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ORIGINAL RESEARCH ARTICLE

Blood cellular, serum biochemical, and organosomatic alterations in albino rats following sub-acute oral administration of varied doses of *Pterocarpus santalinoides* methanolic leaf extract

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ABSTRACT

Background: Leaf extracts of *Pterocarpus santalinoides* are used in ethno-medicine for the treatment of liver diseases, and studies had validated its efficacy.

Aim: This study evaluated the blood cellular, serum biochemical, and organosomatic alterations associated with sub-acute oral administration of *Pterocarpus santalinoides* methanolic leaf extract (PSMLE) to albino rats.

Methods: To be used for the study, 25 female rats were randomly assigned into four groups (1–4) of five each. Group 1 rats (untreated control) were given distilled water placebo, while Groups 2, 3, and 4 were treated orally with 50, 250, and 500 mg/kg PSMLE, respectively. Treatment was done daily for 28 days after which blood samples were collected for hematology and blood biochemistry. The rats were afterwards humanely sacrificed, and the liver, kidneys, heart, and spleen were eviscerated and weighed.

Results: Serum total protein levels of Group 2 and Group 3 rats were significantly ($p<0.05$) higher than that of Groups 1 and 4, while serum globulin levels of Group 2 were significantly higher ($p<0.05$) than that of Groups 1 and 4. All other serum biochemical and all hematological parameters and the relative liver, kidney, spleen, and heart weights did not significantly ($p>0.05$) vary among the groups. Mean body weights of Group 3 rats were significantly ($p<0.05$) higher than that of Groups 1 and 2 on Day 28.

Conclusion: Sub-acute administration of PSMLE to rats led to no significant hematological, serum biochemical, and organosomatic alterations, except for significantly higher serum total proteins and globulins and higher body weights.

KEYWORDS:
hematology; organosomatic indices; *Pterocarpus santalinoides* extract; serum biochemistry; sub-acute toxicity

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INTRODUCTION

Plants have been used in virtually all cultures as a source of medicine, and are considered to be the backbone of traditional medicine (Cragg & Newman, 2001). All over the world, especially in developing countries, herbal drugs play an important role in health care programs because they are readily available, accessible, affordable, and acceptable to consumers (Sofowora, 1985; George, 2011). The use of different parts of medicinal plants to alleviate specific ailments has been in practice since ancient times, and about 80% of the world's population use medicinal plants as their primary source of medication (Cragg & Newman, 2001; Uma et al., 2013). The rationale for the traditional utilization of medicinal plants has rested largely on long-term cultural and clinical experience, with little or no scientific data on their efficacy and safety, as they are mostly presumed safe or non-toxic because they were obtained from "natural" sources (Gesler, 1992; Zhu et al., 2002; Bent & Ko, 2004; Bhowmik et al., 2009). However, these herbal products may contain bioactive principles with potential to cause damage to body organs (Asante-Duah, 2002; Schilter et al., 2003; Bhowmik et al., 2009). With the upsurge in the use of herbal medicines, a thorough scientific investigation of these medicines is imperative, hence the need to validate their folkloric usage and safety (Sofowora, 1989).

Toxicity studies are usually done to determine the safety of new drugs, and such toxicity studies may be acute, sub-acute, or chronic depending on the duration of exposure of experimental animals to the drugs (Parasuraman, 2011; Saganuwan, 2017). Sub-acute toxicity is the toxic effect produced by repeated exposure of experimental animals to the test drug in sub-lethal doses for a period of 14 to 28 days (Parasuraman, 2011). Sub-acute toxicity studies commonly involve determination of the effects of a test drug on biochemical and hematological parameters as well as evaluation of possible organosomatic and histomorphological alterations (Stevens & Mylecraine, 1994; Parasuraman, 2011). Blood constituents and organosomatic indices may change in relation to health conditions and these changes are of value in assessing the response of animals and humans to various physiological and pathological stresses (Bloom, 1993; Burdinsky Jr., 2000; Giannini et al., 2003; Stockham & Scott, 2008). Specifically, serum biochemical alterations are known indicators of the integrity and functionality of vital body organs such as the liver, kidney, heart, and brain (Giannini et al., 2003; Stockham & Scott, 2008). Thus, the evaluation of the effects of medicinal plant extracts on hematology, serum biochemistry, and morphology of vital body organs is crucial when considering the safety of these medicinal plants.

Pterocarpus santalinoides DC (Figure 1) is a plant indigenous to tropical Western and Southern Africa, and South America. It is an evergreen, small to medium sized tree that belongs to the Family *Papilionaceae* (Keay, 1989; Adetunji, 2007). It is commonly known as "red sandal wood" in English language (Adetunji, 2007), and as *nturukpa* in Igbo language of Nigeria (Anowi et al., 2012). The tender leaves of the plant are used as soup vegetable in Eastern Nigeria, and extracts of its leaves, stem bark, and roots are used in ethno-medicine for the treatment of various ailments (Adesina, 1982; Okwu & Ekeke, 2003). Research reports in available literature showed that *P. santalinoides* leaf extract possesses hepatoprotective and antioxidant activities, and glucose and lipid lowering properties (Offor et al., 2015; Ihedioha et al., 2017, 2018 & 2019). No form of toxicity has been reported with the folkloric use of the leaves of *P. santalinoides* as vegetable and ethno-medicine in humans, and as fodder or ethno-medicine in livestock (Poppenga, 2007; Tiwari, 2010; Anowi et al., 2012). Acute and chronic toxicity studies on the leaf extracts by different researchers has not reported any substantial toxicity either (Anowi et al., 2012; Ihedioha et al., 2020), but there have been no reports in available literature on its sub-acute toxicity/safety. Hence the present study, which evaluated blood cellular, serum biochemical, and organosomatic alterations associated with sub-acute administration of varied doses of methanolic leaf extract of *P. santalinoides* in albino rats.

Figure 1 *Pterocarpus santalinoides* tree, with a close focus on the leaves inset on the right.

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

The test reagents for evaluation of the serum activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST), and levels of serum total proteins, albumins, and total cholesterol and creatinine were procured from Quimica Clinica Aplicada (QCA), Spain. That for evaluation of serum bilirubins was sourced from Randox

Laboratories Ltd, County Antrim, United Kingdom, while the test reagents for serum urea evaluation were sourced from DIALAB, Neudorf, Austria. The glucometer and blood glucose test strips used for the determination of fasting blood glucose levels (FBGL) were a product of Roche Diagnostics GmbH, Mannheim, Germany. Thiopentone sodium was procured from Chandra Bhagat Pharma Pvt., Ltd., Mumbai, India. All other reagents, diluting fluids and chemicals used for the study were of analytical grade.

PREPARATION OF PTEROCARPUS SANTALINOIDES METHANOLIC LEAF EXTRACT

Leaves of *Pterocarpus santalinoides* were collected fresh from the tree at Nru in Nsukka, Enugu State Nigeria, in June 2018, for the study. The plant was identified by a plant taxonomist (Mr. A.O. Ozioko), of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, and a voucher specimen (UNH/2018 No. 2) was deposited at the University of Nigeria herbarium. The leaves were air-dried under shade and pulverized to powdered form. Using cold maceration technique, 300 g of the powdered leaves were extracted with 80% methanol. The resulting extract was filtered with a Whatman size 1 filter paper, evaporated to dryness in a rotary evaporator, and stored in a refrigerator at 4°C as *Pterocarpus santalinoides* methanolic leaf extract (PSMLE).

ANIMALS USED FOR THE STUDY

For the study, 20 female albino rats (*Rattus norvegicus*) of 10 weeks of age, with body weight range 163–181 g, were procured from the Laboratory Animal Section of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The rats were kept in stainless steel cages in a fly-proof animal house with room temperature range of 23–28°C, and they were allowed 2 weeks to acclimatize before the study commenced. They were fed commercial rat chow (Grand Cereals Ltd, Jos, Nigeria), and were provided with clean drinking water ad libitum all through the study. The rats were handled humanely all through the study period, and guidelines for humane laboratory animal use in investigations were strictly followed (Ward & Elsea, 1997). The Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, approved the protocol for the laboratory animal study (Approval Reference Number: FVM-UNN-IACUC/2018/0814).

EXPERIMENTAL DESIGN

After acclimatization, the 20 rats used for the study were weighed and randomly assigned into four groups (1–4) of five rats each. Group 1 rats (untreated control) were given distilled water placebo at the dose of 10 ml/kg body weight. Rats in Groups 2, 3, and 4 were treated daily with 50, 250, and 500 mg/kg PSMLE, respectively. Treatments were done orally for 28 days. After the administration of the last dose on Day 28, the rats were weighed again and blood samples for haematology and serum biochemistry were collected from each of them. After the blood sample collection, the rats were euthanized by intra-peritoneal injection of 250 mg/kg thiopentone sodium (AVMA, 2013). The liver, kidney, heart, and spleen of all the rats were carefully eviscerated and weighed. The relative organ weights (organ weight percentage of body weight) of each were calculated.

METHODS

Blood samples were obtained from the rats by the orbital bleeding technique (Bolliger & Everds, 2010). Fasting blood glucose levels (FBGL) were immediately determined on whole blood by the glucose oxidase method (Sacks, 2008), using a glucometer (Roche Diagnostics, Mannheim, Germany). Blood samples for hematology (0.5 ml) was dispensed into sample bottles pre-treated with ethylene diamine tetra-acetic acid (EDTA) to prevent clotting, while the blood samples for serum biochemistry (1.5 ml each) were dispensed into clean test tubes and allowed to stand at room temperature for 45 min to clot; they were then centrifuged at 3000 revolutions per minute for 10 min to separate the serum from the clot. The supernatant (serum) for each sample was aspirated and discharged into clean labeled sample bottle, and used immediately for the serum biochemistry evaluations.

Standard procedures were followed in hematology and serum biochemistry determinations. Packed cell volume (PCV) determination was done by the microhematocrit method (Thrall & Weiser, 2002), while hemoglobin concentration was determined following the cyanomethemoglobin method (Higgins et al., 2008a). Counting of red blood cells (RBC) and total white blood cells (WBC) were done by the hemocytometer method (Thrall & Weiser, 2002). Blood smears for differential WBC counts were made on clean grease-free glass slides, air-dried, and stained by using Leishman technique and the different WBC blood smears were enumerated using the longitudinal counting method (Thrall & Weiser, 2002).

The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using the Reitman–Frankel method, while the serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method (Colville, 2002). Serum total bilirubin determination was done by the Jendrassik–Grof method (Higgins et al., 2008b), while the serum total cholesterol determination was done by using the enzymatic colorimetric method (Rifai et al., 2008). Serum total protein determination was done by the direct Biuret method and serum albumin determination was done by the bromocresol green method (Johnson, 2008). Serum globulin level for each rat was obtained by subtracting the serum albumin level from the serum total protein level (Johnson, 2008). Serum creatinine determination was done by the modified Jaffe method, while serum urea determination was by the modified Berthelot–Searcy method (Lamb & Price, 2008).

STATISTICAL ANALYSIS

Data obtained from the study were analyzed using SPSS version 16.0 for Windows software. The data were subjected to

one way analysis of variance (ANOVA), and the least significant difference (LSD) method was used post-hoc to separate variant means. Significance was accepted at $p<0.05$. Summary of the results were presented as means with standard error of mean in tables and bar chart.

RESULTS

There were no significant ($p>0.05$) variations between the groups in all the hematological parameters assayed even though the lymphocyte counts of the groups treated with PSMLE (Groups 2, 3 and 4) were noticeably higher than that of the untreated control in a dose-dependent manner (Table 1). Also, serum activities of ALT, AST, and ALP did not significantly vary across the groups (Table 2). However, the serum total protein levels of rats in Groups 2 and 3 were significantly higher ($p<0.05$) than those of Groups 1 and 4 (Table 2). There were no significant differences ($p>0.05$) between the groups in their serum albumin levels, but the mean serum globulin level of Group 2 rats was significantly ($p<0.05$) higher than those of groups 1 and 4 rats (Table 2). The blood glucose levels and serum levels of bilirubin (total, direct, and indirect), total cholesterol, creatinine, and urea did not significantly vary ($p>0.05$) among the groups (Table 2).

The mean body weights on Day 0 (baseline) did not significantly vary ($p>0.05$) among the groups, but on Day 28, the mean body weights of Group 3 rats were significantly higher ($p<0.05$) than those of Groups 1 and 2 (Figure 2). No signs of disorder and no mortality were recorded in any of the groups all through the 28-day experimental period. After humane slaughter, there were no significant ($p>0.05$) variations between the groups in their relative liver, kidney, spleen, and heart weights (Table 3).

DISCUSSION

The lack of significant alterations in the hematological parameters of the rat groups treated with PSMLE suggests that the administration of PSMLE as used in the study led to no adverse effects on the blood cellular pools and the bone marrow, which are usually very sensitive to toxic compounds (Bloom, 1993; Burdinsky Jr., 2000). The hematology results obtained in the present study are in agreement with the earlier reports of no significant effects on hematology of rats treated for 30 days with ethanol stem bark extract of *Pterocarpus erinaceus* (a related plant of the same genus as *P. santalinoides*) [Salawu et al., 2008], and also reports of no significant effects on hematology in rats treated sub-chronically for 3 months with *P. santalinoides* methanol leaf extract (Ihedioha et al., 2020). However, there had been an earlier report of increase in PCV and hemoglobin concentration in albino rats treated with ethanol leaf extract of *P. santalinoides* for 2 weeks (Offor & Ogbugo, 2015). The relatively higher lymphocyte counts recorded in the present study also concurs with earlier reports of higher percentages of lymphocytes in rats treated with *P. erinaceus* extracts when compared with that of untreated controls (Salawu et al., 2008).

The findings from the present study showing no significant effects of PSMLE administration on the serum enzyme markers of hepatocellular integrity (ALT and AST) and hepatobiliary function (ALP) is suggestive of the fact that the treatment did not lead to any adverse effects or damage to the hepatocytes, muscles (including heart muscles), and biliary epithelium (Boyd, 1988; Giannini et al., 2003; Sagar et al., 2015). This implies that sub-acute administration of PSMLE as used in the study did not lead to the damage of the vital organs in the body. The findings from this study showing no adverse effects on serum enzyme markers of hepatocellular integrity and hepatobiliary function agrees with earlier reports on rats treated with *P. santalinoides* extracts for 2 weeks (Offor et al., 2015) and 3 months (Ihedioha et al., 2020), respectively. It also concurs with the finding in the present study of no significant effects on relative liver and heart weights.

The significantly higher serum total protein levels recorded in Groups 2 and 3 rats and the significantly higher globulin levels recorded for Group 2 rats strongly suggests that the administration of PSMLE especially at lower doses enhances

Table 1 Hematological profile of albino rats treated with daily oral doses of ***Pterocarpus santalinoides*** methanolic leaf extract (PSMLE) for 28 days (results are presented as means ± standard error)

Parameters	Group 1 (Untreated control)	Group 2 (50 mg/kg PSMLE)	Group 3 (250 mg/kg PSMLE)	Group 4 (500 mg/kg PSMLE)
Packed cell volume (%)	47.83 ± 0.81	48.25 ± 1.11	47.50 ± 0.74	48.13 ± 0.94
Hemoglobin (g/dl)	15.29 ± 0.63	16.61 ± 0.34	16.44 ± 0.19	16.48 ± 0.42
RBC (10^6/µl)	11.08 ± 0.28	11.32 ± 1.09	11.09 ± 0.92	11.65 ± 0.43
Total WBC (10^3/µl)	10.93 ± 1.04	10.59 ± 0.91	10.13 ± 0.80	10.49 ± 0.85
Lymphocytes (10^3/µl)	6.56 ± 0.71	6.71 ± 0.61	7.08 ± 0.74	8.57 ± 0.86
Neutrophils (10^3/µl)	1.91 ± 0.65	2.20 ± 0.20	1.93 ± 0.39	1.44 ± 0.15
Monocytes (10^3/µl)	1.54 ± 0.94	1.03 ± 0.32	0.37 ± 0.02	0.44 ± 0.19
Eosinophils (10^3/µl)	0.89 ± 0.44	0.41 ± 0.09	0.70 ± 0.18	0.38 ± 0.13
Basophils (10^3/µl)	0.00 ± 0.00	0.03 ± 0.03	0.03 ± 0.03	0.00 ± 0.00

No significant variations ($p>0.05$) between the means of the groups [RBC – Red blood cells; WBC – White blood cells].

Table 2 Serum biochemistry profile of albino rats treated with daily oral doses of ***Pterocarpus santalinoides*** methanolic leaf extract (PSMLE) for 28 days (results are presented as means ± standard error)

Parameters	Group 1 (Untreated control)	Group 2 (50 mg/kg PSMLE)	Group 3 (250 mg/kg PSMLE)	Group 4 (500 mg/kg PSMLE)
ALT (IU/L)	26.08 ± 1.32	21.22 ± 0.60	25.67 ± 4.47	26.42 ± 3.94
AST (IU/L)	59.04 ± 0.86	60.27 ± 2.23	60.17 ± 7.89	66.28 ± 5.48
ALP (IU/L)	156.20 ± 12.30	143.48 ± 17.91	150.79 ± 44.08	113.83 ± 28.28
Total protein (g/dl)	6.83 ± 0.36[a]	8.17 ± 0.28[b]	7.89 ± 0.36[b]	6.74 ± 0.48 [a]
Albumin (g/dl)	3.14 ± 0.21	3.12 ± 0.15	3.34 ± 0.11	2.97 ± 0.12
Globulin (g/dl)	3.69 ± 0.51[a]	5.05 ± 0.22[b]	4.55 ± 0.28[ab]	3.76 ± 0.40[a]
Total bil. (mg/dl)	0.83 ± 0.03	0.98 ± 0.05	0.90 ± 0.05	0.89 ± 0.06
Direct bil. (mg/dl)	0.21 ± 0.01	0.22 ± 0.03	0.24 ± 0.01	0.20 ± 0.05
Indirect bil. (mg/dl)	0.62 ± 0.03	0.76 ± 0.02	0.65 ± 0.04	0.69 ± 0.08
Blood glucose (mg/dl)	66.90 ± 3.18	67.83 ± 2.97	65.50 ± 4.12	68.11 ± 4.06
Total chol. (mg/dl)	66.42 ± 3.25	75.58 ± 3.37	71.93 ± 5.99	67.71 ± 3.23
Creatinine (mg/dl)	0.87 ± 0.06	0.78 ± 0.04	0.88 ± 0.05	0.93 ± 0.09
Urea (mg/dl)	34.37 ± 3.99	35.80 ± 2.53	31.69 ± 5.12	29.96 ± 2.25

[a b] Different alphabetical superscripts in a row indicate significant difference ($p < 0.05$) between the means of the groups. ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; ALP – Alkaline phosphatase; bil. – Bilirubin; chol. – Cholesterol

Table 3 Organ weight percentage of body weights (relative organ weights) of albino rats treated with daily oral doses of *Pterocarpus santalinoides* methanolic leaf extract (PSMLE) for 28 days (results are presented as means ± standard error)

Relative organ weights	Group 1 (Untreated control)	Group 2 (50 mg/kg PSMLE)	Group 3 (250 mg/kg PSMLE)	Group 4 (500 mg/kg PSMLE)
Relative liver weight (%)	3.57 ± 0.32	3.70 ± 0.22	3.72 ± 0.38	3.62 ± 0.36
Relative kidney weight (%)	0.32 ± 0.01	0.34 ± 0.02	0.33 ± 0.01	0.30 ± 0.01
Relative spleen weight (%)	0.33 ± 0.01	0.31 ± 0.04	0.29 ± 0.02	0.32 ± 0.02
Relative heart weight (%)	0.40 ± 0.03	0.36 ± 0.01	0.41 ± 0.06	0.37 ± 0.02

No significant variations ($p > 0.05$) between the means of the groups.

protein synthesis and globulin production (Stockham & Scott, 2008; Tothova et al., 2016). This finding is in agreement with reports in which administration of *P. santalinoides* leaf extracts led to higher levels of serum proteins in rats with experimentally induced liver damage (Ihedioha et al., 2017 & 2019; Enemali et al., 2019) and in normal rats (Ihedioha et al., 2020). It is thought that this ability to enhance protein synthesis may be one of the mechanisms by which *P. santalinoides* extracts exercise their hepatoprotective effects in experimental toxic liver damage, as most cases of toxic liver damage are associated with poor hepatic protein synthetic ability (Thapa & Walia, 2007; Tothova et al., 2016). The higher globulin levels recorded for the groups treated with PSMLE concurs with their higher lymphocyte counts relative to the untreated control, as beta lymphocytes are the main producers of immunoglobulins, and immunoglobulins are the most predominant ones of the globulins in blood (Marnila & Korhonen, 2011; Tothova et al., 2016).

The absence of significant alterations in the blood glucose and the serum levels of albumins, bilirubin, total cholesterol, creatinine, and urea in the rats treated with PSMLE in this study suggests that sub-acute PSMLE treatment as used in this study did not lead to any significant alterations on liver and kidney function (Thapa & Walia, 2007; Yap & Aw, 2010; Uchino et al., 2012). These results concur with the lack of significant variation between rat groups in their relative liver and kidney weight percentages of body weight (relative organ weights) in the present study. However, there has been reports of significantly lower serum creatinine levels in rats treated with *P. santalinoides* methanolic leaf extract for 3 months (Ihedioha et al., 2020).

The significantly higher body weights recorded for rats in Group 3 (relative to rats in Groups 1 and 2), which was not significantly different from that of rats in Group 4 suggests that treatment at 250 mg/kg and 500 mg/kg doses led to higher weight gain. It is hypothesized that this higher body weight/weight gain may be the result of the earlier reported presence in extracts of *P. santalinoides* of beneficial micronutrients and phytochemicals (Anowi et al., 2012; Eze at al., 2012; Odeh & Tor-Anyim, 2014; Ihedioha et al., 2019), which may have enhanced growth and weight gain. The higher body weights recorded at Day 28 for the PSMLE-treated groups are in agreement with the earlier reports on body weights of rats treated with *P. santalinoides* leaf extract for 3 months

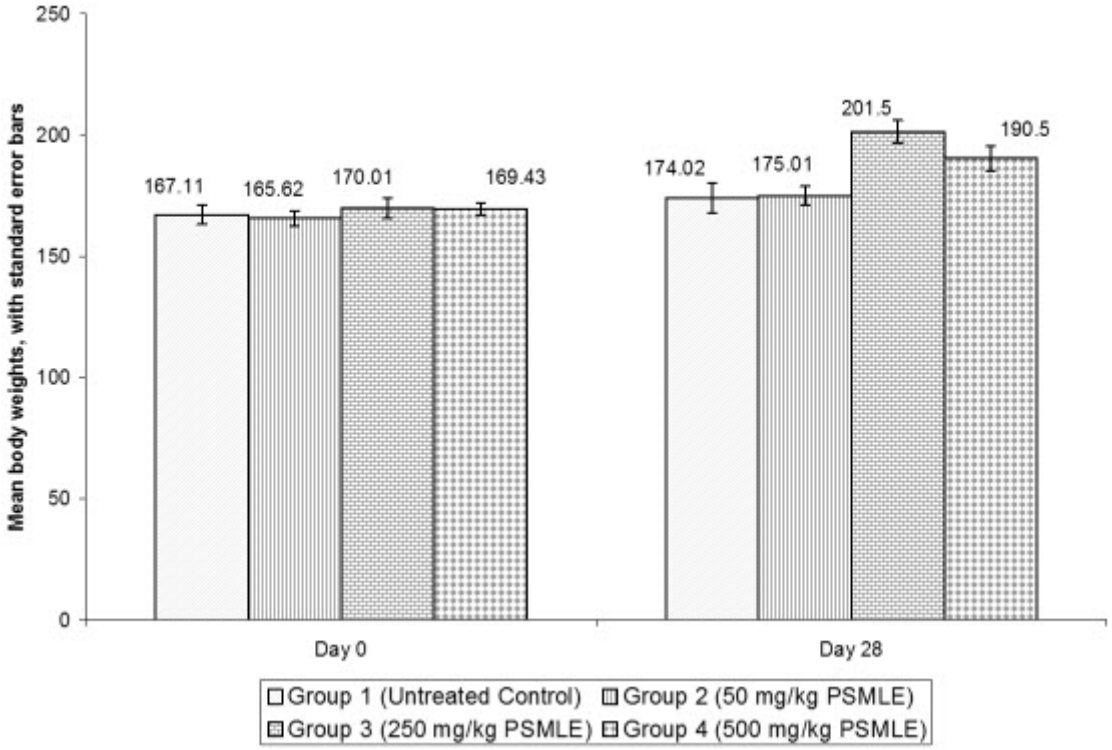

Figure 2 The mean body weight of albino rats before (Day 0) and after (Day 28) the treatment with daily oral doses of *Pterocarpus santalinoides* methanolic leaf extract (PSMLE) for 28 days.

(Ihedioha et al., 2020). The fact that there were no signs of disorder or mortality in the PSMLE-treated rats and that the treated rats gained more weight than the untreated control implies that the PSMLE was well-tolerated by the rats. This is in agreement with earlier reports on the safety of *P. santalinoides* extracts in acute and chronic toxicity studies (Anowi et al., 2012; Obi et al., 2019; Ihedioha et al., 2020).

The lack of significant alterations in the relative liver, kidney, heart, and spleen weights of the treated rat groups in this study suggests that the treatments led to no significant morphological damage of any of these organs, as damage to vital organs are usually associated with either swelling/enlargement and increase in relative weight in acute forms or shrinking and decrease in relative weight in chronic states (Wilkinson, 1981; O'Riordan, 2015; Amin & Siddiqui, 2020). The results of the relative organ weights agreed with the earlier reports (Ihedioha et al., 2020) on the relative liver and kidney weights of rats treated with *P. santalinoides* leaf extract for 3 months, but contrasted with their reports of relatively lower spleen and heart weights in the rats treated for 3 months with *P. santalinoides* leaf extract.

Based on the results of the study, it was concluded that sub-acute oral treatment of albino rats with PSMLE at doses used in this study led to no significant hematological, serum biochemical, and organosomatic alterations, except for higher serum levels of proteins and globulins and higher body weights especially in the groups treated with lower doses (50 mg/kg and 250 mg/kg).

CONFLICT OF INTEREST

There are no conflicts of interests associated with this work.

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