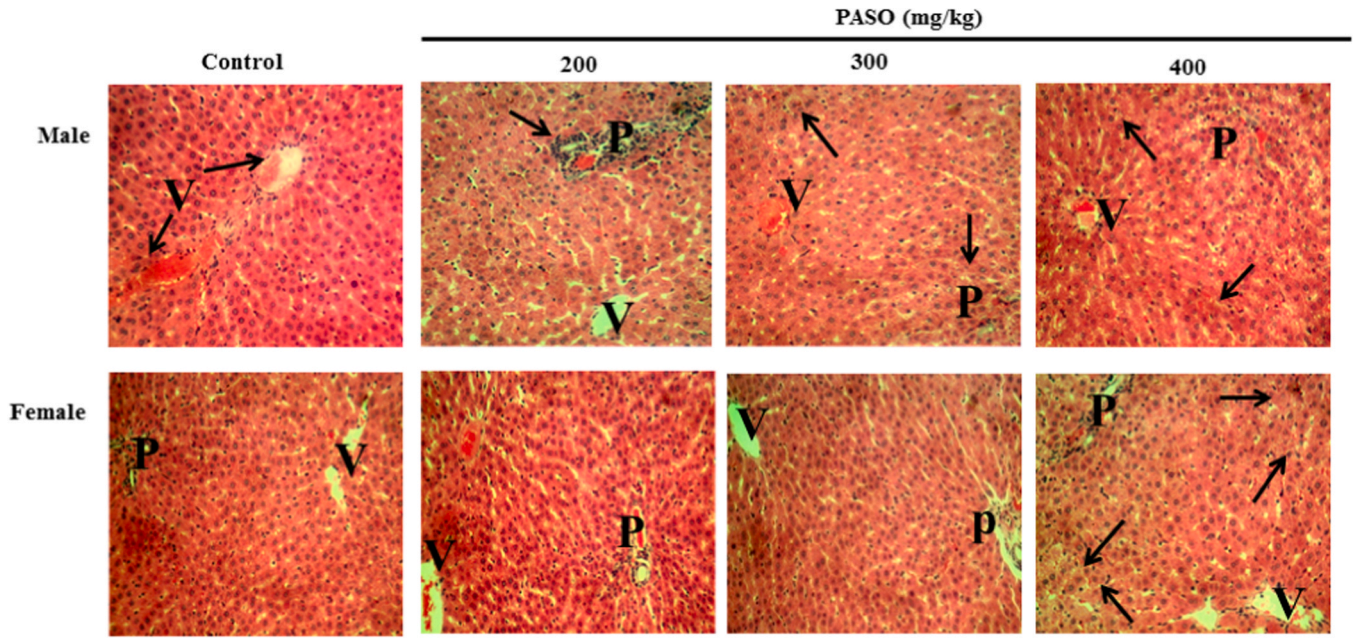

Fig. 2. Effect of PASO on the liver histology of albino Wistar rats. Haematoxylin and eosin staining (H&E), magnification × 100. CV= Central Vessel; P = Portal area. Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively. In the control group, the arrows show normal hepatocytes arranged in interconnecting cords around the central veins (V). In 200 mg/kg PASO, the arrows showed portal areas (P) with mild infiltration of mononuclear inflammatory leukocytes. In 300 and 400 mg/kg PASO, the arrows showed mild cellular swelling involving the hepatocytes around the portal areas (P). The affected hepatocytes appear pale, swollen (arrow) and appear to occlude the adjacent hepatic sinusoids.

Table 6
Effects of PASO extracts on renal function markers of male and female albino Wistar rats.

		PASO (mg/kg)		
Parameters	Control	200	300	400
Male				
Urea (mg/dL)	19.28 ± 0.51[a]	18.63 ± 0.57[a]	20.32 ± 0.93[a]	22.58 ± 0.63[b]
Creatinine (mg/dL)	0.71 ± 0.06[a]	0.80 ± 0.04[a]	0.83 ± 0.03[a]	0.98 ± 0.05[b]
Uric acid (mg/dL)	4.65 ± 0.16[a]	4.78 ± 0.19[a]	5.11 ± 0.10[a]	5.24 ± 0.20[a]
Sodium (Na^{+}) (mEq/L)	129.42 ± 2.21[b]	128.60 ± 1.61[a]	126.48 ± 2.52[a]	126.32 ± 1.79[a]
Potassium (K^{+}) (mEq/L)	4.75 ± 0.21[a]	4.58 ± 0.35[a]	4.38 ± 0.08[a]	4.18 ± 0.06[a]
Chloride (Cl^{-})(mEq/L)	87.80 ± 2.09[b]	86.20 ± 0.91[b]	81.28 ± 2.44[a]	79.70 ± 1.27[a]
Bicarbonate (HCO_3^{-}) (mmol/L)	20.18 ± 0.41[a]	20.46 ± 0.25[a]	21.26 ± 0.40[a]	22.16 ± 0.43[a]
Female				
Urea (mg/dL)	20.76 ± 0.80[a]	20.65 ± 1.71[a]	21.15 ± 0.97[b]	22.23 ± 0.64[b]
Creatinine (mg/dL)	0.74 ± 0.06[a]	0.87 ± 0.03[a]	0.92 ± 0.03[a]	1.09 ± 0.10[b]
Uric acid (mg/dL)	4.60 ± 0.23[a]	4.53 ± 0.23[a]	4.73 ± 0.19[a]	4.64 ± 0.11[a]
Sodium (Na^{+}) (mEq/L)	125.56 ± 2.97[a]	126.40 ± 1.15[a]	129.78 ± 1.40[a]	130.94 ± 1.34[a]
Potassium (K^{+}) (mEq/L)	4.61 ± 0.13[a]	4.55 ± 0.06[a]	4.62 ± 0.10[a]	4.79 ± 0.15[a]
Chloride (Cl^{-}) (mEq/L)	84.18 ± 3.63[a]	87.32 ± 1.22[a]	88.46 ± 1.26[a]	90.44 ± 1.49[a]
Bicarbonate (HCO_3^{-}) (mmol/L)	20.08 ± 0.46[a]	20.64 ± 0.61[a]	20.36 ± 0.36[a]	20.90 ± 0.39[a]

Mean ± SD, n = 6. Within each row, values bearing the same letter show no significant difference (P > 0.05). Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively.

(1–30%) inflammation of the renal tubules both in the cortex and in the outer and inner medullary regions of the renal structure (indicated by an arrow).

Rats administered PASO showed a significant dose-dependent decrease ($P < 0.05$) in RBC, PCV and a dose-dependent increase in WBC, platelet and lymphocyte counts compared to the control groups. However, no significant changes were observed in Hb, mean corpuscular MCH, MCHC, eosinophils, basophils, monocytes or neutrophils in the PASO-treated groups compared to the control groups in both male and female rats (Table 7).

Table 8 illustrates the effects of 14-day treatment with PASO on the lipid profiles of albino rats. The results show a statistically significant ($p < 0.05$) dose-dependent reduction in TC, TAG and LDL-C and increased in HDL-C in the PASO-treated groups compared to the control group at all concentrations (Table 8). The LDL-C/HDL and TC/HDL-C ratios of the PASO-treated groups decreased non-significantly ($p > 0.05$) in a dose-dependent manner compared to the normal control (Table 8). Table 8 also shows the effect of PASO treatment on cardiac and proinflammatory biomarkers in male and female rats. While no significant difference ($p < 0.05$) was observed for LDH, a significant increase ($p < 0.05$) was observed for CPK, cardiac troponin, IL-1β and TNF-α in the rats treated with 400 mg/kg PASO compared to the control groups for both sexes (Table 9). The effects of PASO extract on antioxidant metrics in albino rats are shown in Table 10. A significant decrease ($p < 0.05$) in GSH, SOD and CAT was observed in the 400 mg/kg PASO treated group in both sexes compared to the normal control groups and the other PASO treated groups. In addition, MDA levels showed a significant increase in rats receiving 300 and 400 mg/kg PASO compared to the control group.

4. Discussion

In traditional medicine, the seed powder of *P. americana* is used for the prevention, control and treatment of hypertension and as a cardioprotective supplement. However, the toxicological profile of the seed oil has not been studied. In this study, the phytochemical composition

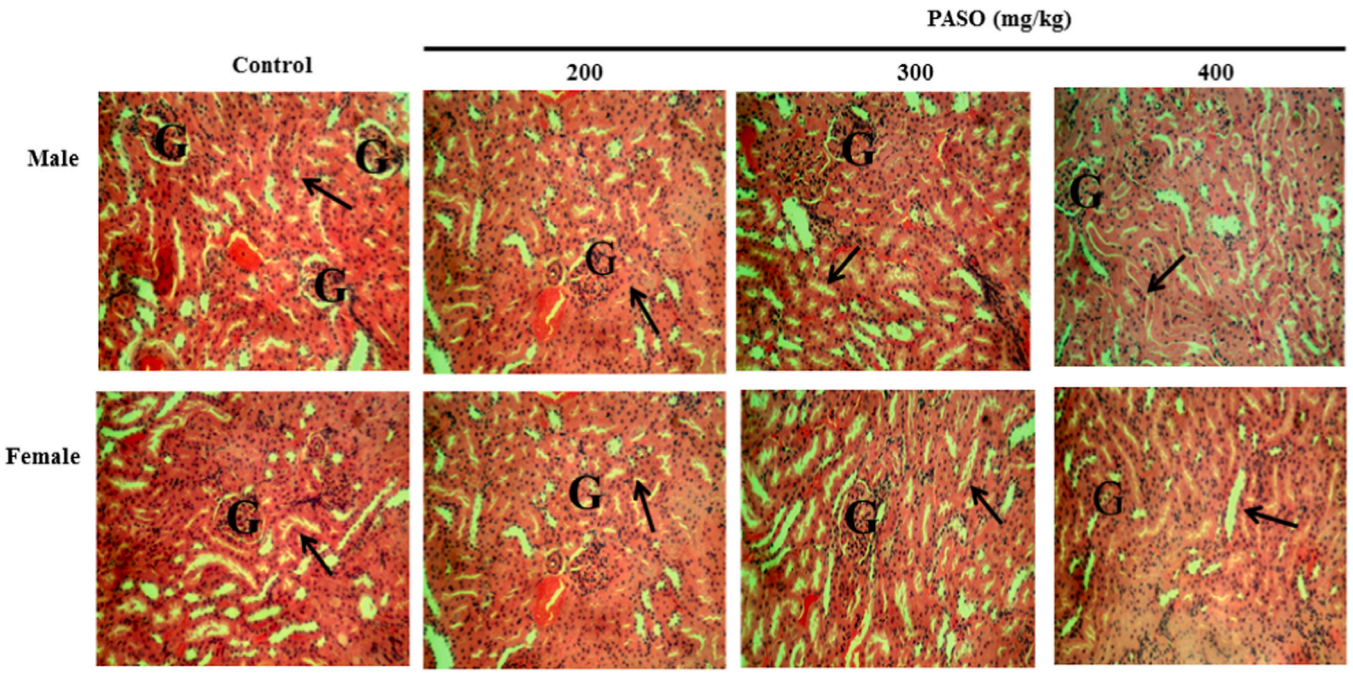

Fig. 3. Effect of PASO on the kidney histology of albino Wistar rats. Haematoxylin and eosin staining (H&E), magnification × 100. G= glomerulus. Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively. In control, 200 and 300 mg/kg PASO, the arrows show normal glomeruli (G) in their respective Bowman's capsules surrounded by a sea of normal renal tubules. In 400 mg/kg, the arrows showed mild inflammation of the renal tubules in the cortex and in the outer and inner medullary areas of the renal architecture.

and toxicity profile (both acute and subacute) of *P. americana* seed oil (PASO) in albino rats were investigated. GC-MS analysis of PASO revealed the presence of various bioactive compounds with different pharmacological properties. The bioactive compounds identified in PASO such as 2,4-di-tert-butylphenol, 9-octadecenoic acid, heptane and hexadecane have antioxidant activity; 17-pentatriacontene, 9,12-octadecadienal, undecane, palmitic acid, oleic acid, 9-octadecenal and hydroxylamine have anti-inflammatory properties [24,25], while 9-octadecenoic acid and 9,12-octadecadienal have anti-cancer activities [26,27], suggesting that PASO contains bioactive compounds with known therapeutic potential.

In the acute toxicity study, our result showed that PASO is slightly or moderately toxic, with an LD_{50} value of 1477.83 mg/kg. Similar studies have been conducted by different research groups and they reported LD_{50} values for ethanolic and aqueous extracts of *P. americana* seeds of 1200.75 mg/kg [28] and 1767 mg/kg [29], respectively. The differences in these results can be attributed to variations in the solvents and extraction methods used. Significant changes in body weight and organ weight of experimental rats after exposure to toxic substances/agents are indicative of a possible toxic effect [6,30], with a weight loss of $>$ 10% indicating a severe toxic effect [31]. In our study, all experimental rats gained weight, however, treatment with PASO resulted in a significant decrease in body weight compared to the normal control groups in both male and female rats.

The liver, a vital organ responsible for various physiological processes such as energy metabolism and detoxification, was evaluated using liver enzyme biomarkers to assess its integrity. Elevated ALT and AST levels are indicative of tissue necrosis, cell damage and cardiovascular problems [32,33]. In this study, treatment with PASO resulted in a significant increase in liver enzymes (AST, ALT and ALP), suggesting that administration of PASO at the doses tested impairs liver function. In addition, pathological changes and lesions were observed in the liver histology of PASO-treated rats, suggesting that treatment with PASO may impair liver function with mild structural damage.

In kidney function tests, increases in creatinine and urea are considered indicators of impaired kidney function [34]. A significant increase in these renal function biomarkers above normal reference values may be an indication of renal dysfunction [30,35]. In our study, treatment with PASO resulted in a dose-dependent increase in creatinine and urea levels compared to normal control rats. Histopathological analysis of the kidney revealed that a dose of 400 mg/kg PASO induced mild inflammation in the renal tubules, suggesting that treatment with higher doses of PASO impairs renal function with mild (1–30%) structural damage of the kidney.

The possible mechanism for increase in liver enzymes (AST, ALP and ALP) and renal function parameter (urea and creatinine) may be attributed to oxidative stress imposed on the liver and kidneys as a result of the metabolism and excretion of PASO by the liver and kidneys respectively. In response to the oxidative stress induced by PASO exposure, these organs may have also enhanced the de novo synthesis of these enzymes. Another potential mechanism may be that PASO caused membrane damage, which permits the leakage of compartmentalized enzymes into the extracellular fluids.

Haematological analysis is an important parameter for evaluating the physiological changes in the blood of humans or animals after exposure to toxic substances [36]. In this study, a significant dose-dependent decrease ($P < 0.05$) in RBC and PCV and a dose-dependent increase in WBC count, platelet count and lymphocyte count were observed compared to the control groups. The observed decrease in RBC and PCV levels may lead to anaemia [9]. Our study suggests that PASO may have immunosuppressive or haemolytic effects, possibly due to mild suppression of erythropoietin production in the kidneys, which could lead to decreased erythropoiesis.

A lipid profile test is a series of clinical chemistry tests used for the comprehensive assessment of lipid metabolism disorders [37]. Total cholesterol, TAG, LDL-C and VLDL-C were significantly lowered after administration of PASO, suggesting that PASO has anti-atherogenic properties. In a related study, Asaolu et al. [38] reported that administration of 200 mg/kg methanol extract of avocado seed in hypercholesterolemic mice resulted in a significant reduction in TC, LDL-C and TAG levels. According to Paha-Ramos et al. [28], methanolic extract of avocado seed also reduced TC and LDL-C levels in hypercholesterolemic

Table 7
Effect of oral administration of PASO extract on hematological parameters of both male and female albino Wistar rats.

	PASO (mg/kg)			
ParametersControl	200	300	400	
Male				
Red blood cell ($x10^{12}$/L)	7.45 ± 0.13^{b}	7.33 ± 0.12^{b}	6.89 ± 0.22^{a}	6.54 ± 0.458^{a}
Packed cell volume (%)	46.00 ± 0.71^{c}	45.20 ± 0.84^{c}	43.00 ± 0.71^{b}	40.60 ± 1.95^{a}
Haemoglobin (g/dL)	16.92 ± 0.26^{a}	16.82 ± 0.36^{a}	16.26 ± 0.24^{a}	15.34 ± 0.53^{a}
White blood cell ($x10^{9}$/L)	9.54 ± 0.83^{a}	10.80 ± 1.53^{a}	11.04 ± 0.75^{a}	12.84 ± 0.63^{b}
Platelets ($x10^{9}$/L)	214.24 ± 2.83^{a}	217.60 ± 3.14^{b}	218.84 ± 5.72^{b}	220.82 ± 4.87^{b}
MCV (fl)	61.78 ± 0.29^{a}	61.65 ± 0.43^{a}	62.42 ± 1.27^{a}	62.18 ± 1.72^{a}
MCH (pg)	3.68 ± 0.02^{a}	3.72 ± 0.04^{a}	3.78 ± 0.03^{a}	3.78 ± 0.11^{a}
MCHC (g/dL)	36.78 ± 0.17^{a}	37.21 ± 0.37^{a}	37.82 ± 0.30^{a}	37.81 ± 1.14^{a}
Neutrophils (%)	34.80 ± 2.59^{a}	34.40 ± 1.95^{a}	32.40 ± 2.88^{a}	30.00 ± 2.92^{a}
Lymphocytes (%)	55.8 ± 2.59^{a}	56.80 ± 1.64^{a}	58.80 ± 2.28^{a}	64.80 ± 1.92^{b}
Monocytes (%)	5.80 ± 0.45^{a}	5.00 ± 0.71^{a}	4.60 ± 0.55^{a}	4.80 ± 0.45^{a}
Eosinophils (%)	3.20 ± 0.45^{a}	3.60 ± 0.55^{a}	3.60 ± 0.55^{a}	3.80 ± 0.45^{a}
Basophils (%)	0.40 ± 0.55^{a}	0.20 ± 0.45^{a}	0.60 ± 0.55^{a}	0.60 ± 0.55^{a}
Female				
Red blood cell ($x10^{12}$/L)	7.09 ± 0.13^{b}	6.77 ± 0.32^{ab}	6.66 ± 0.17^{ab}	6.11 ± 0.45^{a}
Packed cell volume (%)	43.80 ± 0.84^{b}	42.20 ± 2.05^{b}	41.40 ± 0.89^{a,b}	39.60 ± 2.30^{a}
Haemoglobin (g/dL)	16.12 ± 0.31^{a}	15.42 ± 0.40^{a}	15.40 ± 0.39^{a}	14.20 ± 0.60^{a}
White blood cell ($x10^{9}$/L)	9.30 ± 0.70^{a}	8.16 ± 0.81^{a}	8.61 ± 0.86^{a}	11.48 ± 1.15^{b}
Platelets ($x10^{9}$/L)	213.00 ± 11.75^{a}	213.40 ± 13.41^{a}	215.00 ± 14.02^{a}	217.20 ± 10.26^{b}
MCV (fl)	61.81 ± 0.49^{a}	62.31 ± 0.65^{a}	62.17 ± 0.93^{a}	64.93 ± 1.66^{b}
MCH (pg)	22.75 ± 0.20^{a}	22.79 ± 0.54^{a}	23.13 ± 0.66^{a}	23.30 ± 0.81^{a}
MCHC (g/dL)	36.80 ± 0.34^{a}	36.57 ± 0.90^{a}	37.21 ± 0.94^{a}	35.89 ± 1.05^{a}
Neutrophils (%)	35.40 ± 2.51^{b}	31.80 ± 2.39^{a}	31.80 ± 2.68^{a}	31.00 ± 3.94^{a}
Lymphocytes (%)	57.20 ± 2.28^{a}	60.80 ± 2.17^{b}	60.80 ± 3.77^{b}	62.20 ± 3.11^{b}
Monocytes (%)	4.80 ± 0.45^{a}	4.60 ± 0.55^{a}	4.40 ± 0.55^{a}	4.20 ± 0.45^{a}
Eosinophils (%)	2.60 ± 0.55^{a}	2.60 ± 0.55^{a}	2.60 ± 0.55^{a}	2.20 ± 0.45^{a}
Basophils (%)	0.00 ± 0.00^{a}	0.20 ± 0.45^{a}	0.40 ± 0.55^{a}	0.40 ± 0.55^{a}

Mean ± SD, n = 6. Within each row, values bearing the same letter show no significant difference (P > 0.05). Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively. MCV= mean corpuscular volume, MCHC= mean corpuscular haemoglobin concentration, MCH= mean corpuscular haemoglobin

mice. HDL inhibits LDL [39], thus reducing atherogenic potential of LDL. The observed increase in HDL and decrease in LDL-C/HDL-C and TC/HDL-C ratios suggest that PASO may exhibit anti-arteriosclerotic activity by increasing HDL-C, leading to a decrease in blood cholesterol levels especially LDL in PASO-treated rats (Table 8).

This study showed that when 400 mg/kg PASO was administered to rats, the levels of TNF-α, IL-1β, cardiac troponin and CPK were higher in both male and female rats than in the control groups. The observed increase in these proinflammatory biomarkers in this study may indicate

Table 8
Effects of PASO extract on the serum lipid markers on male and female albino Wistar rats.

		PASO (mg/kg)		
Parameters	Control	200	300	400
Male				
Total cholesterol (mg/dL)	119.80 ± 1.20^{b}	116.82 ± 0.93^{b}	115.96 ± 1.45^{b}	102.30 ± 3.76^{a}
Triglycerides (mg/dL)	87.20 ± 1.59^{b}	83.10 ± 1.51^{a}	82.66 ± 2.11^{a}	79.32 ± 1.61^{a}
HDL-C (mg/dL)	71.98 ± 3.54^{a}	72.70 ± 2.68^{a}	74.24 ± 3.71^{b}	76.82 ± 2.80^{b}
LDL-C (mg/dL)	30.38 ± 2.23^{c}	27.00 ± 2.47^{b}	25.20 ± 1.89^{b}	9.70 ± 1.08^{a}
VDL-C (mg/dL)	17.44 ± 1.22^{a}	16.62 ± 1.10^{a}	16.53 ± 1.17^{a}	15.86 ± 1.32^{a}
LDL-C/HDL-C	0.42 ± 0.02	0.37 ± 0.11	0.34 ± 0.05	0.13 ± 0.01
TC/HDL-C	1.66 ± 0.20	1.60 ± 0.05	1.56 ± 0.02	1.33 ± 0.16
Female				
Total cholesterol (mg/dL)	140.08 ± 1.34^{b}	137.20 ± 1.07^{b}	120.88 ± 3.14^{a}	110.87 ± 3.20^{a}
Triglycerides (mg/dL)	90.76 ± 3.12^{b}	87.12 ± 2.36^{b}	82.70 ± 1.42^{b}	74.90 ± 2.53^{a}
HDL-C (mg/dL)	72.15 ± 2.56^{a}	73.25 ± 2.87^{a}	75.43 ± 3.37^{b}	76.92 ± 3.18^{b}
LDL-C (mg/dL)	49.70 ± 2.24^{c}	46.52 ± 2.30^{c}	28.91 ± 1.61^{b}	18.97 ± 1.33^{a}
VDL-C (mg/dL)	18.15 ± 0.49^{a}	17.42 ± 0.47^{a}	16.54 ± 0.28^{a}	14.98 ± 0.51^{a}
LDL-C/HDL-C	0.68 ± 0.08^{b}	0.64 ± 0.10^{b}	0.38 ± 0.06^{a}	0.25 ± 0.02^{a}
TC/HDL-C	1.94 ± 0.12	1.87 ± 0.24	1.60 ± 0.06	1.44 ± 0.30

Mean ± SD, n = 6. Within each row, values bearing the same letter show no significant difference (P > 0.05). Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively. HDL= high-density lipoprotein, VLDL= very low-density lipoprotein, LDL= low-density lipoprotein.

Table 9
Effect of n-hexane extract of PASO on the cardiac and pro-inflammatory biomarkers of male and female albino Wistar rats.

		PASO (mg/kg)		
Parameters	Control	200	300	400
Male				
LDH (U/L)	282.80 ± 11.45^{a}	286.20 ± 14.29^{a}	283.20 ± 11.03^{a}	284.20 ± 6.65^{a}
CPK (U/L)	190.20 ± 8.59^{a}	195.00 ± 10.89^{b}	199.60 ± 11.70^{b}	199.20 ± 7.50^{b}
Cardiac troponin (ng/ml)	80.80 ± 4.03^{a}	79.20 ± 5.26^{a}	79.00 ± 3.39^{a}	87.0 ± 3.54^{b}
IL-Ib (pg/ml)	0.41 ± 0.03^{a}	0.41 ± 0.02^{a}	0.44 ± 0.02^{a}	0.70 ± 0.10^{b}
TNF-α (pg/ml)	20.24 ± 1.43^{a}	20.12 ± 1.56^{a}	22.86 ± 1.61^{b}	27.50 ± 1.77^{c}
Female				
LDH (U/L)	263.80 ± 10.69^{a}	259.20 ± 16.90^{a}	265.20 ± 15.94^{a}	273.80 ± 16.27^{a}
CPK (U/L)	197.20 ± 9.45^{a}	192.80 ± 25.31^{a}	202.80 ± 15.02^{a}	241.60 ± 17.10^{b}
Cardiac troponin (ng/ml)	80.00 ± 3.67^{a}	78.40 ± 5.90^{a}	79.60 ± 8.08^{a}	89.00 ± 2.92^{b}
IL-Ib (pg/ml)	0.48 ± 0.06^{a}	0.46 ± 0.04^{a}	0.46 ± 0.05^{a}	0.94 ± 0.14^{b}
TNF-α (pg/ml)	17.52 ± 1.40^{a}	17.02 ± 2.13^{a}	17.88 ± 2.48^{a}	25.26 ± 1.92^{b}

Mean ± SD, n = 6. Within each row, values bearing the same letter show no significant difference (P > 0.05). Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively. LDH= Lactate Dehydrogenase; CPK= Creatine Phosphokinase; IL-1β= Interleukin 1 beta; TNF-α= Tumor necrosis factor alpha.

Table 10
Effect of PASO extract on Antioxidants parameters of male and female albino Wistar rats.

Parameters	Control	PASO (mg/kg) 200	300	400
Male				
Glutathione (U/L)	45.76 ± 2.11[b]	45.48 ± 1.56[b]	45.90 ± 1.33[b]	42.86 ± 1.93[a]
SOD (U/L)	22.94 ± 1.66[b]	22.02 ± 1.45[b]	21.72 ± 0.72[b]	20.86.12[a]
Catalase (U/L)	23.58 ± 0.68[b]	22.64 ± 1.07[b]	23.32 ± 0.89[b]	20.22 ± 2.21[a]
Malondialdehyde (mmol/L)	0.38 ± 0.03[a]	0.39 ± 0.05[a]	0.56 ± 0.04[b]	0.60 ± 0.04[b]
Female				
Glutathione (U/L)	40.44 ± 1.81[c]	38.14 ± 1.19[b]	37.32 ± 1.19[b]	34.34 ± 2.12[a]
SOD (U/L)	35.06 ± 1.65[c]	31.20 ± 1.81[b]	30.96 ± 1.72[b]	28.58 ± 1.78[a]
Catalase (U/L)	24.70 ± 1.90[b]	24.12 ± 2.45[b]	22.40 ± 1.48[a]	21.20 ± 0.85[a]
Malondialdehyde (mmol/L)	0.47 ± 0.04[a]	0.52 ± 0.05[a]	0.60 ± 0.07[b]	0.64 ± 0.06[b]

Mean ± SD, n = 6. Within each row, values bearing the same letter show no significant difference (P > 0.05). Control represents Group 1 while 200, 300 and 400 mg/kg PASO represent Groups II, III and IV respectively. SOD= Superoxidase dismutase

possible damage to the heart or muscles of the rats [31,40]. Both male and female rats in this study showed lower levels of GSH, SOD and CAT after administration of 400 mg/kg PASO.

In addition, treatment with 300 and 400 mg/kg PASO significantly increased MDA in contrast to the control groups. According to Ismail et al. [7], exposure to hazardous compounds probably leads to a significant increase in MDA and a decrease in antioxidant enzyme activity. Decreased levels of antioxidant enzymes are indicative of increased oxidative radical formation. Malondialdehyde, a biomarker used to assess lipid peroxidation, may be elevated as a result of pathological conditions or hazardous exposure [7]. In this our study, treatment with PASO increased oxidative stress and free radicals, as shown by increased MDA and decreased antioxidant enzymes.

5. Conclusion

Analysis of PASO by GC-MS showed that PASO contains bioactive compounds with potential therapeutic effects, such as antioxidant, anticancer and anti-inflammatory activities. Our study showed that the LD_{50} of PASO is 1477.83 mg/kg body weight, which classifies it as a slight or moderately toxic substance. In the subacute toxicity study, administration of PASO caused a significant increase in liver enzymes (ALT, AST and ALP), renal function parameters (urea and creatinine) and inflammatory markers (CPK, cardiac troponin, IL-1β and TNF-α). In addition, treatment with PASO reduced RBC and PCV levels as well as antioxidant enzymes (GSH, SOD and CAT). There was also an increase in MDA. These results showed that PASO mildly impaired liver and kidney functions of the experimental rats. In contrast, our result showed that administration of PASO reduced TC, TAG, LDL-C, and VLDL-C, suggesting that PASO may have anti-atherogenic activities. Overall, the results of both the acute and subacute studies indicate that PASO exhibits mild toxicity following oral administration, suggesting that PASO may not be safe for therapeutic purposes at the doses tested. However, further studies are needed to investigate the chronic toxicity profile of PASO to better understand its safety profile for therapeutic or cosmetic applications.

6. Authors statement

The authors valued the editor's and reviewers' comments and have responded to all of them appropriately. All the recommendations and suggestions made by the editor and reviewers have been implemented and highlighted in red in the main manuscript.

CRediT authorship contribution statement

Ugbogu Eziuche Amadike: Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **Amuji Doris Nnenna:** Writing – original draft, Methodology. **Dania Omor-emime Elizabeth:** Writing – original draft, Methodology. **Okoro Benedict Chukwuebuka:** Writing – original draft, Software, Methodology, Investigation, Data curation. **Okore Finian Uchenna:** Writing – original draft, Software, Methodology, Investigation, Data curation. **Iweala Emeka Joshua:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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