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Research Paper

Biochemical and Histopathological Assessment of Some Organs in Rats Administered Picralima Nitida Aqueous Fruit-Pulp Extract

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Abstract

Picralima nitida (Apocynaceae) is frequently used in ethnomedicine to manage various illnesses. The potential and safety of P. nitida aqueous, unripe fruit-pulp extract were assessed. Unripe P. nitida fruit pulp was cleaned, dried to a persistent mass, and crushed to powder. Then, it was immersed in distilled water for 72 h, filtered, and freeze-dried. The study involved six groups of rats, each with an average weight of 160–185 g, who were given 200–3000 mg/kg of pulp's aqueous extract daily for 35 days, with weekly measurements of weight, fasting blood glucose levels, and feed intake. Blood samples were obtained for biochemical tests, and certain organs underwent histopathological examination. A considerable ($P > 0.05$) increase in body weight was accompanied by a significant ($P > 0.05$) decrease in blood glucose and cholesterol levels. The relative organ weights did not differ significantly at a significance level of $P > 0.05$. ALT increased as doses of the extract rose. Serum electrolytes were altered at higher extract concentrations. The urea and creatinine concentrations were not appreciably changed. The hematological assessment revealed no variations in the quantity of leukocytes (total and differential), but there was a significant increase in hemoglobin at low doses. Histopathological studies revealed heart myocarditis, kidney tubular necrosis, liver hepatitis, pancreatitis, and bronchiolar mucosa ulceration in unripe P. nitida fruit pulp extract at a dosage of 3000 mg/kg body weight. The findings revealed that unripe P. nitida fruit pulp extract is relatively safe at low concentrations.

Keywords: *Picralima; fruit; biochemical; histopathological; Toxicity.*

INTRODUCTION

Picralima nitida (P. nitida) (Stapf.) is a West Central African native understory tree that is a member of the Apocynaceae family. The various tree components—stem bark, seeds, roots, and leaves—have been employed by traditional medical practitioners to treat and cure various ailments such as malaria, gastrointestinal problems, jaundice, fever, and hypertension (Falodun et al., 2006; Kouitcheu et al., 2008; Teugwa et al., 2013; Erharuyi et al., 2014). Activity against bacteria, fever, pain, malaria, inflammation, anticancer, antioxidant, and diabetes were among the numerous pharmacological properties of P. nitida seeds that have been demonstrated (Erharuyi et al., 2014). Pulp fruit is utilized in ethnomedicine to treat diabetes, dysmenorrhea, and malaria. P. nitida leaf extracts in acetone, ethanol, and aqueous media contain saponins and tannins, which are linked to glucosidase and alpha-amylase inhibitory properties (Kazeem et al., 2013). In a diabetic rat model, Teugwa et al. (2013) observed that administering 300 mg/kg body weight of P. nitida methanolic

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leaf extract effectively lowered the glycemic index.

The emergence of multidrug-resistant organisms (MDROs) has prompted extensive research into alternative agents, particularly plant-based compounds (Sulong et al., 2024). Plants with medicinal value have been used to manage and treat various diseases since ancient times (Karthikeyan et al., 2009; Yakubu et al., 2018; Glen et al., 2023). Rahman et al. (2022) identified 134 medicinal plant species as potential therapeutic substances. The Santal community's Soren clan uses 53 medicinal plants (categorized into 32 families) for various medical purposes. These plants treat various illnesses, including diabetes, urinary problems, gastrointestinal disorders, sexual dysfunctions, STDs, helminthiasis, leprosy, filariasis, tuberculosis, chronic heart disease, snake bites, pain, epilepsy, and paralysis (Hasan et al., 2012; Rahmatullah et al., 2012; James et al., 2023). The bioactive compounds present in medicinal plants in underdeveloped nations are always of great importance to health care. Herbal medicines have a similar mechanism of action to conventional medications and may have potential negative side effects. Safety is crucial when choosing herbal remedies for health care, because plant extracts are safe to consume and effective. Both plant extracts and conventional medications may have similar effects (Bulus et al., 2011). Using histopathology of the heart, liver, lungs, spleen, pancreas, and kidney, as well as some biochemical parameters, this study aimed to assess the toxicity of subchronic doses of unripe P. nitida aqueous fruit pulp extract in normal Wistar Albino rats.

RESEARCH METHOD

Plant material

P. nitida fruit was purchased from New Benin Market in Benin City, Nigeria and was identified at the University of Benin's Plant Biology and Biotechnology Department. Unripe fruits were cleaned, peeled, and dried for 56 days, resulting in 20-kg pulp. A mechanical grinder [model: SY-18B Industrial Dry Herbs Grinder, China] was used to grind the dried pulp into a fine powder. The mixture was continuously stirred for 72 h while being soaked in distilled water (1:10 kg/L) (De Campos et al., 2020). The slurry was sieved through filter paper, cotton wool, and muslin cloth. To create a freeze-dried extract, the filtrate was freeze-dried using a Biobase BK-FD10s Freeze Dryer (Xi'an, China). 637.6 g (12.6%) of the total yield was obtained. The freeze-dried extract was stored at 40 °C in an airtight container until use. The freeze-dried sample of 12.6% unripe P. nitida aqueous fruit pulp extract was evaluated to contain 39.24 ± 0.45mg GAE/g extract total phenol and 24.39±0.74 mg QE/g extract flavonoids (Ilenowa et al, 2024).

Experimental Animals

Wistar rats, adult males weighing 160–185 g, originated at the University of Benin's Department of Anatomy in Benin City, Nigeria's animal house. Following protocol evaluation, the study was approved (number CMS/REC/2022/281) by the University of Benin College of Medical Sciences Ethical Committee in Benin City, Nigeria. The Committee's guidelines for maintaining the control and supervision of animal experiments were followed (Tejano et al., 2020; Donovan & Brown, 2013; Ago et al., 2002; O'Malley et al., 2022). With wood shavings as bedding and a 12/12-h light/dark cycle, the animals were kept in hygienic plastic enclosures. Animals were placed in weight-based groups, and their bedding was replaced daily. Top Feeds, Ibadan, Nigeria, supplied pelleted poultry finisher's mash feed for their livestock to feed. The animals were acclimatized for 14 days.

Subchronic evaluation of toxicity

Weight (160.0–185.0 g) was used to allocate the animals into six groups of five (5) each. The oral gavage technique (Diehl et al., 2001; Turner et al., 2011a; Turner et al., 2011b) was used in this

study to introduce the aqueous fruit pulp extract of P. nitida at doses ranging from 200 to 3000 mg/kg body weight per day in rats. The animals in the control group had unrestricted access to distilled water. Throughout the trial, signs of poisoning and animal death were observed. Using a weighing balance, the animals' weights and feed intakes were recorded every week during the feeding study. [Adventurer: OHAUS CORP, China]. Following a 12-h fast, the animals were euthanized on the 35th day via cervical dislocation and then dissected through a midline incision. Blood samples were collected in appropriate containers for biochemical analyses. The pancreas, kidneys, heart, lungs, spleen, and liver were removed, cleaned of adhering tissues, weighed, and preserved in plain bottles containing 10% buffered formalin for histological evaluation.

Blood glucose evaluations

On days 0, 3, 6, 9, 12, 15, 18, 21, and 35, glucometer readings were obtained after an overnight fast (ACCU-Check, Roche, Germany). A pinprick was used to obtain blood samples from the ends of the rats' tails, and the glucose levels were recorded.

Serum biochemical analyses

Hematological Analysis

The study includes various tests. An automatic blood analyzer (URIT-3010 Automated Hematology Analyzer, Gullin, Guangxi, China) was used to measure various blood parameters such as WBC, hematocrit, platelet counts, RBC, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin.

Lipid Profile

The study utilized commercial kits from Randox Laboratories Ltd., Crumlin, UK were used to measure total cholesterol, plasma triglyceride levels, and high- and low-density lipoprotein cholesterol (Friedewald et al., 1972).

Assessment of hepatic serum markers

Serum from each group was tested for enzymes using commercial kits following the manufacturer's instructions, including aspartate aminotransferase [Randox, UK. Cat. No. AS 101], total protein [Randox, UK. Cat. No. TP 245], alkaline phosphatase [ALP: Teco Diagnostics, USA. A506], and alanine aminotransferase [ALT: Randox, UK. Cat. No. AL 100].

Determination of Electrolytes, Creatinine and Urea

An OPTITM LION Electrolyte Analyzer [OPTI Medical Systems, Inc., Roswell, USA] was used to measure the electrolytes Na+, K+, HCO3-, and Cl- in serum. Commercial kits were used to measure urea (Randox, UK. Cat. No. UR 1068) and creatinine (CREA: Randox, UK. Cat. Nos. CR 510 and CR 524) quantities in the serum, following the manufacturer's instructions.

Histopathological analysis

After removal from the body and clearing of any adhering tissue, the liver, lungs, heart, spleen, kidney, and pancreas were placed in sterile containers containing 10% buffered formalin. (Palanivel et al., 2008). The tissues were sliced into smaller pieces to fit inside the cassettes, exposing the processing-relevant portions. The tissue was processed by being removed from buffered formalin and subjected to a spectrum of alcohol concentrations (beginning at the lowest: 70%, 90%, 95%, and absolute), and dehydration was achieved. Xylene was used to remove the alcohol from the tissues, and paraffin wax was then replaced by impregnation. Hematoxylin and eosin staining was performed, and a microtome was used to cut sections at 5 and 0 microns.

Microscopically, the treated tissues on the slides were examined.

Statistical analysis

The data were analyzed using ANOVA and Duncan's multiple-range test to identify significant variations. ($P > 0.05$) and statistically significant differences across groups.

FINDINGS AND DISCUSSION

Subchronic Toxicity

Clinical observations

A 35-day period of daily oral administration of unripe P. nitida aqueous fruit pulp extract to rats showed no overt symptoms of toxicity or mortality at the tested doses (200, 500, 1000, 2000, and 3000 mg/kg body weight).

Organ weight

The administration of P. nitida extract did not cause any discernible difference in organ weight ($P > 0.05$).

Table 1. Organ weight (g) of experimental rats administered with unripe P. nitida fruit pulp extract.

Organs	Control	200 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg
LIVER	7.12±0.43 ^{a}	6.38±0.58^{a}	6.44±0.58^{a}	5.74±0.33^{a}	6.70±0.30^{a}	6.66±0.34 ^{a}
KIDNEYS	1.08±0.04^{a}	1.24±0.07^{a}	0.98±0.06^{a}	1.08±0.14^{a}	1.22±0.18^{a}	1.08±0.23^{a}
PANCREAS	0.24±0.05^{a}	0.38±0.06^{a}	0.30±0.05^{a}	0.30±0.03^{a}	0.36±0.04^{a}	0.48±0.14^{a}
HEART	0.58±0.05^{a}	0.70±0.08^{a}	0.56±0.07^{a}	0.84±0.22^{a}	0.76±0.07^{a}	0.78±0.05^{a}
LUNGS	1.26±0.05^{a}	1.24±0.26^{a}	1.42±0.18^{a}	1.26±0.09^{a}	1.30±0.13^{a}	1.30±0.08^{a}
SPLEEN	0.82±0.07^{a}	0.64±0.07^{a}	0.64±0.04^{a}	0.78±0.22^{a}	0.74±0.11^{a}	0.72±0.09^{a}

Means with different superscript letters are statistically significant at P<0.05. Values are reported as mean ± SEM (n = 5).

Subchronic investigations are intended to detect unobservable harmful effects and to offer information on the toxicity of target organs (NRC, 2006). This can disrupt the function of several organs, and this method is used in animal models to estimate the potentially harmful condition of botanical extracts (Aniagu et al., 2005). A 35-day treatment of the extract did not significantly change feed consumption. Crude plant extract may be harmful by products that can affect gastric function when taken in greater dosages (Chokshi, 2007). In this study, the experimental animals given the extract tolerated the feed. P. nitida did not lead to any modifications in carbohydrate, protein, or fat metabolism or nutritional benefits, proving how useful it is to weigh organs in

toxicity tests because it can predict acute injury, toxicity, and enzyme induction (Michael et al., 2007). The findings of this investigation demonstrated that the extract was effective at the indicated treatment doses. Dosages did not significantly change kidney, pancreas, liver, spleen, lungs, or heart weight ($P > 0.05$).

Biochemical assessment

When compared with their controls, following the treatment of rats' blood glucose with varying dosages of extracts, the blood glucose levels in the groups that received extract doses ranging from 200 to 3000 mg/kg were significantly lower ($P > 0.05$).

Table 2. Blood glucose concentrations in experimental rats administered with unripe P. nitida fruit pulp extract (Mg/dl)

Days	Control	200 mg	500 mg	1000 mg	2000 mg	3000 mg
Day 0	109.60±4.38^{b}	76.20±5.49^{a}	84.20±4.71^{a}	83.40±4.15^{a}	100.60±3.41^{b}	76.00±4.70^{a}
Day 3	96.00±1.70^{b}	62.60±2.01^{a}	56.20±6.22^{a}	61.20±1.46^{a}	61.80±4.69^{a}	66.80±2.75^{a}
Day 6	98.40±3.85^{c}	53.60±3.88^{a}	60.20±4.09^{ab}	65.40±1.47^{b}	56.20±3.75^{ab}	57.40±1.54^{ab}
Day 9	101.80±1.93^{c}	51.00±5.34^{a}	66.60±3.66^{b}	52.00±2.61^{a}	55.60±3.75^{a}	50.40±3.76^{a}
Day 12	101.60±1.29^{c}	62.20±1.56^{ab}	61.80±0.97^{ab}	67.80±2.50^{b}	57.40±3.36^{a}	58.60±3.91^{a}
Day 15	100.80±1.46^{b}	62.00±2.10^{a}	57.60±4.91^{a}	61.40±4.61^{a}	56.40±4.46^{a}	60.40±1.86^{a}
Day 18	100.40±1.03^{c}	51.00±2.55^{a}	47.00±2.43^{a}	51.20±2.44^{a}	51.20±3.31^{a}	77.80±3.60^{b}
Day 21	100.00±1.48^{b}	62.40±2.75^{a}	58.20±4.78^{a}	65.00±2.02^{a}	59.20±4.07^{a}	63.20±3.95^{a}
Day 35	99.80±0.86^{d}	64.20±2.44^{abc}	67.60±2.38^{c}	65.60±2.32^{bc}	59.00±3.71^{ab}	57.20±3.18^{a}
Percentage Reduction	8.44±3.21	13.94±7.36	19.02±4.03	20.12±6.33	41.38±3.02	23.54±6.28

The data are presented as mean ± SEM for a total of five samples, with statistical significance at P<0.05.

In this study, rats were administered daily dosages of 200–3000 mg/kg body weight of P. nitida unripe fruit pulp aqueous extract for 35 days to assess subchronic toxicity. A hypoglycemic effect was noted in normal rats administered the aqueous P. nitida fruit pulp extract for 35 days. The extract showed no toxicity or mortality in rats given different doses (200 mg/kg to 3000 mg/kg body weight), with the highest percentage reduction achieved at 2000 mg/kg body weight, making it the most effective dose. No clinical indicators were found. (Table 2).

Hematological parameters

Hematological assessment revealed that white blood cell (WBC) and lymphocyte (LYM) counts did not significantly increase at 500 mg/kg and did not significantly decrease at 200, 1000, 2000, and 3000 mg/kg. The study found no significant decrease in granulocyte and monocyte levels, but a significant increase in hemoglobin at 1000 and 2000 mg/kg and a decline in the treated group's hematocrit. No significant ($P > 0.05$) decreases in red blood cells (RBC), monocytes (MCHC), or platelets (PLT) were observed.

Table 3. Effects of different doses of P. nitida extract on the hematological parameters of rats

Parameters	Control	200 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg
WBC ($x10^6$ U/L)	10.92±2.01^a	7.56±0.57^a	16.00±3.04^a	10.92±1.91^a	9.00±1.78^a	9.42±1.51^a
LYM (%)	9.72±1.81^a	6.64±0.53^a	14.80±2.99^a	9.96±1.81^a	8.08±1.55^a	8.64±1.41^a
MID (%)	0.68±0.12^a	0.48±0.08^a	0.68±0.05^a	0.60±0.17^a	0.44±0.10^a	0.56±0.10^a
GRAN (%)	0.52±0.10^a	0.44±0.10^a	0.52±0.10^a	0.36±0.10^a	0.48±0.14^a	0.48±0.10^a
LYM# ($x10^9$ /L)	176.76±3.46^a	176.04±4.49^a	182.44±3.33^a	180.72±5.52^a	179.08±1.05^a	170.56±7.79^a
MID# ($x10^9$ /L)	12.96±1.72^a	11.92±1.24^a	9.24±1.22^a	12.28±3.60^a	11.08±0.71^a	11.16±1.06^a
GRAN# (%)	10.28±1.82^a	11.92±3.31^a	8.32±2.13^a	7.00±1.98^a	9.84±0.95^a	10.28±1.22^a
RBC ($x10^{12}$ /L)	9.21±0.78^a	7.39±0.23^a	7.89±0.15^a	8.10±0.82^a	6.89±0.34^a	7.59±0.32^a
HGB (g/dL)	16.84±1.31^a	13.60±0.37^a	15.12±0.26^a	50.64±4.87^c	41.00±2.58^b	14.20±0.54^a
HCT (%)	53.40±4.49^c	44.36±1.58^a	49.48±1.07^{ac}	15.24±1.60^b	12.72±0.51^b	45.68±2.53^a
MCV (fL)	116.28±0.81^b	119.92±0.65^{ab}	125.56±3.30^c	125.04±1.03^c	119.04±1.94^b	120.08±2.06^{ab}
MCH (Pg)	36.72±0.67^a	36.80±0.26^a	38.32±0.91^a	37.84±0.92^a	37.08±0.64^a	37.40±0.35^a
MCHC (g/dL)	63.16±0.80^a	61.24±0.52^a	61.08±0.45^a	60.64±1.28^a	62.40±1.58^a	62.44±1.52^a
RDW-CV (%)	34.72±1.24^a	35.08±0.72^a	35.72±1.67^a	35.64±0.80^a	36.56±1.18^a	33.96±1.24^a
RDW-SD (fL)	67.12±2.49^a	70.00±1.20^a	74.72±4.55^a	74.12±1.31^a	72.20±1.26^a	67.68±1.51^a
PLT ($x10^9$ /L)	625.60±48.73^a	845.20±64.67^{bc}	819.20±54.39^{abc}	1012.00±100.95^c	915.60±38.96^{bc}	733.20±53.06^{ab}
MPV (fL)	15.08±0.29^a	15.56±0.60^a	15.92±0.22^a	15.28±0.30^a	15.00±0.38^a	14.92±0.27^a
PDW (fL)	29.24±0.20^a	29.28±0.16^a	29.16±0.15^a	29.48±0.10^a	29.08±0.22^a	29.04±0.07^a
PCT (%)	4.73±0.41^a	6.62±0.75^{bc}	6.50±0.38^{bc}	7.68±0.70^c	6.91±0.46^{bc}	5.48±0.34^{ab}
P-LCR (%)	24.68±2.31^a	25.68±3.52^a	28.16±1.68^a	24.28±2.00^a	21.64±2.52^a	20.84±1.59^a

The values are presented as mean ± SEM means, and at $P<0.05$, various superscripts indicate statistical significance.

The effects of plant extracts on blood function can be assessed using hematological measures (Yakubu et al., 2007). Variations in plasma component values may indicate hemotoxicity (Dioka et al., 2002). Blood parameters, including platelet count (PLT), hematocrit (HCT), hemoglobin (HGB), and white blood cell (WBC), can be used to assess the well-being of a person or animal (Schlam et al., 1975). Consuming toxic plants can change these values to their typical ranges (Abatan & Arowolo, 1989). Blood markers were assessed after the oral intake of Picralima nitida extract for 35 days. Neither the lymphocyte (LYM) count nor the white blood cell count significantly increased at 500 mg/kg, but there were no significant decreases at 200, 1000, 2000, and 3000 mg/kg. There was also no significant decrease in granulocytes (GRAN) or monocytes (MID), but there was a significant increase in hemoglobin from 1000 to 2000 mg/kg and a significant drop in hematocrit (HCT) in the treated group, indicating that the shape, osmotic fragility, and erythropoiesis of red blood cells may have been affected by the extract (Guyton & Hall, 2000). Since the histological evaluation did not reflect the observed changes, except at 3000 mg/kg (plates 6, 12, 18, 24, and 30), the observed significant increase in platelets from 200 mg/kg to 3000 g/kg of the extract is possibly suggestive of hematopoietic potential in the extract but not necessarily predictive of harmful hematological alterations. There was no discernible decrease in RBC and MCHC and no significant ($P < 0.05$) increase in MCH. Furthermore, there was a substantial rise ($P > 0.05$) in both MCV and PLT. The extract had very little effect on the size of the RBC, according to this study. HGB weight per blood count, as shown by the estimated RBC indices, which were not significantly changed by the extract. Consequently, various inflammatory cells in the WBC are involved in the inflammatory process, which is the unique feature of this system (Kytridis & Manetas, 2006). When exposed to a life-threatening environment, WBC and certain indices associated with it, including lymphocytes, typically exhibit increased activity (Robins, 1974). The extract may not have significantly impacted the immune system of animals, as it did not significantly alter lymphocytes and WBC, the immune system's main effector cells.

Lipid profile

The study found no significant differences in triglycerides and total cholesterol, but a significant increase in HDL cholesterol and a decrease in LDL cholesterol across different doses. (Table 4).

Table 4. Effects of oral P. nitida extract at varying dosages on rats' lipid profile measures

Mg/dl	Control	200 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg
Total cholesterol	108.42±6.14^{a}	110.08±5.10^{a}	85.74±13.85^{a}	108.32±8.06^{a}	100.46±9.28^{a}	92.00±5.45^{a}
Triglyceride	149.50±29.47^{a}	167.12±25.59^{a}	144.70±25.62^{a}	157.28±13.60^{a}	150.92±24.39^{a}	173.72±15.42^{a}
HDL-cholesterol	22.38±1.19^{a}	46.36±8.15^{b}	27.12±6.88^{ab}	38.22±7.17^{ab}	47.14±3.04^{b}	34.36±3.84^{ab}
LDL-cholesterol	60.86±9.52^{b}	31.48±5.37^{a}	29.68±5.94^{a}	38.62±7.16^{a}	23.26±4.67^{a}	23.08±4.73^{a}

Means with different superscript letters are statistically significant at P<0.05. Values are reported as mean±SEM (n=5).

This study investigated the impact of sub-chronic ingestion of Picralima nitida fruit pulp extract for 35 days on blood lipid levels. High lipid levels are linked to atherosclerosis, heart disease, and stroke. While triglycerides and total cholesterol levels did not differ significantly, a significant increase in HDL cholesterol and a drop in LDL cholesterol were observed across different doses. Higher HDL cholesterol levels are typically associated with lower cardiovascular risk. This might be due to the alkaloids found in Picralima nitida's unripe fruit pulp extract, which have cholesterol-lowering properties. The capacity of Picralima nitida aqueous unripe fruit pulp extract to prevent atherosclerosis was demonstrated by the observed significant ($P > 0.05$) reduction in the extract from 200 mg/kg to 3000 mg/kg in contrast to the control group. According to Brown et al. (2006), LDL is the primary source of blood cholesterol. Constituting more than 50% of the total lipoprotein in the plasma, low-density lipoprotein (LDL) serves as the primary carrier of cholesterol and cholesteryl esters (Astrup, 2005). Elevated LDL cholesterol levels in the plasma can progress to atherosclerosis and coronary heart disease (Taskinen, 2003). Therefore, a large safety margin was suggested for the Picralima nitida unripe fruit pulp extract based on the considerable reduction in blood LDL-cholesterol seen in this investigation. Awodele et al., (2019) reported a negligible decline in total and low-density lipoprotein cholesterol levels in Picralima nitida seed extract.

Enzyme markers of Liver function

The study found no substantial change in ALP levels across all groups given the P. nitida extract, but increased ALT values in serum and decreased AST values in the test groups, with no significant difference in ALP levels. (Table 5).

Table 5. Impact of extract concentration on serum liver enzyme levels following oral administration.

Parameter	Control	200 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg
ALP (U/L)	26.10±4.51^{a}	30.80±3.52^{a}	23.72±2.27^{a}	27.64±2.52^{a}	25.08±1.41^{a}	24.44±2.45^{a}
AST (U/L)	135.76±19.99^{b}	58.10±9.69^{a}	73.48±7.01^{a}	65.54±9.74^{a}	59.52±5.35^{a}	43.08±5.88^{a}
ALT(U/L)	10.68±1.98^{c}	25.58±5.75^{a}	17.32±2.49^{abc}	11.26±2.62^{bc}	20.94±3.49^{ab}	21.72±0.25^{a}

$P<0.05$ indicates statistical significance for means with distinct superscripts. Values are reported as mean±SEM (n=5).

To identify potential hepatic dysfunction and tissue injury, liver function was assessed by prolonged exposure to an aqueous, unripe fruit pulp extract of Picralima nitida. When determining liver necrosis and inflammation, serum ALT and AST are helpful markers (Tilkian et al., 1979). Serum AST and ALT levels have been shown to dramatically increase in an unsafe setting (Adam, 1998). The organ with the highest ALT concentration was the liver, whereas the kidneys and skeletal muscles exhibit lower levels of the enzyme's activity. ALT levels are particularly unique to the liver (Whitby et al., 1989). The aqueous, unripe fruit pulp extract of Picralima nitida may have the potential to affect hepatic function by significantly increasing blood alanine aminotransferase (ALT) levels. This enzyme causes aberrant liver cell membrane permeability, which is linked to its existence. However, the significant decrease in serum AST levels in this study indicated a nonhepatic potential effect of an aqueous, unripe fruit pulp extract from Picralima nitida. Additionally,

histopathology analysis showed that neither necrotic lesions nor inflammation were seen in the livers of rats treated with graded doses of P. nitida fruit pulp extract (200 mg, 500 mg, 1000 mg, and 2000 mg/kg body weight), with the exception of rats that received 3000 mg/kg extract by body weight. Singh and Devkota's (2003) investigation found no significant change in serum lactate dehydrogenase, AST, ALP, and ALT levels in rats administered Piper methysticum aqueous extract. Alkaline phosphatase activity is elevated in liver and bone disorders (Wolf, 1978). The primary liver condition, if not the only one that causes elevated plasma alkaline phosphatase activity is cholestasis (Tilkian et al., 1979). The extract's metabolites may interact with hepatocytes, potentially leading to a non-significant change in ALP values compared with the control group.

Total protein, albumin, Creatinine, and urea

The study found that oral P. nitida extract treatment at varying dosages did not significantly alter serum albumin and total protein levels in contrast to the control group, and the same dosages did not affect serum urea and creatinine values. (Table 6).

Table 6. Differences in the serum levels of total protein, albumin, urea, and creatinine of experimental rats

Parameter	Control	200 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg
Total protein (g/dL)	70.76±1.40[a]	66.52±1.09[a]	68.86±2.14[a]	69.68±2.94[a]	64.68±2.39[a]	66.86±1.97[a]
Albumin (g/dL)	38.86±2.07[a]	36.94±3.91[a]	40.64±1.83[a]	37.14±3.45[a]	40.04±2.03[a]	40.28±1.20[a]
Creatinine	1.28±0.17[a]	1.34±0.07[a]	1.22±0.11[a]	1.22±0.09[a]	1.22±0.07[a]	1.26±0.11[a]
Urea	30.96±5.06[a]	29.92±3.10[a]	37.82±3.79[a]	35.32±2.70[a]	36.28±1.13[a]	33.90±1.71[a]

P<0.05 indicates statistical significance for means with distinct superscripts. Values are reported as mean±SEM (n=5).

Plasma concentrations of proteins may decrease because of overhydration or deficient synthesis of proteins resulting from starvation, intestinal problems, or hepatic illness (Whitby et al., 1989). The investigation found that the extract did not significantly affect the liver's hepatic secretory activities, as evidenced by a non-significant drop in serum total protein levels and no significant differences in albumin levels between the treated and control groups.

Renal dysfunction can be evaluated by measuring urea, creatinine, and electrolytes (Akindele et al., 2014). Protein metabolism produces waste products such as urea and creatinine, which are transported to the kidneys for filtering. Renal failure is indicated by elevated levels of these substances. Creatinine concentration remains unchanged in a normal setting unless a change in the glomerular filtration rate (GFR) is caused by impaired renal function, making it an effective indicator for determining GFR (Whitby et al., 1989). The current study found that renal function remained unaffected after the administration of an aqueous extract of Picralima nitida fruit pulp, with no significant increase in creatinine levels. However, plasma urea concentration, which depends on liver function, protein consumption, and oxidation, is not a better indicator of GFR than creatinine because it is absorbed into renal tubular cells (Tilkian et al., 1979).

Serum electrolytes

The treatment group exhibited a significant increase in bicarbonate and sodium levels, whereas potassium concentrations decreased with each dose. Chloride concentrations did not significantly decrease except at 3000 mg/kg body weight as compared with the control. (Table 7).

Table 7. Effects of serum electrolytes on P. nitida fruit pulp extract administered to experimental rats.

Parameters	Control	200 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	3000 mg/kg
Bicarbonate	6.13±0.44^{b}	11.04±1.49^{a}	14.50±1.73^{a}	11.61±1.46^{a}	12.45±1.11^{a}	14.82±2.18^{a}
Sodium	136.52±1.12^{c}	141.92±0.74^{ab}	139.20±1.04^{a}	139.98±0.94^{a}	142.74±0.32^{b}	141.74±0.83^{ab}
Potassium	8.86±0.74^{b}	6.62±0.71^{a}	5.98±0.62^{a}	6.78±0.91^{a}	5.13±0.38^{a}	5.67±0.19^{a}
Chloride	105.00±1.04^{b}	103.94±0.69^{b}	102.76±0.56^{ab}	102.94±0.49^{ab}	103.20±0.35^{b}	100.80±0.84^{a}

P<0.05 indicates statistical significance for means with distinct superscripts. Values are presented as mean±SEM (n=5).

The electrolyte levels of blood, including sodium, potassium, chloride, and bicarbonate, are determined by the regulation of ionic charges and osmotic balance (Tilkian et al., 1979). The adrenal glands and kidneys regulate sodium levels, water distribution, and osmotic pressure. Acid-base imbalances can occur due to renal failure and are presented as tubular acidosis, hyperkalemic acidosis, and ketoacidosis. In rats administered the aqueous fruit pulp extract of Picralima nitida, serum sodium and bicarbonate ion concentrations increased significantly, but this increase was not dependent on the dose. Reduced potassium levels are associated with adrenal cortical hyperactivity, gastrointestinal fluid loss, malnutrition, and a negative nitrogen balance (Tietz & Saunders, 1976). The aqueous, unripe fruit pulp extract of Picralima nitida may have harmful physiological effects, potentially altering the kidney and other organs' ability to function with electrolytes. The stability of bicarbonate, potassium, sodium, and chloride levels in blood is a reliable indicator of the heart and kidney performance, and a substantial change in these electrolyte concentrations indicates impaired or poor renal function (Imo & Uhegbu, 2015).

Macro pathology and Histopathology

The cardiac photomicrograph revealed normal cardiac muscle fibers and interstitial spaces. However, perivasculitis was noted in the coronary artery at 3000 mg/kg. The liver had a normal architecture, but inflammation around the portal triad was stimulated at 3000 mg/kg. The kidneys showed normal glomeruli, but tubular necrosis occurred at 3000 mg/kg. The pancreas showed normal blood vessels, exocrine glands, islets, and pancreatic ducts at different concentrations of extract. The 2000 mg/kg extract displayed dilated blood vessels, whereas the 3000 mg/kg extract revealed mild inflammation, indicating pancreatitis. The lungs showed whitish spaces in the alveoli, terminal bronchioles, and bronchial arteries. The 200–2000 mg/kg extract showed

normal bronchioles, arteries, and alveoli. However, treatment with 3000 mg/kg resulted in ulcerated bronchioles and damage to lung tissues. The spleen revealed an increase in erythrocytes. Sequestration, lymphocyte follicles, and splenic histiocytes were observed across doses, activating the spleen's immune activities and red blood cell sequestration, as observed across doses.

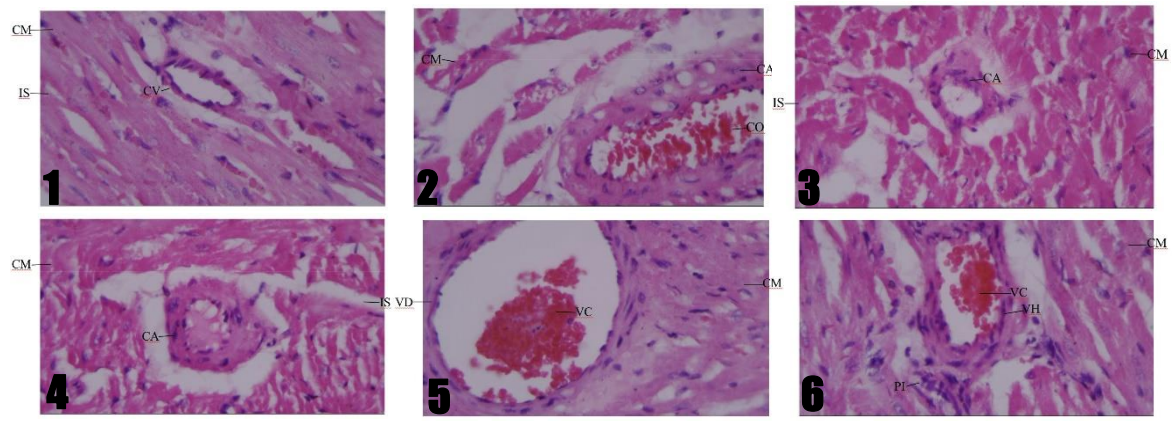

Figure 1. Photomicrograph of a rat heart slice treated for 35 days at 200, 500, 1000, 2000, and 3000 mg/kg body weight/day (plates 1-6) in unripe Picralima nitida aqueous fruit pulp extract compared with plate 1 as the control. Plates 1-6 were stained using H&E x 400.

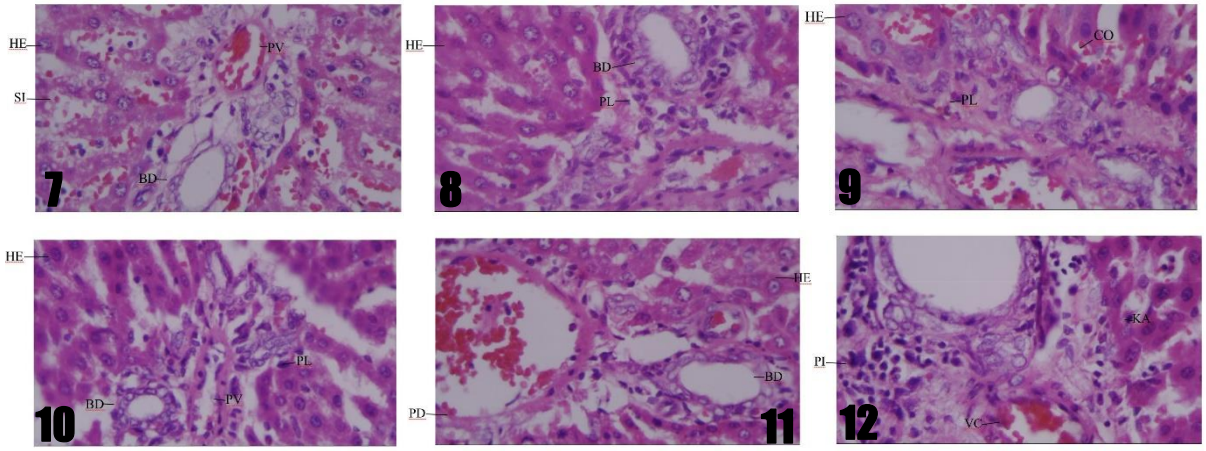

Figure 2. Plates 8–12 show a photomicrograph of a rat liver slice treated for 35 days with several dosages of the aqueous fruit pulp extract of Picralima nitida (200, 500, 1000, 2000, and 3000 mg/kg body weight/day) compared with plate 7, which was the control. A H&E x 400 stain was applied to the sections.

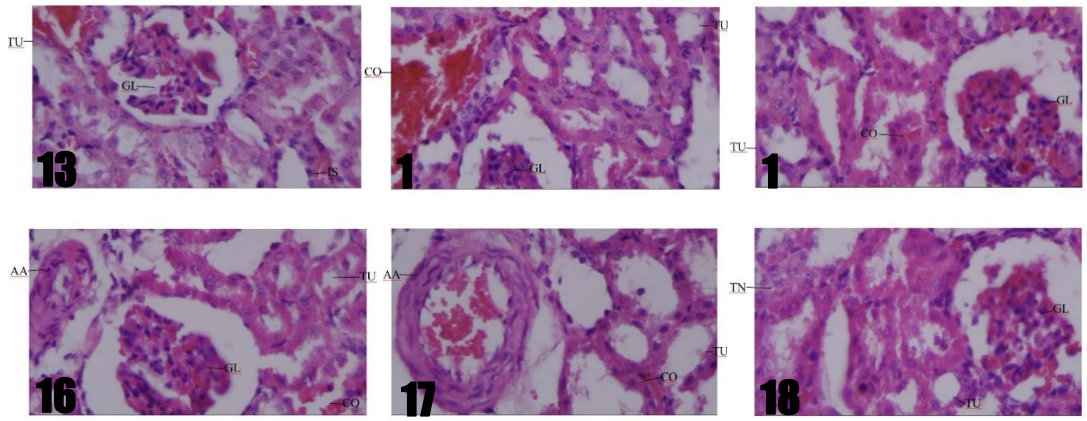

Figure 3. A comparison of the rat kidney section (photomicrograph 14–18) to the control (plate 13) after 35 days of treatment with an aqueous fruit pulp extract of Picralima nitida at doses of 200, 500, 1000, 2000, and 3000 mg/kg body weight/day. Plates 13–18 were stained using H&E x 400.

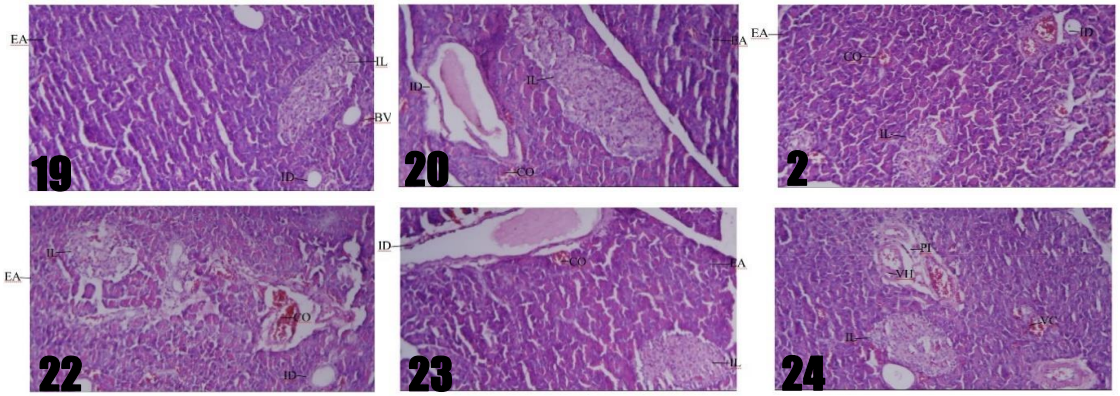

Figure 4. Rat pancreatic segment photomicrograph after 35 days of treatment with Picralima nitida aqueous fruit pulp extract at concentrations of 200, 500, 1000, 2000, and 3000 mg/kg body weight/day (plates 20–24, respectively) compared with the control (plate 19). Plates 19–24 were stained with H&E x 100.

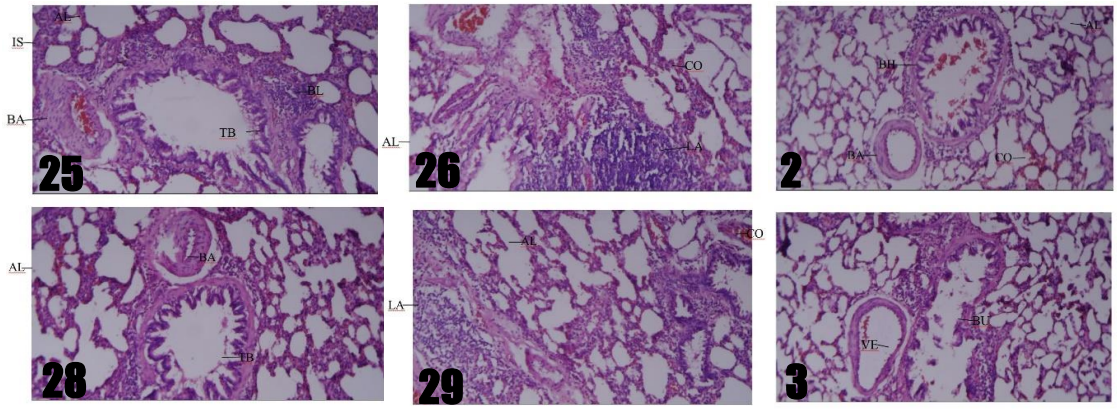

Figure 5. Rat lung segment photomicrograph after 35 days of treatment with Picralima nitida aqueous fruit pulp extract at concentrations of 200, 500, 1000, 2000, and 3000 mg/kg body weight/day (plates 26–30, respectively) compared with the control (plate 25). H&E x 100 was used to stain sections (plates 25–30).

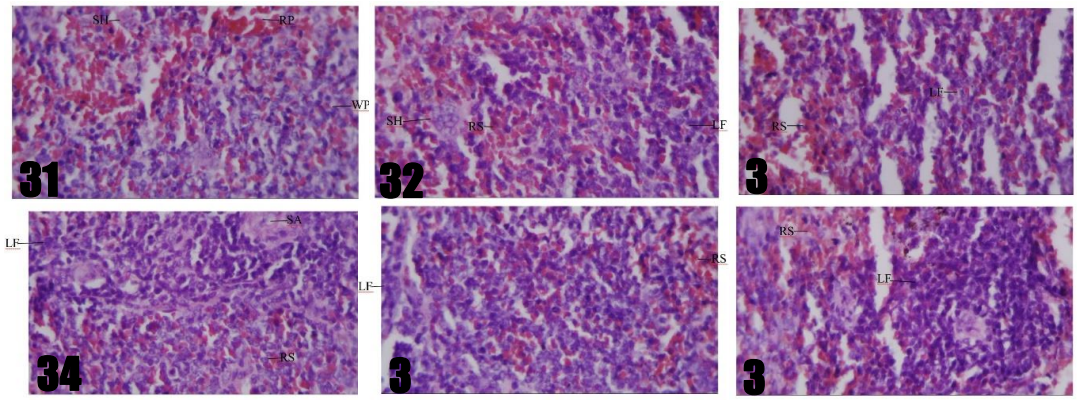

Figure 6. Rat spleen segment photomicrograph after 35 days of treatment with Picralima nitida aqueous fruit pulp extract at concentrations of 200, 500, 1000, 2000, and 3000 mg/kg body weight/day (plates 32–36, respectively) compared with plate 31 (control). H&E x 400 was used to stain sections (plates 31–36).

The heart muscle was examined for cardiac muscle fibers and interstitial spaces containing cardiac vessels. The extract shows a normal coronary artery and myocardium as well as a normal round and patent coronary artery. At 2000 mg/kg, the coronary artery appeared normal but dilated due to vasodilatation. Widening blood vessels reduces vascular resistance, increases cardiac output, and reduces blood pressure. An extract dose of 3000 mg/kg of body weight caused hypertrophy of the artery, indicating blood vessel congestion. Inflammatory cells and blood vessel congestion, known as myocarditis, are associated with this condition.

Photomicrographs of the liver reveal the cytoplasm of hepatocytes, sinusoids, and the portal vein, which supply 75%–80% of the blood to the hepatic system and bile duct. The portal triad zone around these vessels is characterized by plasma cells, which are smaller than polymorphs and stimulated by active inflammation. The liver's innate immune system has lymphocytes around it, but seeing more plasma cells around the portal triad indicates a boost in the liver's immune system at 200 mg/kg of the extract. The 500–2000 mg/kg extract showed normal liver architecture and no inflammation around the portal triad. However, the 2000 mg/kg extract dilated the blood vessels, similar to heart vessels. The 3000 mg/kg extract stimulated inflammation around the portal triad, with most inflammatory cells being polymorphonuclear leukocytes. This condition is known as portal hepatitis, and it is an inflammation that occurs in the portal zone of the liver.

The renal photomicrograph shows normal glomeruli, arteries, and tubules with normal tissues. The 500–1000 mg/kg extract showed a normal glomerulus, but at 200 mg/kg, it showed a glomerulus with well-opened tubules and active interstitial congestion. At 2000 mg/kg, the extract increased blood flow and opened tubule lumens, a condition used in hypertension treatment. At 3000 mg/kg, some tubules around the glomerulus were degenerated.

Plate 19 shows the Langerhan islets containing endocrine cells, blood vessels, and pancreatic ducts. The 200 mg/kg extract exhibited the largest endocrine cell aggregation and dilated pancreatic ducts. The 500–1000 mg/kg extract showed normal blood vessels, exocrine glands, Langerhan islets, and pancreatic duct. However, 2000 mg/kg dilated the blood vessel. The 3000 mg/kg extract caused mild inflammation around blood vessels, indicating pancreatitis. The islets and endocrine glands were not affected.

The photomicrograph of the lungs shows alveoli spaces, terminal bronchioles, and bronchial arteries. The 200–2000 mg/kg extract showed normal terminal bronchioles, bronchial arteries, and alveoli. However, 200 mg/kg of activated lymphoid aggregate, 2000 mg/kg of activated blood vessels, and 3000 mg/kg of activated bronchioles produced ulcerated bronchioles.

The spleen contains Sirius, a mononuclear phagocyte system, and red blood cells. Extracts containing 200–2000 mg/kg of extracts showed increased red blood cell sequestration, lymphocyte follicles, and splenic histiocytes. Extracts containing 3000 mg/kg showed normal lymphocyte follicles and red blood cell sequestration.

CONCLUSIONS

The unripe P. nitida aqueous fruit pulp extract is relatively safe at lower concentrations, but at higher concentrations, the organs exhibit various toxicity effects, including heart myocarditis, liver hepatitis, kidney tubular necrosis, pancreatitis, and lung bronchiolar mucosa ulceration. The extract also significantly reduced blood glucose and cholesterol levels in the model animals studied. P. nitida aqueous unripe fruit pulp extract can be used at low doses as an adjuvant to moderate blood glucose and cholesterol levels. However, long-term studies should be encouraged, as should the identification of active components in the extract.

AUTHOR CONTRIBUTION

All authors have accepted responsibility for the entire content of this submitted manuscript and have approved the manuscript.

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CONFLICT OF INTEREST

No funding organization(s) participated in the study design; collection, analysis, and interpretation of data; writing of the report; or decision to submit the report for publication.

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