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Original Research Article

Sub-Chronic Toxicity: Biochemical, Hematological and Histopathological Effects of *Picralima nitida* (Apocynaeceae) Root Bark

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Abstract

Purpose: *Picralima nitida*, commonly called Akuamma, is a plant native to West Africa, and has been traditionally used for its medicinal properties. This study aimed to investigate the sub-chronic toxicity, with specific focus on biochemical, haematological and histopathological effects of the root bark of *Picralima nitida*.
Methods: Adult male Wistar rats were randomly divided into four groups: Group 1 (control) received distilled water only, Groups 2 – 4 (treatment groups) were administered 50, 100, and 200 mg/kg of *P. nitida* root bark methanol extract, respectively orally once daily for 28 days. On the 29th day, rats were sacrificed, blood samples were collected for biochemical and haematological analyses, while vital organs (liver, kidney, and heart) were excised and used for histopathological analysis.
Results: Results showed that the sub-chronic administration of *P. nitida* root bark did not cause significant changes in serum biochemical and haematological parameters in rats. However, the extract produced significant ($P<0.05$) increases in mid cells (MID), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) when compared to the control. No significant increase ($P>0.05$) was observed in white blood cell (WBC) count, red blood cell (RBC) count, and hemoglobin (HGB) concentration. Histopathological examination revealed normal tissue architecture, although the presence of immunological cells, and vasodilatation may indicate toxicity.
Conclusion: Findings from the study suggest that the root bark extract of *P. nitida* is nontoxic, and relatively safe on sub-chronic administration. However, further studies are needed with higher doses and longer duration of administration to fully understand the plant's toxicity.

Keywords: Medicinal plants, *Picralima nitida*, Toxicity, Haematology, Histopathology, Biochemical.

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INTRODUCTION

Medicinal plants play a crucial role in traditional medicine, serving as the foundation for many traditional healing practices.[1] Historically, traditional medicine systems have relied on medicinal plants to treat a wide range of health conditions, from common ailments to severe diseases.[1,2] Indigenous people and local communities have developed a deep understanding of the therapeutic properties of specific plants, using them to produce remedies such as decoctions, infusions, poultices, and salves.[3] This knowledge is often closely tied to the cultural practices and beliefs of a particular community or ethnic group, passed down through generations.[4]

The use of medicinal plants in traditional medicine is closely connected to the local biodiversity of a region.[5] The diversity of plant species in an area contributes to the rich repertoire of medicinal plants available to traditional healers. Different ecosystems may have unique plants with specific medicinal properties.[5] Traditional medicine relies on the bioactive compounds found in medicinal plants, which can have various effects on the body, such as anti-inflammatory, analgesic, antimicrobial, or antioxidant properties.[6]

In recent years, there has been a growing interest in integrating traditional medicine and modern medicine, particularly in the use of medicinal plants as a source of pharmaceutical drugs.[4,6,7]

Plants produce a variety of chemical compounds for various purposes, including defense and protection against insects, fungus, diseases, and herbivorous mammals.[8] These compounds can have a range of effects on the human body, such as anti-inflammatory, analgesic, antimicrobial, or antioxidant properties, making them valuable resources for traditional and modern medicine.[8]

Scientists have identified and isolated active compounds from medicinal plants to produce modern medicines, highlighting the potential of traditional medicine to contribute to modern healthcare.[9] However, the use of medicinal plants in modern medicine is not without risks. The lack of standardization and regulation in the production and distribution of medicinal plants and their products can lead to inconsistencies in quality, purity, and safety. Therefore, it is essential to establish rigorous quality control measures and regulatory frameworks to ensure the safety and efficacy of medicinal plants and their products.

Picralima nitida commonly called Akuamma is a medium-sized tree native to West Africa, particularly in countries like Nigeria, Ghana, Uganda, Ivory Coast, Gabon, and Cameroon. The root bark of the tree is the most commonly used part in traditional medicine. It is harvested from mature trees, dried, and ground into powder, which is then used to prepare herbal remedies for various health conditions, including malaria, hypertension, abscesses, hepatitis, pneumonia, diabetes, and gastrointestinal disorders.[10] Several scientific investigations have been conducted on *P. nitida*, highlighting its potential therapeutic benefits. For instance, a study found that *P. nitida* root bark extract exhibited antioxidant and antimicrobial properties, suggesting its potential use in treating oxidative stress-related disorders and infections.[11] Another study reported that *P. nitida* root bark extract exhibited anti-inflammatory and analgesic effects, supporting its traditional use in treating inflammation and pain.[12] Previous studies have demonstrated that different extracts derived from this plant contain a rich array of phytochemicals such as glycosides, alkaloids, triterpenes, flavonoids, polyphenols, saponins, and tannins.[13] Despite its widespread use, there is limited scientific evidence on the safety and toxicity of *P. nitida* root bark, especially its long-term effects on health. A few studies have reported the toxicity of *P. nitida*, primarily in animal models.[11,14] Based on the wide-spread ethnomedicinal use of *P. nitida*, and the limited information on its toxicity, it is necessary to evaluate the long-term toxic effect, especially the effect on the function of vital organs such as the liver, kidneys, and heart. This study therefore aims to evaluate the sub-chronic toxicity of *Picralima nitida* in order to assess its safety profile for human consumption and therapeutic use.

MATERIALS AND METHODS

Chemicals and reagents

Methanol (99.8%, Loba Chemie®), India), distilled water, chloroform (MolyChem®, India), absolute ethanol (Loba Chemie®), formalin (Sigma Aldrich, Germany). The equipment used include refrigerator (LG®, South Korea), rotary evaporator (Stuart®, UK), vacuum pump (Stuart®, UK), electronic weighing balance (Ohaus®, USA), haematology analyzer (ERMA PCE 210, ERMA, Japan), microscope (Olympus 230 V 50/60 He, Germany), and camera (Eakins 12Mega pixels, UK).

Plant collection, identification, and preparation

Fresh roots of *P. nitida* were procured from a local market (New Benin Market, Oredo Local Government Area, Benin City, Nigeria (GPS: 6°20'00"N, 5°37'20"E) in February, 2024. The plant material was identified at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria with the voucher number UBH-P424. The root barks were chopped and dried in an oven. The dried root bark was powdered, and stored in an air-tight container until ready for use.

Extraction of plant material

The powdered root bark (0.5 kg) was extracted by maceration in 1.5 L of absolute methanol at room temperature for 72 h. The crude extract was filtered, and the filtrate was concentrated *in vacuo* using a rotary evaporator (Stuart, UK) at 45°C. The concentrated extract was stored in an air-tight container and refrigerated.

Animals

Twenty-four (24) male Wistar rats (99 – 160 g) were sourced from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The rats were kept in plastic cages and acclimatized to the laboratory conditions for one week. The rats were fed with pelletized finisher feeds and had access to drinking water *ad libitum*. The bedding materials (wood shavings) of the cages were changed daily.[15]

Ethical considerations

Ethical approval with reference number ADM/E 22/A/VOL VII/1047 was granted by the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria. All experiments were carried out in accordance with the National institute of Health Guidelines for the care and use of Laboratory Animals.[15]

Experimental design

The rats were randomly divided into four groups of six animals per group, and treated as follows:

Group 1: Control group, was administered distilled water only

Group 2: Treated with 50 mg/kg body weight of *P. nitida* extract

Group 3: Treated with 100 mg/kg body weight of *P. nitida* extract

Group 4: Treated with 200 mg/kg body weight of *P. nitida* extract

The treatments were administered via oral route with the aid of an oral intubation tube. The treatments were given once daily for 28 days. On the 29th day, blood samples were collected from the lateral tail vein for biochemical and haematological analyses. Thereafter, the rats were sacrificed under chloroform anaesthesia, vital organs (heart, kidneys, and liver) were harvested and used for histopathological analysis.

Measurement of body weight

The body weights of the rats were measured before extract administration, and at the end of the treatment period, the organ weights were also measured to assess the effect of *P. nitida* extract on body/organ weight.

Biochemical and hematological analyses

Blood sample was taken from each rat using a 5 mL syringe into a plain sample bottle, and allowed to stand on the laboratory bench in an inclined position for 15 minutes and then centrifuged at 3000 rpm for 10 minutes. The resulting serum was transferred into an appropriately labelled bottle for biochemical and hematological evaluations using the Automated Haematology System analyser (ERMA PCE 210, ERMA, Japan). The biochemical parameters investigated include (i) Liver function parameters: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB), Total Protein (TP), Albumin (ALB), and Globulin (GLO); (ii) Renal function parameters: Electrolytes (Na^+, K^+, Cl^-, HCO_3^-), urea, and creatinine; (iii) Lipid profile: Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL). The haematological parameters analysed include; White blood cell (WBC), lymphocyte (LYM), mid-cell (MID), granulocyte (GRAN), red blood cell (RBC), hemoglobin (HBG), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-standard deviation (RDW-SD), red blood cell distribution width-coefficient of variance (RDW-CV), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet crit (PCT), and platelet larger cell ratio (P-LCR).

Histopathological analysis

After euthanasia, a midline incision was made on the ventral aspect starting from the base of the neck down to the umbilical. The liver, heart, and kidneys were carefully cut off, washed with normal saline solution and put in Universal bottles containing formalin. The tissues were fixed in normal formal saline for 72 hours. These tissues were completely dehydrated in ascending concentration of ethanol (70, 90, 96 and 100%). They were further treated with paraffin wax, and allowed to solidify prior to sectioning. Tissue sections were cut with a microtome to 4 µm size. These were then fixed on a slide, and allowed to dry. The samples were subsequently stained in hematoxylin-eosin and examined under a microscope (Olympus 230 V 50/60 He, Germany) and camera (Eakins 12Mega pixels, UK) at x400 magnification.

Photomicrographs of the samples were taken and recorded.

Statistical analysis

All data were presented as mean ± standard error of mean (SEM). Data were analyzed by one-way analysis of variance (ANOVA) using Statistical Analysis System (Graph pad prism version 6. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Medicinal plants are the most accessible and cheapest sources of medicines for preventive, curative or protective purposes from early civilization till date.[16] It is generally believed that medicinal plants are safer than orthodox medicines, hence they are increasingly being used as complementary and alternative medicines to synthetic drugs.[16] However, studies have shown that not all medicinal plants are safe. Therefore, to develop safer natural products from plants, preliminary toxicological studies are necessary to investigate potential risks, and as such, many examinations are performed on medicinal plants to prevent their toxicities.[17-19] If the toxic effects are not prevented, important organs like the kidneys and liver are exposed to the toxic effects of these plants.

Picralima nitida is widely used in African traditional medicine, especially in West Africa for the treatment of various ailments.[10] The present study investigated the sub-chronic oral toxicity of the methanol root bark extract of *P. nitida* in order to determine its safety profile. In this study, neither mortality nor significant signs of toxicity were observed in rats after sub-chronic oral administration of *P. nitida* root bark extract at doses of 50, 100, and 200 mg/kg.

Effect of *P. nitida* root bark extract on body and organ weights of rats

Results showed that the weights of the rats increased significantly over the full course of the experiment (Figure 1). Although the control group showed a greater increase in weights, the treatment groups all had a significant increase. The organ weights of the rats in the treatment groups were compared to that of the control group, and the results showed that there was no significant difference in the weight of the organs (liver, kidneys, and heart) among the various groups (Figure 2). Body weight serves as a general indicator of the overall health and well-being of test animals. A decline in body weight may suggest systemic toxicity, metabolic disturbances, or impaired nutrient absorption, whereas stable or increasing body weight typically indicates normal physiological function.[20]

Substances with toxic effects can lead to changes in metabolic processes, organ function, or energy balance, resulting in alterations in body weight.[21] A significant decrease in body weight may indicate systemic toxicity, organ damage, or physiological stress induced by the test substance.[21]

From the results of the study, there was no significant differences in the body weight, and weight of the organs (liver, kidneys, and heart) between the extract-treated groups and the control group (Figures 1 and 2). The present observation is in contrast with the findings from a previous study that reported that *P. nitida* root bark extract caused a significant decrease in body weight and food intake in rats.[14]

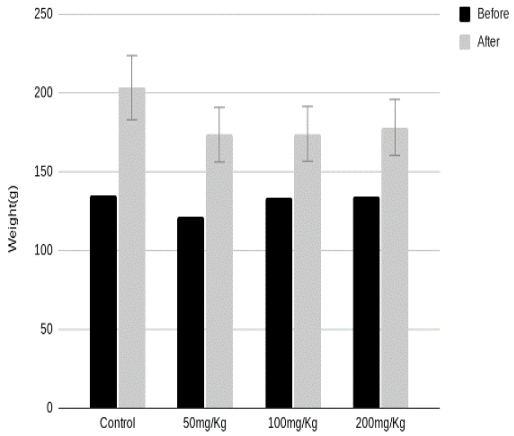

Figure 1: Effect of methanol extract of *Picralima nitida* root bark on body weights of rats. Data represent mean ± standard error of mean (SEM), n = 6.

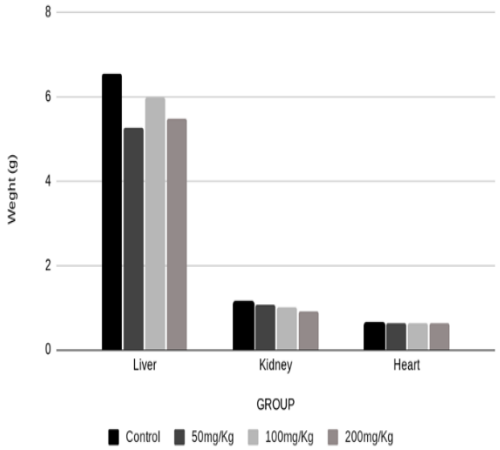

Figure 2: Effect of methanol extract of *Picralima nitida* root bark on organ weights of rats

Effect of *P. nitida* root bark extract on biochemical parameters

The effects of *Picralima nitida* root bark extract on liver and renal function parameters as well as lipid profile are presented in Tables 1 – 3.

The results showed that there was no significant difference in the levels of ALT and AST between the treatment groups and that of the control group. However, ALP was significantly elevated ($p < 0.05$) in the 100 mg/kg dose group compared to the

control group (Table 1). This is consistent with the work of Awodele *et al*. (2019).[22] Elevation of ALP could have resulted from the fact that ALP can be excreted from the bones and other tissues probably due to external causes. Therefore, elevated ALP levels can be indicative of liver disorders.[22] Hepatotoxic substances damage liver cells, leading to leakage of liver enzymes into the bloodstream. Elevated levels of liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) observed in liver function tests may indicate hepatotoxicity.[22] There were no significant changes in serum creatinine, blood urea nitrogen (BUN), and electrolyte levels in the treatment groups and that of the control group (Tale 2). This indicate that *P. nitida* treatment did not affect renal function of the rats. This observation is similar to findings from previous studies on the seeds of *P. nitida*, in which no significant changes in the renal function parameters of treatment groups and that of the control was observed.[22,23]

The kidneys are vital organs responsible for maintaining the body's internal balance by regulating fluid and electrolyte levels, filtering metabolic waste products from the blood, and excreting them through urine.[24] Nephrotoxicity refers to kidney injury resulting from exposure to various substances, including drugs and environmental chemicals, either directly or indirectly.[25] Elevated levels of serum creatinine may indicate impaired kidney function, as the kidneys are responsible for removing creatinine from the bloodstream.[25] BUN levels may increase in conditions such as decreased renal function, dehydration, or increased protein breakdown.[25] Electrolyte levels, including sodium, potassium, chloride, and bicarbonate, are closely regulated by the kidneys.[23]

Table 1: Effect of methanol extract of *Picralima nitida* root bark on liver function parameters

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dL)	TP (g/dL)	ALB (g/dL)	GLO (g/dL)
Control	138.00 ± 7.40	75.25 ± 4.72	391.50 ± 31.80	0.23 ± 0.02	7.45 ± 0.20	3.2 ± 0.06	4.00 ± 0.09
50 mg/kg	151.60 ± 8.88	68.20 ± 3.15	372.80 ± 11.91	0.24 ± 0.02	7.22 ± 0.23	3.16 ± 0.05	4.14 ± 0.23
100 mg/kg	146.50 ± 19.43	69.26 ± 5.25	474.50 ± 75.21	0.25 ± 0.02	7.20 ± 0.20	3.16 ± 0.05	4.03 ± 0.18
200 mg/kg	119.00 ± 11.20	54.26 ± 3.01	301.83 ± 23.56	0.22 ± 0.01	7.47 ± 0.12	3.20 ± 0.07	4.27 ± 0.18

Values are mean ± standard error of mean (SEM), n = 6. AST - Aspartate Aminotransferase, ALT - Alanine Aminotransferase, ALP - Alkaline Phosphatase, TB - Total Bilirubin, TP - Total Protein, ALB - Albumin, GLO – Globulin

Table 2: Effect of methanol extract of *Picralima nitida* root bark on renal function parameters

Group	Urea (mmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)	HCO_3^- (mmol/L)	Cl^- (mmol/L)	Creatinine (mg/dL)
Control	44.00 ± 1.37	139.75 ± 1.71	4.10 ± 0.06	21.75 ± 0.98	103.50 ± 1.47	0.48 ± 0.03
50 mg/kg	42.00 ± 2.01	139.40 ± 1.49	4.00 ± 0.12	26.20 ± 2.30	102.00 ± 1.02	0.42 ± 0.02
100 mg/kg	40.33 ± 1.63	140.67 ± 1.58	4.00 ± 0.15	21.167 ± 0.82	101.17 ± 1.75	0.42 ± 0.03
200 mg/kg	36.33 ± 1.19	140.67 ± 1.15	3.90 ± 0.14	19.83 ± 0.26	102.33 ± 0.94	0.45 ± 0.02

Values are mean ± standard error of mean (SEM), n = 6.

Table 3: Effect of methanol extract of *Picralima nitida* root bark on lipid profile

Group	TC (mg/dL)	TG (g/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	95.75 ± 5.67	99.75 ± 11.68	31.75 ± 7.21	49.00 ± 7.78
50 mg/kg	107.40 ± 5.67	96.80 ± 9.18	36.80 ± 5.94	51.40 ± 5.02
100 mg/kg	89.67 ± 5.92	119.17 ± 14.33	36.50 ± 3.46	34.00 ± 6.12
200 mg/kg	110.67 ± 5.65	96.83 ± 9.18	44.00 ± 5.75	47.17 ± 2.93

Values are mean ± standard error of mean (SEM), n = 6. TC: Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein

Abnormalities in electrolyte levels may indicate kidney dysfunction or electrolyte imbalances secondary to toxicity or renal disease.[25]

For the lipid profile, the result showed no significant difference in total cholesterol, triglyceride, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol in the treatment groups and that of the control group (Table 3). Based on these observations, administration of *P. nitida* root bark extract has no risk of drug-induced dyslipidemia.

Hematological effects of *P. nitida* root bark extract

Table 4 below illustrates the effects of *P. nitida* root bark extract on the hematological parameters in Wistar rats. The result shows that there were significant increases in the concentrations of MID (mid-cell), MCV, and MCH in the extract-treated groups compared to the control group. Other parameters showed no significant changes. There was an increase in WBC of the 100 mg/kg and 200 mg/kg extract-treated groups, although not significant, indicating that the extract has the potential of enhancing the immune system and hence its function of defending the body against infectious diseases and foreign substances. This agrees with the study on the effect of *P. nitida* seed extract on hematological parameters of mice, where an increased WBC was observed.[26] Group 2 (treated with 50 mg/kg of *P. nitida* extract) had lower WBC than that of the control and other groups. This suggests that lower dose of *P. nitida* root bark extract may suppress the immune system. Mid-cell (MID%) indicates the combined value of the other types of white blood cells not classified as lymphocytes or granulocytes.

Table 4: Effect of *Picralima nitida* root bark extract on hematological parameters

Parameter/group	Group 1	Group 2	Group 3	Group 4	P-value
WBC (10^3/uL)	4.43 ± 1.20	4.12 ± 1.07	5.07 ± 1.95	4.80 ± 1.16	0.77
LYM (%)	86.93 ± 3.03	90.04 ± 3.02	89.92 ± 2.02	90.58 ± 2.32	0.26
MID (%)	8.95 ± 2.68	5.84 ± 1.89	5.77 ± 0.41	5.75 ± 1.17	0.04
GRAN (%)	4.13 ± 0.43	4.10 ± 1.74	4.32 ± 1.98	3.67 ± 1.25	0.92
LYM 2 (10^3/uL)	3.88 ± 1.07	3.72 ± 1.00	4.58 ± 1.78	4.38 ± 1.15	0.75
MID (%)	0.40 ± 0.10	0.24 ± 0.08	0.28 ± 0.13	0.27 ± 0.05	0.02
GRAN (10^3/ul)	0.15 ± 0.09	0.16 ± 0.05	0.20 ± 0.12	0.15 ± 0.08	0.79
RBC (10^6/uL)	7.23 ± 0.46	7.69 ± 0.38	7.33 ± 0.64	7.55 ± 0.54	0.63
HGB (g/dL)	14.43 ± 1.26	16.16 ± 0.77	15.53 ± 1.41	16.42 ± 1.05	0.12
HCT (%)	38.93 ± 4.13	44.12 ± 2.33	41.28 ± 3.81	44.40 ± 3.49	0.14
MCV (fL)	53.78 ± 2.21	57.46 ± 1.74	56.38 ± 2.06	58.92 ± 1.46	0.01
MCH (pg)	19.88 ± 0.75	20.98 ± 0.66	21.15 ± 0.87	21.75 ± 0.91	0.04
MCHC (g/dL)	37.08 ± 1.25	36.60 ± 1.03	37.62 ± 1.58	37.00 ± 1.33	0.73
RDW-SD (fL)	42.73 ± 1.52	44.44 ± 2.49	45.58 ± 1.60	46.32 ± 1.60	0.07
RDW-CV (%)	17.85 ± 0.41	17.64 ± 0.85	18.37 ± 0.64	18.00 ± 0.67	0.45
PLT (10^3/uL)	802.00 ± 177.42	741.20 ± 156.27	805.33 ± 253.54	898.50 ± 266.02	0.77
MPV (fL)	10.93 ± 0.81	10.06 ± 0.20	10.33 ± 1.06	10.68 ± 0.99	0.54
PDW (%)	13.68 ± 0.54	13.66 ± 0.88	13.72 ± 2.15	14.85 ± 1.59	0.57
PCT (%)	0.88 ± 0.23	0.74 ± 0.16	0.85 ± 0.31	0.98 ± 0.32	0.64
P-LCR (%)	29.48 ± 4.97	25.05 ± 3.94	27.63 ± 3.95	27.67 ± 7.53	0.78

WBC: White blood cell; LYM: lymphocyte; MID: mid-cell; GRAN: granulocyte; RBC: red blood cell; HBG: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red blood cell distribution width-standard deviation; RDW-CV: red blood cell distribution width-coefficient of variance; PLT: platelet; MPV: mean platelet volume; PDW: platelet distribution width; PCT: platelet crit; P-LCR: platelet larger cell ratio.

The lower or higher than normal values of MID cells indicate infections, certain medical conditions, or immune disorders.[27] Among the extract-treated groups, MID values were not significantly different ($p > 0.05$). The MID value in the control group was higher and significantly different ($p < 0.05$) from those of the extract-treated groups.

Red blood cell (RBC) count indicates the amount of red blood cells contained in the systemic circulation.[28] The result showed that all extract-treated groups had higher RBC count than the control group. This evidence shows that treating rats with graded doses of *P. nitida* root bark extract exhibits increase in RBC count and proves that the extract has protective effect on the integrity of the red blood cells.

Mean Corpuscular Volume (MCV) measures the average size of red blood cells. MCV was analyzed within the range 80.0 - 99.0 fL. Small red blood cells are not ideal for good health as this might decrease oxygen carrying capacity. All the extract-treated groups had a significant increase ($p < 0.05$) in MCV and Mean Corpuscular Hemoglobin Concentration (MCHC) than the control group. The study carried out by Adeneye *et al*. (2010)[29] on the hematopoietic properties of *Hunteria umbellata* confirms that certain medicinal plants can increase MCV. The results of the hematological studies of this research work agree with the findings of Odeghe *et al*. (2012)[30] who reported that graded doses of *Anthocleista grandifora* stem bark extract administered to mice showed significant ($p < 0.05$) increase in PCV, Hb, WBC and platelet counts compared to the control.

Effect of *P. nitida* root bark extract on histology of the liver, kidneys, and heart

Histopathological examination revealed normal tissue architecture of the liver, kidneys, and the heart (Figures 3 – 5), although the presence of immunological cells, and vasodilatation may indicate toxicity. Also, the recruitment of Kupffer cells in the liver of rats in the extract-treated groups could suggest potential hepatotoxicity as Kupffer cells are known to be present in liver injury and hepatocellular necrosis.

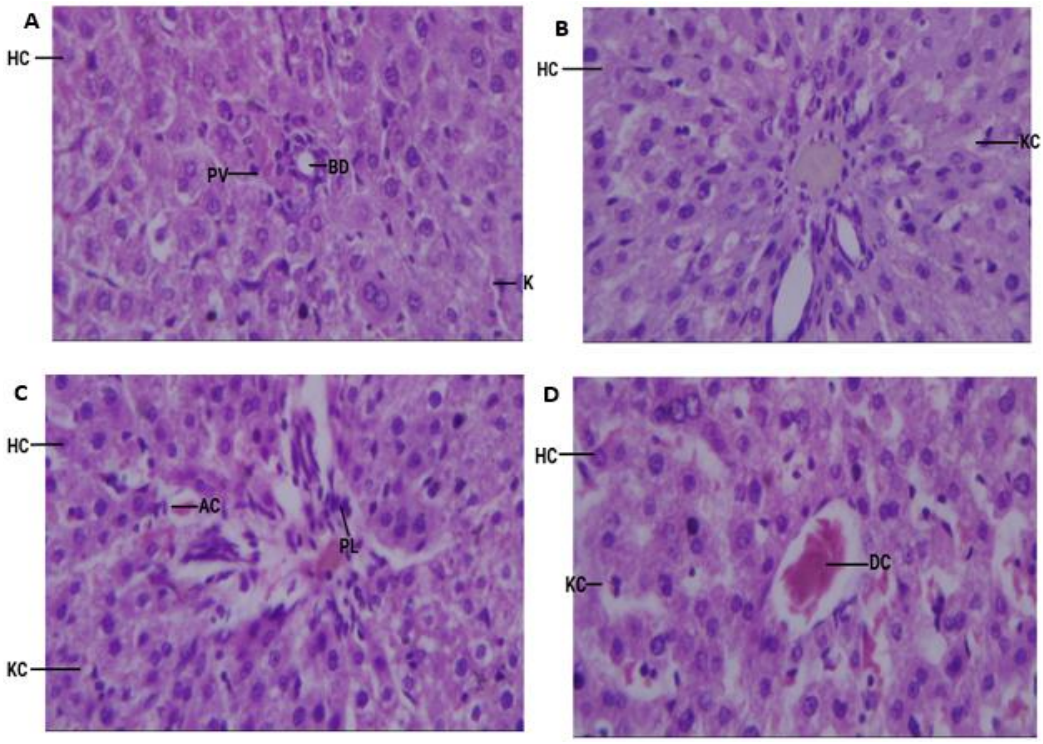

Figure 3: Photomicrograph of rat liver. **(A)** Control group showing normal hepatocytes (HC), portal vein (PV) and bile duct (BD), Kupffer cell mobilization (KM); **(B)** 50 mg/kg extract group showing normal hepatocytes (HC), recruitment of sinusoidal Kupffer cells (KC); **(C)** 100 mg/kg extract group showing normal hepatocytes (HC), recruitment of sinusoidal Kupffer cells (KC), active vascular congestion (AC) and mild periportal mobilization of lymphocytes (PL); **(D)** 200 mg/kg extract group showing normal hepatocytes (HC), active vascular congestion and vasodilatation (DC), recruitment of sinusoidal Kupffer cells (KC). H&E x 400.

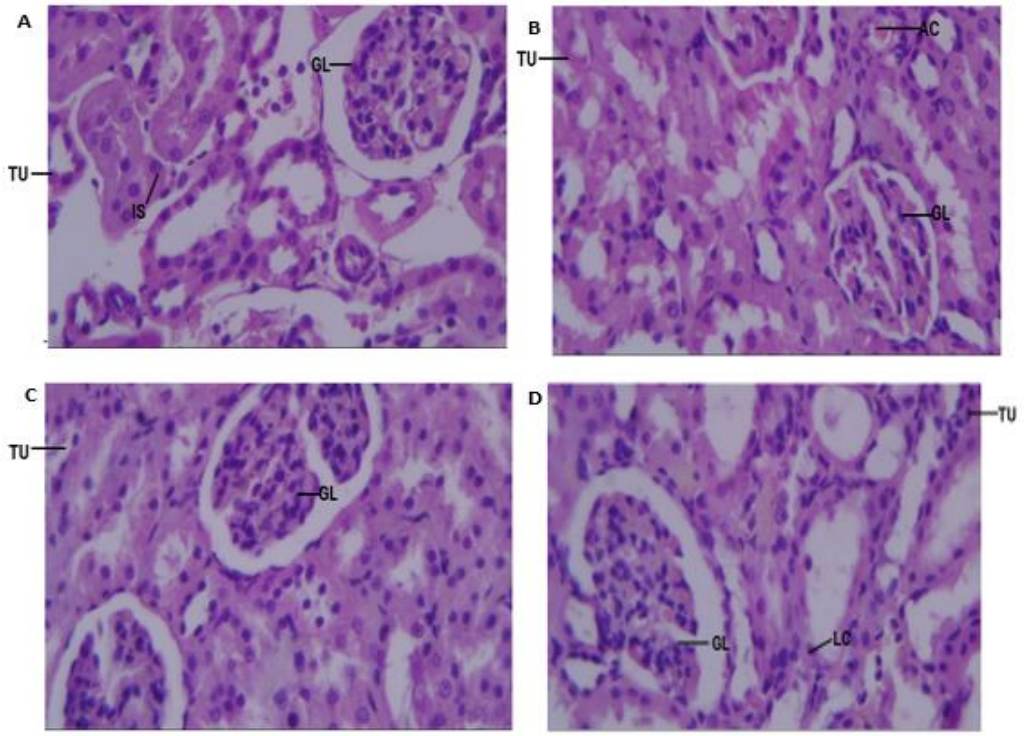

Figure 4: Photomicrograph of rat kidneys. **(A)** Control group showing normal architecture composed of tubules (TU), glomerulus (GL) and interstitial space (IS) **(B)** 50 mg/kg extract group showing normal tubules (TU), active interstitial congestion (AC) and glomeruli (GL); **(C)** 100 mg/kg extract group showing normal tubules (TU) and glomeruli (GL); **(D)** 200 mg/kg extract group showing normal architecture tubules (TU), glomerulus (GL) and interstitial mobilization of lymphocytes (LC). H&E x 400.

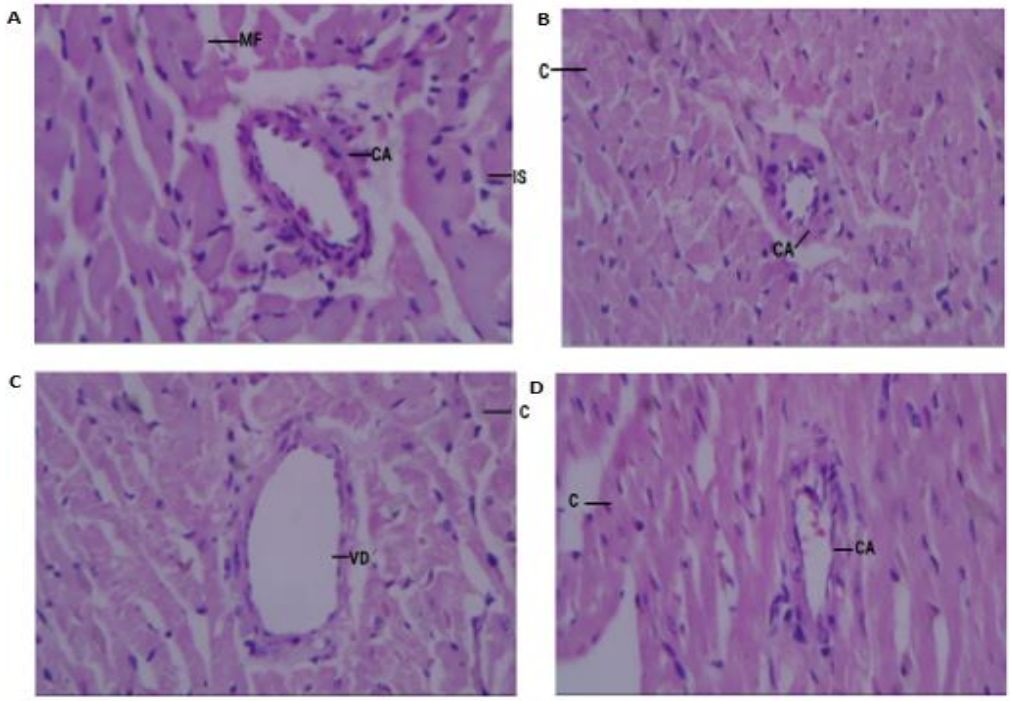

Figure 5: Photomicrograph of rat heart. **(A)** Control group showing normal architecture composed of bundles of myocardial fibres (MF), interstitial space (IS) and coronary artery (CA); **(B)** 50 mg/kg extract group showing normal bundles of cardiomyocytes (CM) and coronary artery (CA); **(C)** 100 mg/kg extract group showing normal bundles of cardiomyocytes (CM) and coronary vasodilatation (VD); **(D)** 200 mg/kg extract group showing normal architecture: bundles of cardiomyocytes (CM) and coronary artery (CA). H&E x 400.

The photomicrograph of the liver of the rats in the control group showed normal hepatocytes. Treatment with *P. nitida* root bark extract did not cause significant abnormality in the liver tissue architecture, except for the recruitment of Kupffer cells in all the extract-treated groups (Figure 3).

Histology of the kidneys showed normal glomerulus and tubules in the control and treatment groups. However, at 200 mg/kg, the interstitial spaces showed the presence of lymphocytes (Figure 4). This is consistent with the study of Ojewole (2000)[11], who found that high doses of *P. nitida* root bark extract caused liver and kidney damage in mice.[11]

Histology of the heart showed normal histoarchitecture of the heart in the control and treatment groups. The coronary artery was shown to be normal in all the groups. However, there was vasodilation in the different treatment groups (Figure 5). This vasodilation seems to increase in a dose-dependent manner. Overall, these findings demonstrate that the root bark extract of *P. nitida* is non-toxic, and relatively safe on sub-chronic administration.

CONCLUSION

The findings from the present study suggest that *Picralima nitida* root bark extract is non-toxic as no significant alterations in the biochemical and haematological parameters, as well as histopathological features of the liver, kidneys, and heart were observed in rats following the sub-chronic administration of the extract. Therefore, *Picralima nitida* root bark extract could be said to be relatively safe on sub-chronic administration. However, further studies are necessary to explore its long-term toxicity, and safety profile in different experimental animal models.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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