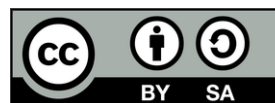




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Research Article

Sub-acute study of different processing methods of *Momordica charantia* leaf extract on healthy albino rats: Body chemistry, oxidative stress and histology study

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Abstract

Safety evaluation of medicinal plant extracts after preparation and before consumption is paramount. In this study, *Momordica charantia* leaf extracts were prepared using different processing techniques and the safety of each extract was evaluated in experimental rats. Preparation methods include fresh preparation of *M. charantia* (FPMC), parboiled *M. charantia* (PBMC) and air-dried *M. charantia* (ADMC). A total of 50 male rats were administered doses of FPMC, PBMC and ADCMC. After 21 days of administration, animals were sacrificed, and samples were obtained for biochemical analysis. Findings revealed progressive increase in rat's body weight with highest percentage increase of $23.45 \pm 2.01\%$ and $22.69 \pm 3.33\%$ observed in rats that were administered 100 mg.kg⁻¹ of PBMC and ADCMC. Except for significant increase that was observed in AST across tested animals, other hepatic markers were not affected. Only PBMC and FPMC caused significant increase in kidney function parameters. With the exception of PBMC that demonstrated poor lipoprotein activities, there was significant ($p < 0.05$) decrease in triglyceride across tested doses. While there are improvements in liver antioxidants, high doses of extracts caused noticeable changes in organs architecture. Conclusively, the administration of FPMC, PBMC and ADCMC does not elicit any adverse effects in experimental animals at low doses.

Keywords

kidney, oxidative stress, parboiled, safety, uric acid

Abbreviations

ALT – alanine aminotransferase; ALP – alkaline phosphatase; AST – aspartate aminotransferase; CAT – catalase; CHOL – cholesterol; CREA – creatinine; GGT – gamma glutamyl-transpeptidase; HDL-C – high density lipoprotein–concentration; MDA – malondialdehyde level; GSH – reduced glutathione concentration; SOD – superoxide dismutase; TPC – tissue protein concentration; TRIG – triglyceride

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Introduction

Traditional medicine includes practices and knowledge indigenous occupants of the globe use to maintain their health and prevent/treat physical and mental illness. This orthodox means of treatment is dependent on several theories, beliefs and experiences of different cultures (WHO 2005). For instance, herbs from medicinal plants are generally perceived to be safe owing to their natural state. However, this is seldom true especially when herbs are not properly processed to remove inherent toxins, consumed or abused with over-the-counter medications (Loya et al. 2009; Cohen and Ernst 2010).

Medicinal plants are consumed as infusions, syrups, essential oils, or whole herbs (Wachtel-Galor and Benzie 2011). Herbal extracts include tinctures, hot water extracts, decoctions, and macerated extracts, processed based on extraction solvent, temperature, and time. Processing aims to enhance palatability, reduce toxicity and side effects, and boost therapeutic efficacy. Despite huge importance, different preparation techniques for the same plant species may show diversity in terms of quality and therapeutics since the different treatments variably influence their chemical constituents (WHO 2018). Earlier reports have revealed the decomposition of chemical bonds in esters or glycosides, decreased concentrations of essential oil and changes in the solubility or properties of bioactive compounds such as alkaloids and phenolic compounds (Liu 2011). Common examples of medicinal plants alongside desirable changes after processing include raw aconite (*Aconitum carmichaelii*, Debeaux species), *Rehmannia glutinosa* root, *Corydalis yanhusuo* rhizomes (WHO 2018).

Momordica charantia is a multipurpose medicinal herb that is universally known as bitter melon. It is cultivated in several parts of the globe and different ethnic groups use its parts for the treatment of different diseases (Marr et al. 2004). The medicinal role of *M. charantia* includes treatment of stomach upset, intestinal gas expulsion, demulcent, and wounds (Sofowora 2006). Experimental validation of its antioxidant, antibacterial, antidiabetic, anticancer, anti-inflammatory and anti-malaria activities has also been reported by different

researchers (Patil and Patil 2011). Addition to its excellent chronicles, the leaves serve as warehouse for many important polyphenolic compounds such as chlorogenic acid, catechin, vanillic acid, gallic acid and coumaric acid (Kubola and Siriamornpun 2008). Studies on various processing techniques of *M. charantia* leaves revealed changes in the polyphenolic content and antioxidant potential. Ng et al. (2011) reported an increase in total phenol and a decline in the radical scavenging ability of *M. charantia* leaves after cooking under pressure and or after microwave treatment.

Momordica charantia leaves are commonly processed by squeezing the fresh leaves between fists to make juice, parboiling the leaves and air-drying the leaves to make powder for soup. Despite these processing techniques confer noticeable advantages to most herbs in terms of increasing therapeutic efficacy, adverse effects could also arise due to inappropriate preparation. Previous study by Husna et al. (2013) revealed that high dosage (2000 mg.kg⁻¹) of *M. charantia* extract could provoke toxic effects on blood, tissue and vital organ especially liver. Accidental side effects may also arise owing to paucity of knowledge on the toxic components in plant materials, and the ineffective removal of contaminants such as heavy metals, toxins and infectious microbes (Zhang et al. 2012; Olaniyan et al. 2016). This study aimed to process *M. charantia* leaves using fresh, parboiled, and air-dried methods, and assess the safety of each extract in normal albino male rats through a subacute study.

Materials and Methods

Collection of *Momordica charantia* leaves. Fresh leaves were collected in Igodan, Okitipupa local government, Ondo State, Nigeria between May and August 2022. The leaves were identified and authenticated at the University Herbarium with the herbarium number OAUSTECH/H/0657. The leaves were sorted, destalked and washed thoroughly under running water and separated into three portions; freshly prepared *M. charantia* (FPMC), parboiled *M. charantia* (PBMC) and air-dried *M. charantia* (ADMC).

Extract preparation and concentration. FPMC was prepared by slicing the leaves and homogenizing 1000 g with 2500 mL of distilled

water using an electric blender (VTCL mixer and Blender, Gurudev Appliances, India). PBMC was prepared by parboiling sliced leaves (1000 g) with distilled water (2500 mL) for 10 min in a regulated water bath (85°C), cooled and gently homogenized. Leaf sample for ADMC was air-dried for 2 weeks and thereafter pulverized to coarse powder. Portion (1000 g) of the ADMC was soaked in distilled water (2500 mL). After each treatment, samples were allowed to stand for 24 h in an airtight volumetric flask with intermittent agitation. Each sample was filtered using double-cheese cloth to obtain filtrates and concentrated at 45°C using rotary evaporator (IKA Rotary Evaporator RV 10 digital V, Delson Pascal Ventures, Akure, Nigeria).

Experimental animals and study design. Total of fifty (50) male Wistar rats (120-150 g) were obtained from the animal house, Ladoke Akintola University of Science and Technology, Ogbomosho, Osun state, Nigeria. They were kept in cages and acclimatized for 2 weeks in a well-ventilated room. The animals were allowed free access to rat chow and water *ad libitum*. Treatment of the animals was in accordance with the principle of laboratory animal care (NIH Publication 85-93, revised 1985) and OAUSTECH ethical approved committee standards with reference code (OAUSTECH/ETHC-BCH/2022/05). After acclimatization, animals were allotted into four groups (n=5) and administered varying doses of FPMC, PBMC and ADMC. Animals in group 1 (control) received 0.5 mL of distilled water while group 2, 3 and 4 were respectively administered 100, 500 and 1000 mg extracts/kg body weight. The extract doses for this study were selected to consider the lower limit, median and upper limit dose range following OECD guideline no. 408 as described by Chung (2022). Extracts were orally administered daily (8.00–10.00 am) for 21 days using gavage and weekly body weight was monitored at intervals.

Sample collections and preparations. After 21 days of administration, rats were fasted overnight, sacrificed for blood and tissue samples collection. Blood samples were centrifuged using tabletop electric centrifuge (80-2 Winfield medical, UK) for 15 min at $3000 \times g$ to obtain plasma. Excised tissues were rinsed in physiological saline, dried between layers of Whatman filter paper, weighed

and homogenized with 0.1 M phosphate buffer (pH 7.4) at $4000 \times g$ for 20 min to obtain the supernatants. Portions of excised tissues were fixed in 10% buffered formaldehyde for histology.

Blood biochemical assays. Body chemistry parameters such as plasma protein concentration (PC), albumin (ALB), alanine and aspartate aminotransferase, alkaline phosphatase (ALP), gamma glutamyl-transpeptidase (GGT), uric acid (UC) and creatinine (CREA) were performed using Fortress diagnostic kits (Fortress Diagnostics, Antrim, United Kingdom). Triglyceride (TRIG), cholesterol (CHOL), high density lipoprotein-concentration (HDL-C) and low-density lipoprotein-concentration (LDL-C) using Randox kit (Randox Laboratories LTD, United Kingdom). Reaction mixtures were quantified with a 752N UV-VIS spectrophotometer (BOSCH, UK).

Analysis of oxidative stress indices

Tissue protein concentration (TPC). Total protein concentration in homogenate was determined using biuret method (Wu 2006). The principle is based on the formation of colored complex when protein peptide interacts with cupric ions in an alkaline medium. Sample (or standard solution) (0.01 mL) was mixed with diluted (1:4 v/v distilled water) biuret reagent (100 mM sodium hydroxide, 16 mM sodium-potassium tartrate, 15 mM potassium iodide and 6 mM copper sulphate). The mixture was incubated at 28°C for 30 min and the absorbance read at 550 nm against the reagent blank.

Reduced glutathione (GSH). Reduced glutathione was estimated by Ellman (1959) method. Aliquot (0.5 mL) of tissue homogenate was added 1.5 mL 10 % TCA and centrifuged. One milliliter (1 mL) of resulting supernatant was withdrawn and mixed with 0.5 mL of Ellman's reagent and 3 mL of phosphate buffer. The colour developed were read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3 mL of buffer.

Catalase (CAT). Catalase was determined according to the method of Cohen et al. (1970). The principle is based on the measurement of H_2O_2 decomposition rate after the addition of enzyme solution and further reaction with excess $KMnO_4$.

The final residual KMnO_4 is now measured spectrophotometrically at 480 nm. Firstly, the reaction mixture was initiated by sequentially adding at fixed intervals of 5 mL cold 30 mM H_2O_2 Phosphate buffer (pH 7.4) and thoroughly mixed by inversion. After 3 min, the reaction was stopped sequentially by adding 1 mL of 6 M H_2SO_4 and mixing by inversion. Finally, 7 mL of 0.01 M KMnO_4 was added to the test and blank tubes one at a time and were mixed twice by inversion. Sample (0.5 mL) was added to ice cold test tube and 0.5 mL of distilled water was added into another ice-cold tube as blank. The reference was prepared by adding 7 mL of 0.01 M KMnO_4 to a mixture of 5.5 mL phosphate buffer (pH 7.4) and 1 mL of 6 M H_2SO_4 solution. The absorbance was read at 480 nm within 30 to 90 sec.

Glutathione peroxidase (GPx). GPx was determined following Nyman (1959) method. The principle is due to the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting in a deep brown color disposition that is kinetically monitored. Aliquot (5.0 mL) of phosphate-buffered hydrogen peroxide (H_2O_2) was premixed with pyrogallol (1.5 mL), inverted and equilibrated to 20°C. Thereafter, portion (0.2 mL) of tissue homogenate was introduced to the mixture, mixed by inversion and absorbance (at 430 nm) of solution was monitored every 30 sec for 2 min.

Reduced glutathione was estimated by Ellman (1959) method. Zero point five milliliters (0.5 mL) of tissue homogenate was added 2 mL 10% TCA and centrifuged. One milliliter (1 mL) of supernatant was treated with 0.5 mL of Ellman's reagent and 3 mL of phosphate buffer. The color developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 mL of buffer.

Superoxide dismutase (SOD). SOD was determined according to the method of Misra and Fridovich (1972) based on the ability of the enzyme to inhibit adrenaline autoxidation. Sample (0.4 mL) was added to ice cold test tube containing 5 mL of 0.05 M carbonate buffer (pH 10.2) to which the reaction was started by the addition of 0.6 mL of freshly prepared 0.3 mM epinephrine as the substrate to the buffered supernatant mixture which

was quickly mixed by inversion. The blank or reference contained 5 mL of 0.05 M carbonate buffer (pH 10.2), 0.4 mL distilled water and 0.6 mL of freshly prepared 0.3 mM epinephrine as the substrate. The increase in absorbance at 480 nm was monitored every 30 seconds for 120 sec.

Malondialdehyde (MDA) level. Lipid peroxidation (LPO) was estimated to be following thiobarbituric acid colorimetric reaction method (Ohkawa et al. 1979). One milliliter of the sample was added to 2 mL LPO reagent (1:1:1 of 0.37% Thiobarbituric acid, 15% Trichloroacetic acid and 0.24 N Hydrochloric acid). The tubes were capped and immersed in boiling water for 15 min and swirled slightly at intervals. The mixture was cooled and centrifuged for 10 min at 5000 rpm. The absorbance was read at 532 nm using the reagent blank.

Histopathological examination. Excised tissues were fixed in 10% buffered formaldehyde in labelled bottles, processed, stained with hematoxylin and eosin, mounted on glass slides and examined under a standard light microscope for histopathological conditions.

Statistical analysis. Data are expressed as the mean $\pm$ standard error of the mean. Analyses were performed using one-way analysis of variance and differences in mean values were considered to be statistically significant at $p < 0.05$ using the SPSS statistical package. Graphical representation of the results was performed using the GraphPad Prism 5.01 package.

Results and Discussion

Weekly body weight. Medicinal plants are usually processed in different ways with the aim of enhancing their therapeutic values, palatability and decreasing their toxic effects. Animals that were administered varying doses of FPMC, PBMC and ADCMC showed a steady increase in body weight until the last day of administration (Fig. 1). The increasing body weights of animals could presumably be due to retention, increased bioavailability of residual nutrients and improved caloric value (Akintimehin and Onoagbe 2023) following the different preparations (FPMC, PBMC and ADCMC).

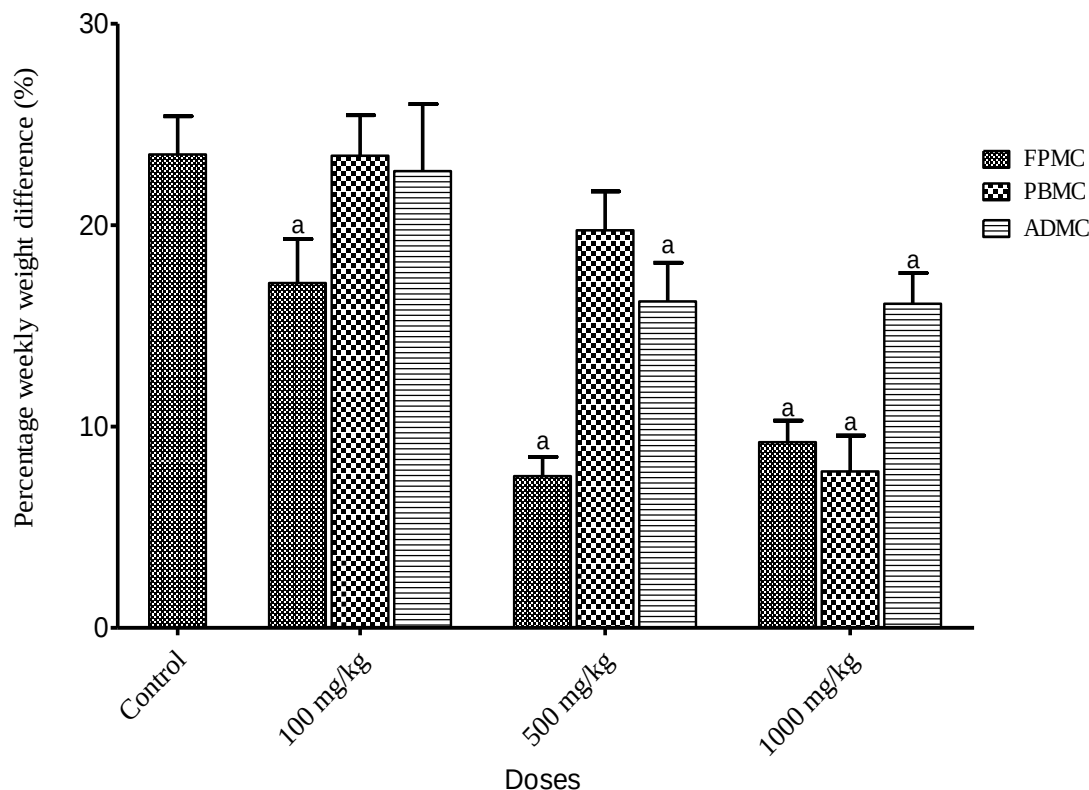


Figure 1. Percentage weekly body weight difference of animals that were administered extracts of *M. charantia* (FPMC, PBMC and ADMC). Values were the mean $\pm$ standard error of mean ^a $p < 0.05$, significant when extracts treated groups were compared to control groups. Control, administered distilled water; FPMC, freshly prepared *Momordica charantia*; PBMC, parboiled extract of *Momordica charantia*; ADMC, air-dried *Momordica charantia*

This finding could suggest that the different preparations did not adversely influence extracts palatability nor do extracts from each preparation induced appetite suppression in animals. Body weight and internal organs are sensitive indices of toxicity following acute or chronic exposure to xenobiotics (Tan et al. 2008).

Blood biochemical assays. The results of protein concentration, albumin and hepatic indices of rats that were administered doses of *M. charantia* extracts are presented in Table 1. Animals that were administered 100 and 500 mg.kg⁻¹ of FPMC showed no changes in PC while animals in other groups demonstrated a significant decrease. The significant decrease in PC of animals that were administered PBMC and ADMC might not directly link to toxic response of the extracts since protein concentration in liver and kidney were not affected (Table 2). This noticeable reduction could be due to

activities of plant metabolites that are involved in the degradation, conjugation or elimination of circulatory protein. On the other hand, albumin concentration in all treated groups showed no significant difference compared to control except for animals that were administered a high dose of FPMC which demonstrated a significant ($p < 0.05$) increase. Impairments in the hepatocellular synthesis of protein and albumin are reported to be early indications of mild to severe liver injury (Olaniyan et al. 2016). To predict the safety of a newly formulated drugs before consumption, toxicity data are required and are usually obtained by evaluating the level of biomarkers in the body fluid or tissues (Chanda et al 2015). ALT, ALP and GGT of most treated groups were not adversely affected by FPMC, PBMC and ADMC administrations. Conversely, AST activities in all treated groups were significantly ($p < 0.05$) elevated.

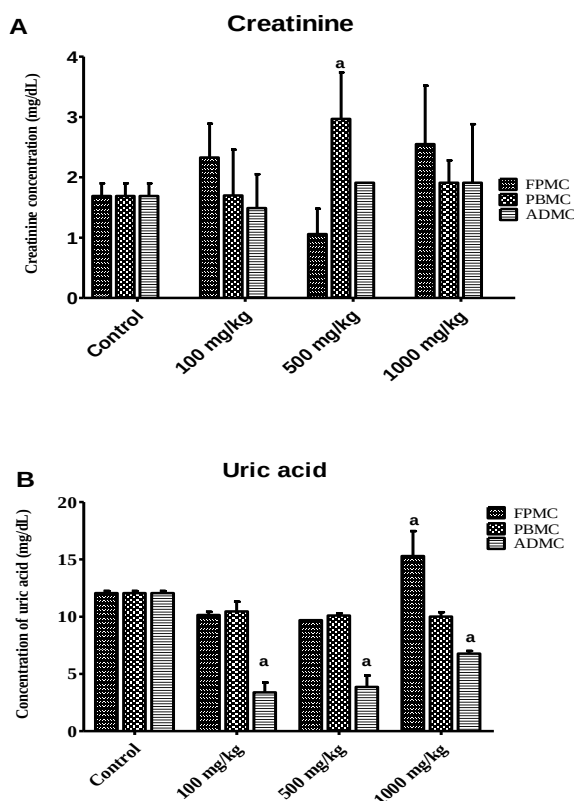


Figure 2. The kidney function (a) creatinine (b) uric acid of FPMC, PBMC and ADMC administered rats. Values were the mean $\pm$ SEM ^a $p < 0.05$, significant when treated groups were compared to control. Control, administered distilled water; FPMC, freshly prepared *Momordica charantia*; PBMC, parboiled extract of *Momordica charantia*; ADMC, air-dried *Momordica charantia*

This observation is somewhat puzzling and could partly suggest mild hepatotoxicity or absence of hepatic injury since other hepatic markers (GGT and ALP) including liver specific transaminase (ALT) were not adversely affected (Abdillah et al 2020).

Literature has revealed that variations in hepatic markers are occasionally expected since some transaminases (such as AST) resides in the cytoplasm and mitochondria of several cells (Al-Mammary et al. 2002). Findings from kidney indices revealed that only animals that received

500 mg PBMC.kg⁻¹ demonstrated a significant ($P < 0.05$) increase in creatinine (Fig. 2a). Rats that received only 1000 mg FPMC.kg⁻¹ demonstrated a significant increase in uric acid while other FPMC doses and all PBMC doses caused non-significant changes compared to control. UC of animals that received ADMC reduced significantly (Fig. 2b). In renal dysfunction, metabolic wastes that are expected to undergo clearance through acidification into urine are usually concentrated in the blood. Creatinine and UC are by-products of creatine phosphate and purine respectively, and they commonly serve as markers for assessing glomerular function (Damiasi 2019). The noticeable increase in creatinine of rats that received 500 mg PBMC.kg⁻¹ might not necessarily indicate kidney damage since their counterparts (1000 mg PBMC.kg⁻¹) demonstrated non-significant changes (Fig. 2a). Interestingly, the value of creatinine in rats that received 500 mg PBMC.kg⁻¹ was not twice the value obtained for the control. The lack of uniformity in creatinine concentration of PBMC treated animals could be linked to factors such as glomerular makeup of animals in the group and muscle bulk probably due to protein content in the extract (Gounden et al. 2022). Accumulation of both urate and uric acid in humans are common causes of calculi in the joints and consequently result in gout, hyperuricemia, arthritis and rheumatic pain. The significant increase of UC concentration in animals that received a high dose (1000 mg.kg⁻¹) of FPMC could be due to ample release of purine molecules from the plant genome during FPMC preparation and increased activity of xanthine oxidase. A recent review revealed that a high dietary intake of purines in plant materials (vegetables and fruits) could result in the bioaccumulation of UC (Hafez et al. 2017). The unperturbed UC level in PBMC treated animals and significant decrease in ADMC treated animals could possibly be due to loss of purine molecules during processing and impeding action of bioactive constituents in PBMC and ADMC against xanthine oxidase activities (Hafez et al. 2017).

The triglyceride concentration was significantly reduced in all treated groups compared to the control (Fig. 3 TRIG).

Table 1. Result of total protein concentration, albumin and liver marker enzymes in FPMC, PBMC and ADMC administered rats

Treatment	PC, g.L ⁻¹	ALB, g.dL ⁻¹	AST, U.L ⁻¹	ALT, U.L ⁻¹	ALP, U.L ⁻¹	GGT, U.L ⁻¹
Control (Dist. H ₂ O)	38.44 ± 2.05	3.40 ± 1.15	46.83 ± 1.64	135.07 ± 5.09	53.37 ± 3.04	268.74 ± 5.25
100 mg.kg⁻¹						
FPMC	32.49 ± 2.70	4.84 ± 0.83	106.33 ± 1.45 ^a	62.73 ± 9.80 ^a	59.41 ± 3.90	128.75 ± 18.01 ^a
PBMC	8.42 ± 1.68 ^a	1.81 ± 0.38	100.00 ± 2.08 ^a	95.53 ± 2.60 ^a	58.28 ± 7.09	89.50 ± 8.99 ^a
ADMC	14.25 ± 0.67 ^a	3.58 ± 0.97	104.00 ± 5.13 ^a	126.40 ± 12.14	50.20 ± 2.38	268.00 ± 20.56
500 mg.kg⁻¹						
FPMC	37.43 ± 3.05	1.83 ± 0.39	117.67 ± 0.60 ^a	112.80 ± 12.04	55.50 ± 6.74	121.59 ± 4.30 ^a
PBMC	15.52 ± 0.23 ^a	3.54 ± 0.40	121.00 ± 3.77 ^a	152.27 ± 4.91	66.43 ± 3.88	92.33 ± 0.25 ^a
ADMC	17.73 ± 0.34 ^a	4.97 ± 0.98	155.83 ± 2.33 ^a	141.60 ± 7.15	57.28 ± 7.52	143.23 ± 6.98 ^a
1000 mg.kg⁻¹						
FPMC	17.16 ± 1.01 ^a	5.69 ± 0.86 ^a	123.33 ± 0.44 ^a	151.13 ± 2.48	68.28 ± 10.09	109.62 ± 1.54 ^a
PBMC	22.23 ± 0.61 ^a	2.76 ± 0.62	103.83 ± 4.60 ^a	107.60 ± 9.74 ^a	63.61 ± 7.72	169.74 ± 1.98 ^a
ADMC	15.96 ± 0.77 ^a	4.74 ± 0.34	98.00 ± 1.32 ^a	142.80 ± 7.66	81.55 ± 7.05 ^a	132.95 ± 10.56 ^a

*Values are expressed as the mean ± standard error of mean ^a p<0.05, significant when extracts treated groups were compared to control group. Control, administered distilled water; FPMC, freshly prepared *Momordica charantia*; PBMC, parboiled extract of *Momordica charantia*; ADMC, air-dried *Momordica charantia*.

The percentage reduction of triglyceride in animals that were administered doses of FPMC were 84.07, 39.82 and 30.09%, respectively. The percentage reduction in PBMC doses (100, 500 and 1000 mg.kg⁻¹) treated rats were 43.36, 69.03 and 56.64% respectively while ADMC doses caused percentage reduction of 59.29, 79.65 and 54.87% respectively. Except for a significant increase that was observed in animals that received 100 mg FPMC.kg⁻¹, doses of other processing methods caused no significant changes in cholesterol concentration (Fig. 3 CHOL). The percentage increase in cholesterol of animals that were administered 100 mg FPMC.kg⁻¹ was 12.44% relative to the control. Animals that were administered 100 mg.kg⁻¹ FPMC, PBMC and ADMC demonstrated a significant reduction in

HDL-C. While 500 mg.kg⁻¹ and 1000 mg.kg⁻¹ PBMC caused a significant reduction in HDL-C, FPMC and ADMC were indistinguishable relative to the control (Fig. 3 HDL-C). LDL-C significantly increased in animals that received 100 mg.kg⁻¹ of FPMC, PBMC and ADMC. Except for the 500 mg.kg⁻¹ and 1000 mg.kg⁻¹ PBMC which showed a significant increase in LDL-C, FPMC and ADMC treated rats showed no significant difference compared to the control (Fig. 3 LDL-C). A high concentration of lipids in the blood has been identified as a major risk factor that contributes to the incidence and severity of atherosclerosis and other associated hyperlipidemic complications (Wang et al. 2010). The triglyceride and cholesterol lowering effects by *M. charantia* leaf

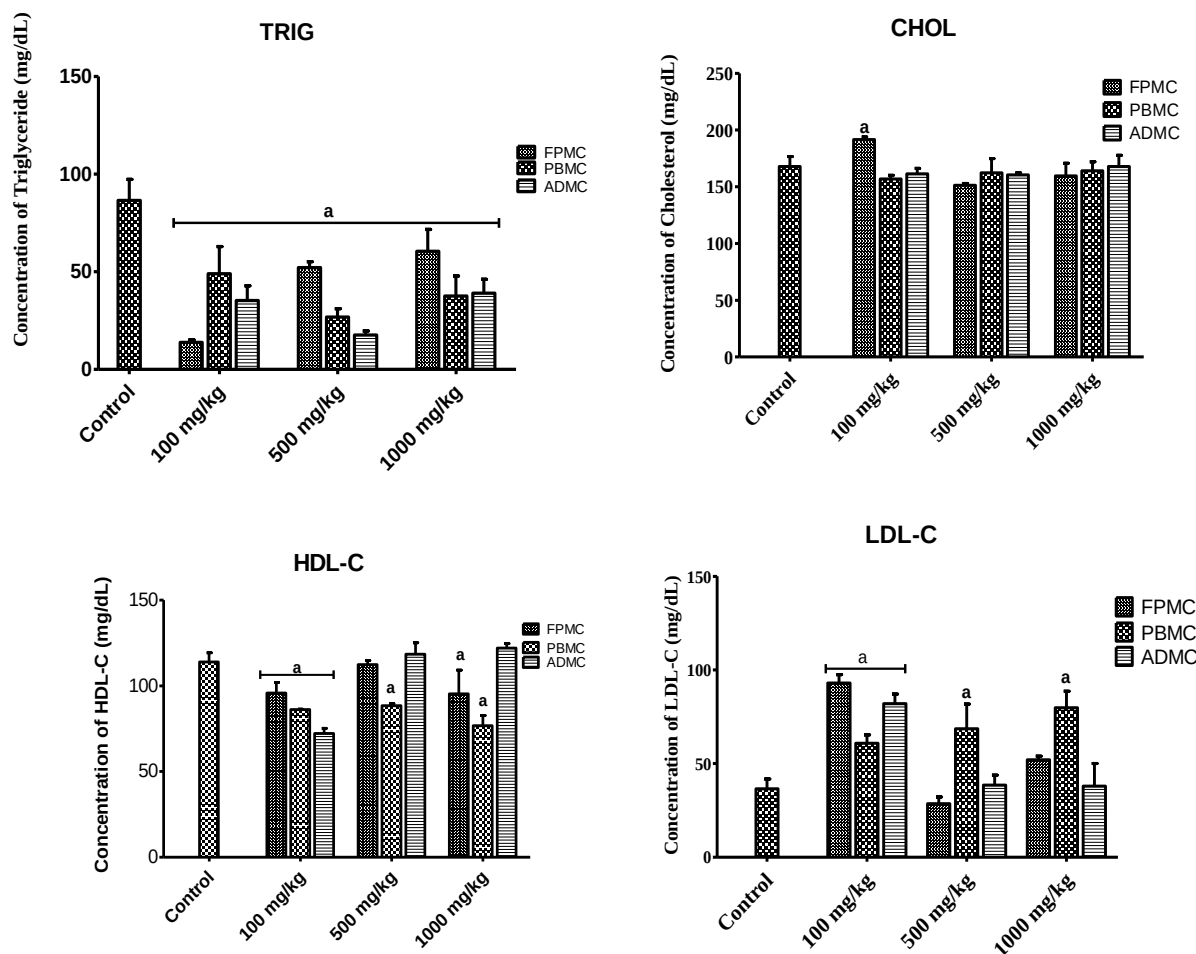


Figure 3. Effects of varying doses of FPMC, PBMC and ADMC on lipid profile of experimental rats. Values were the mean $\pm$ standard error of mean ^a $p < 0.05$, significant when extracts treated groups were compared to control group. Control, administered distilled water; FPMC, freshly prepared *Momordica charantia*; PBMC, parboiled extract of *Momordica charantia*; ADMC, air-dried *Momordica charantia*

extracts in this study is similar when compared to other documented medicinal plants. Possible explanation for these tremendous effects could be delayed absorption of dietary lipids by fiber constituents in the processed extracts and inhibition of pancreatic lipase by the extract's active constituents. Previous report has revealed that *M. charantia* extracts can lower blood lipid using different mechanisms such as increase expression of lipoprotein lipase, adipogenesis via the insulin sensitivity pathway and increased lipid storage (Oyelere et al. 2022). The ratio of lipoproteins (LDL/HDL) is also an important factor to be considered when monitoring the risk of atherogenicity. This study revealed that low

dose of FPMC and ADMC as well as doses of PBMC demonstrated poor cholesterol transport across the body organs.

Oxidative stress indices. The sub-acute administration of FPMC and ADMC for 21 days caused plausible changes in liver and kidney TPC while PBMC caused no significant difference only in liver TPC (Table 2). The level of protein in both liver and kidney could suggest that the herbal preparations might not elicit extract's toxicity. Previous report revealed that low levels of protein in organs could reflect impairment in synthesis due to organ damage (Olaniyan et al. 2016; Adetuyi et al. 2023). Body cells are equipped and

Table 2. Effects of FPMC, PBMC and ADMC on liver and kidney oxidative stress indices

Treatment	TPC, g.L ⁻¹	CAT, U.g ⁻¹ tissue	SOD, U.g ⁻¹ tissue	GPx, μmol.mg ⁻¹ protein	GSH, μmol.g ⁻¹ protein	MDA, μmol.mg ⁻¹ protein
LIVER						
Control (Dist. H ₂ O)	33.19 ± 6.40	3.43 ± 5.06	0.30 ± 0.03	15.47 ± 2.90	1.94 ± 0.24	0.87 ± 0.09
100 mg.kg⁻¹						
FPMC	42.83 ± 7.18	14.61 ± 3.23 ^a	0.34 ± 0.03	13.68 ± 1.98	1.32 ± 0.40	0.25 ± 0.05 ^a
PBMC	59.75 ± 7.56 ^a	11.82 ± 2.03 ^a	0.33 ± 0.02	14.83 ± 5.48	0.40 ± 0.16 ^a	0.42 ± 0.04
ADMC	20.65 ± 2.24	19.67 ± 4.07 ^a	0.41 ± 0.03 ^a	14.05 ± 6.20	2.55 ± 0.52	0.71 ± 0.26
500 mg.kg⁻¹						
FPMC	60.04 ± 2.14 ^a	16.71 ± 0.93 ^a	0.26 ± 0.02	17.00 ± 4.55	1.06 ± 0.13	0.31 ± 0.09 ^a
PBMC	27.53 ± 6.46	10.83 ± 1.34 ^a	0.35 ± 0.05	10.11 ± 1.98	1.38 ± 0.59	0.56 ± 0.14
ADMC	47.88 ± 8.91	6.21 ± 1.04	0.34 ± 0.02	23.71 ± 7.56	0.74 ± 0.47 ^a	0.37 ± 0.08 ^a
1000 mg.kg⁻¹						
FPMC	57.06 ± 3.88 ^a	18.35 ± 2.97 ^a	0.33 ± 0.02	16.80 ± 0.61	0.86 ± 0.18 ^a	0.34 ± 0.12 ^a
PBMC	30.72 ± 4.94	15.02 ± 2.80 ^a	0.36 ± 0.03	11.82 ± 1.05	1.60 ± 0.16	0.59 ± 0.32
ADMC	89.49 ± 0.44 ^a	21.36 ± 1.45 ^a	0.30 ± 0.01	11.31 ± 0.93	0.28 ± 0.06 ^a	0.07 ± 0.02 ^a
KIDNEY						
Control (Dist. H ₂ O)	15.01 ± 0.96	10.80 ± 0.74	1.54 ± 0.06	38.14 ± 0.95	3.17 ± 0.48	0.57 ± 0.22
100 mg.kg⁻¹						
FPMC	17.48 ± 0.38 ^a	18.70 ± 0.74 ^a	1.63 ± 0.06	27.44 ± 2.20 ^a	2.53 ± 0.34	0.16 ± 0.01
PBMC	16.97 ± 0.23 ^a	12.86 ± 1.73	1.81 ± 0.10	56.26 ± 0.30 ^a	3.02 ± 0.39	1.18 ± 0.64
ADMC	18.00 ± 0.06 ^a	25.85 ± 1.09 ^a	1.92 ± 0.06 ^a	63.42 ± 2.33 ^a	2.31 ± 0.76	0.20 ± 0.02
500 mg.kg⁻¹						
FPMC	16.66 ± 0.06 ^a	6.30 ± 1.16 ^a	1.35 ± 0.07	26.43 ± 5.61 ^a	4.84 ± 0.89 ^a	0.15 ± 0.01
PBMC	17.42 ± 0.79 ^a	2.98 ± 1.00 ^a	1.88 ± 0.30	29.13 ± 2.76 ^a	1.86 ± 0.73	0.90 ± 0.52
ADMC	19.44 ± 0.64 ^a	13.74 ± 1.85	1.36 ± 0.07	34.87 ± 1.42	2.61 ± 0.30	0.19 ± 0.02
1000 mg.kg⁻¹						
FPMC	16.97 ± 0.82 ^a	9.38 ± 0.15	1.66 ± 0.09	16.30 ± 1.58 ^a	5.01 ± 0.31 ^a	0.14 ± 0.01
PBMC	18.30 ± 0.06 ^a	4.70 ± 1.12 ^a	1.70 ± 0.11	35.50 ± 1.70	3.57 ± 0.49	0.61 ± 0.15
ADMC	18.43 ± 0.22 ^a	19.38 ± 2.00 ^a	1.30 ± 0.10	23.52 ± 3.06 ^a	1.40 ± 0.19 ^a	0.15 ± 0.02

*Values are expressed as the mean ± standard error of mean ^a p<0.05, significant when extracts treated groups were compared to control group. Control, administered distilled water; FPMC, freshly prepared *Momordica charantia*; PBMC, parboiled extract of *Momordica charantia*; ADMC, air-dried *Momordica charantia*.

frequently protected from the damaging effects of radicals by several enzymic and non-enzymic antioxidants. Natural antioxidants such as amines, betalains, phenolic acids, terpenoids and vitamins

from plant origin have been reported to significantly boost the activities of *in-vivo* antioxidants (Manjula and Ammani 2012). There was a significant increase ($p<0.05$) in the liver

catalase of animals that administered doses of FPMC, PBMC and ADCM while GPx activities in all treated groups remained unchanged. Except for the group that received 100 mg ADCM.kg⁻¹ which demonstrated a significant increase in liver SOD, doses of FPMC and PBMC caused insignificant difference in SOD activities relative to control. The significant increase ($p<0.05$) in liver catalase as well as indifferences in SOD and GPx activities of treated animals could suggest that active compounds in extracts do not adversely impair the

liver cellular defense mechanisms.

Except for PBMC that caused steady increase in liver GSH, FPMC and ADCM caused a dose-dependent decrease in liver glutathione with significant decrease only observed at high dose 1000 mg.kg⁻¹. A possible explanation for these changes could be linked to varying level and activities of bioactive compounds (such as phenols and vitamins C and E) in the extracts, caused by the different preparations (Akintimehin et al. 2024).

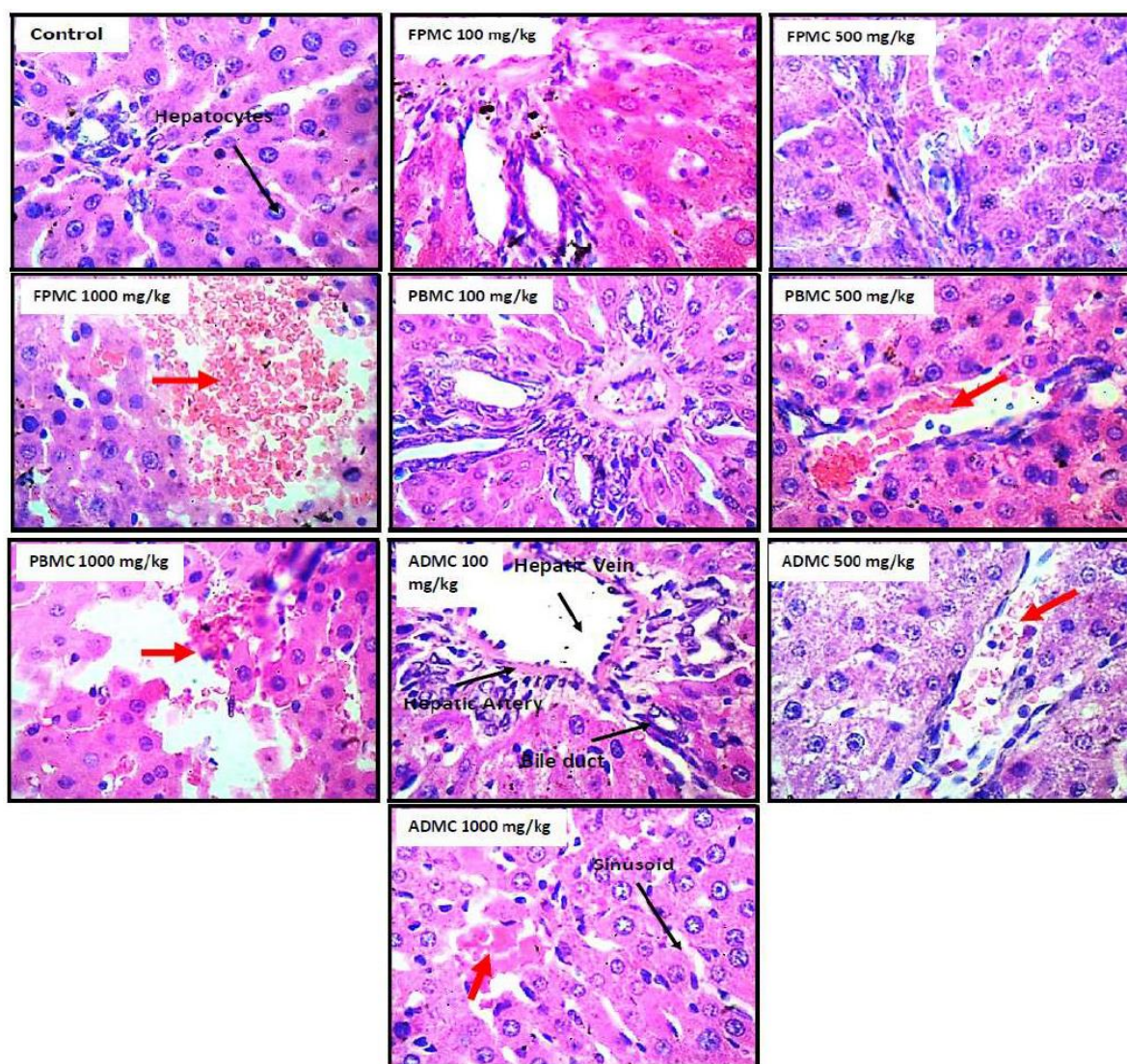


Figure 4. Liver micromorphological section demonstrated by haematoxylin and eosin staining at high magnification ($\times 400$). The hepatocytes, sinusoids and the portal triad (hepatic vein, hepatic artery and bile duct) were all visible across the various groups

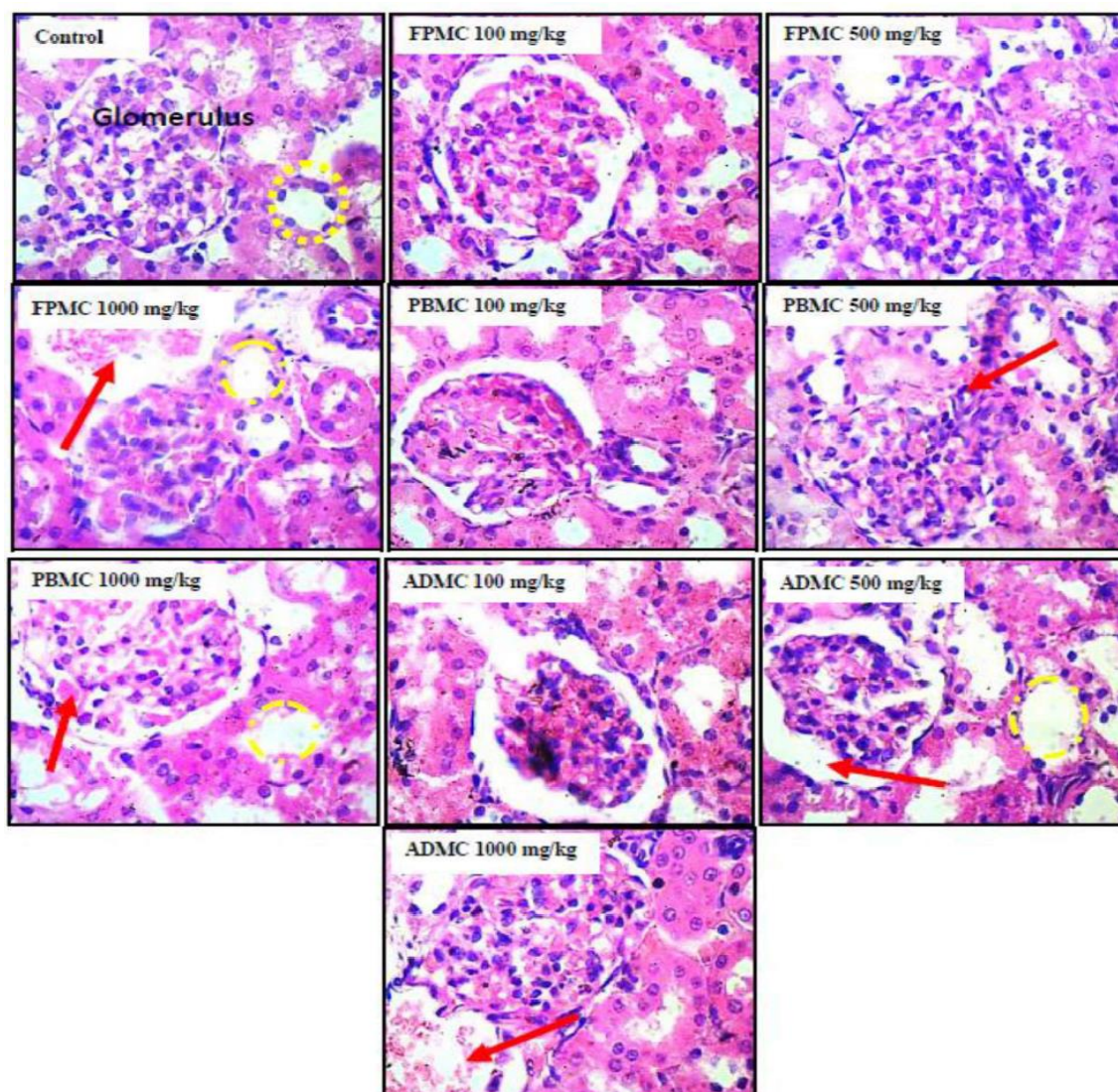


Figure 5. Magnified views of kidney micromorphological sections demonstrated by hematoxylin and eosin staining at high magnification ($\times 400$). The renal cortex, renal tubules (yellow dotted circle), glomeruli and mesangial cells as well as proximal and distal renal convoluted tubules are all visible across the various groups

Boiling has been reported to increase phenolic compounds in medicinal plants as well as disintegration of compounds such as vitamin C and tocopherol (López-García et al. 2018; Arias-Rico et al. 2020). The phenolic contents in the boiled sample might have presumably contributed to the increased GSH by constantly donating protons for interconversion of oxidized glutathione to GSH (Akintimehin et al. 2024). The reduction that was observed in GSH of animals that received FPMC and ADMC might be due to increased antioxidant

activities of vitamin C and E. Skeletal radicals that are produced from dietary ascorbate and tocopherol are reported to constantly regenerate to their active states by dissipating GSSG/GSH ratio, consequently causing GSH reduction (Akintimehin et al. 2021).

Only FPMC and ADMC caused significant decrease in MDA levels at 500 mg.kg⁻¹ and 1000 mg.kg⁻¹ compared to the control. The significant reduction observed in liver MDA could suggest

hepatoprotection and non-toxic effects of extracts as well as sustenance of membrane integrity. Animals that received ADMC demonstrated an excellent kidney catalase and GPx activities (Table 2). However, the activities decreased in animals that received 500 mg.kg⁻¹ and 1000 mg.kg⁻¹ of FPMC and PBMC. All the extracts did not perturb the activities of kidney SOD. Except 1000 mg ADMC.kg⁻¹ that demonstrated a significant ($p<0.05$) decrease in GSH, doses for FPMC, PBMC and ADMC did not affect GSH levels. The levels of Malondialdehyde did not change in the kidneys of animals that were administered FPMC, PBMC and ADMC. The weak ability of FPMC and PBMC to effectively scavenge hydrogen peroxide at high doses is quite puzzling despite no observed changes in kidney SOD, GSH and MDA. The significant reduction in catalase activities of FPMC and PBMC could either be due to interference of extract's active metabolites and catalase activity or regeneration of peroxides from their byproducts. Furthermore, overburdened elimination process of the nephron owing to the frequency and duration of extracts and their reactive metabolites could also play a major role. A previous report has revealed that active compounds from medicinal plants could release reactive metabolites after interacting with the body system, consequently causing a slight surge in the oxidative microenvironment of cells (Oso et al. 2019).

Histology. Histological examination revealed that only high dose of extracts altered the architecture of the organs. Plates without red arrows in the micromorphological liver section revealed normal central venules without congestion, normal hepatocytes morphology and sinusoids without infiltration. Plates with red arrows show dilated central venules with congestion and mild pyknotic hepatocytes (Fig. 4).

The infiltration of the nuclear cytoplasm by fibrosis, the presence of inflammatory red cells that was observed across profiles could be due to activities of reactive metabolites of the extracts. The mild sinusoids infiltration and haemorrhage that was observed could be associated with stress response of the liver probably due to daily dosing of extracts or detoxification process of the active metabolites. From the kidney sections (Fig. 5), plate without red arrows shows normal architecture

as revealed by the normal renal cortex, glomeruli with mesangial cells and capsular spaces. The renal tubules also appeared normal and not congested with a well-defined interstitial space, similar to the control. Plates with red arrows show degenerative changes, characterized by congested renal tubules (proximal and distal convoluted tubules), infiltrated renal parenchyma by red inflammatory cells, dilated renal tubules, and widened capsular margins. The signs of glomerulosclerosis, glomerulonephritis and hemorrhage in the kidney of animals that received high doses of extracts could be attributed to overwhelming elimination of reactive metabolites in the extract (Akintimehin and Onoagbe 2023).

Conclusions

In conclusion, the results from this finding clearly demonstrate that the different preparation modes do not elicit adverse reactions in the body chemistry and organ functionality of the tested animals. However, despite the different preparation methods are relatively safe, the slight changes in the structural architecture of organs across experimental animals at high doses could suggest consciousness of the dose.

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Author Contributions

Conceptualization, E.S.A. and K.O.K.; methodology, E.S.A., K.O.K. and I.O.O.; formal analysis E.S.A., and K.O.K.; investigation, E.S.A., I.A.E., and K.O.K.; resources, K.O.K., F.O.A., I.O.O., and E.S.A.; data curation, E.S.A., and I.A.E.; writing – original draft preparation, I.A.E., and E.S.A.; writing - I.A.E., E.S.A., and K.O.K., review and editing, K.O.K., F.O.A., and I.O.O.; supervision, F.O.A., and I.O.O.; All authors have read and agreed to the published version of the manuscript.

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The experimental protocol and treatment of the animals was in accordance with the principle of laboratory animal care (NIH Publication 85-93, revised 1985) and OAUSTECH ethical approved committee standards with reference code (OAUSTECH/ETHC-BCH/2022/05)

Informed Consent Statement

Not applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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