Toxicity assessment of methanol extract of *Parinari curatellifolia* Planch ex. Benth

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**Abstract**: *Parinari curatellifolia* is mostly employed in the treatments of leukemia, anemia and malaria. The study was to determine the hematological, biochemical and histopathological effects of methanol stem bark extract of *Parinari curatellifolia* (PCME) on liver and kidney of adult female Wistar rats. The oral acute (Lorke’s method) and sub-chronic toxicity of PCME were evaluate. Adult female Wistar rats were grouped into group I (n=6), normal control (5mL/kg of distilled water) and groups II-IV (100, 200 and 400mg/kg/day of PCME, n=6 each) for 30 days. On 31st day, biochemical, hematological and histopathological parameters were assessed. The LD₉₀ was found greater than 5000mg/kg. In hematological parameters, RBC showed an increase in the treatment groups, however, the increment was not significant. HCT, PLT, MCH and MCHC levels were significantly increased (p<0.05) while WBC levels in all PCME groups were reduced (p<0.05). Amongst the liver biochemical parameters, only the ALP activity was significantly (p<0.05) raised. In kidney biochemical parameters, serum potassium and chloride were significantly (p<0.05) reduced. Histopathological findings on the liver showed mild infiltrating leukocytes, vascular congestion and piecemeal necrosis compared to the normal anatomic features while that of the kidney appeared normal. In conclusion, PCME may be slightly toxic to the liver on repeated administration.

**Keywords**: *Parinari curatellifolia*, liver, kidney, histopathology, biochemical, hematology, toxicity.

**INTRODUCTION**: *Parinari curatellifolia*, liver, kidney, histopathology, biochemical, hematology, toxicity.

**INTRODUCTION**

Medicinal plants (MP) are major sources of treatments for emerging diseases. Owing to people’s quests for healing and other health-related issues, a high percentage of global population particularly in the rural communities patronise herbal medicine for healthcare needs without being aware of their possible adverse effects (Dougnon et al., 2021). The common perception that MP being natural in origin are without adverse effects is untrue and misleading (Gamde et al., 2019). Safety of MP is a major issue in traditional medicine. Ascertaining their toxicity profile is of paramount importance (Ugwah-Oguejiofor et al., 2019).

*Parinari curatellifolia* Planch. ex Benth, also known as Mobola plum, Fever tree or hissing tree belongs to the family Chrysobalanaceae. *P. Curatellifolia* is an evergreen medium to large tropical tree found in many deciduous woodlands of Africa including Nigeria where the indigenous names in Hausa, Yoruba, Fulani and Tiv languages are Rura, Idofun, Nawarre-badi and Ibua respectively (Halilu et al., 2018). Ethnobotanical uses of *P. curatellifolia* include anemia, leukemia, diarrhea and malaria (Kundishora et al., 2020). Previous scientific researches have shown that *P. curatellifolia* possesses cytotoxic activities (Anesu et al., 2020), anticonvulsant and sedative activities (Mshelia et al., 2019), antimicrobial (Halilu et al., 2018), anti-snake venom and analgesic activities (Halilu et al., 2020). Some phytochemicals identified in *P. curatellifolia* which accounted for its medicinal properties were cardiac glycosides, tannins, anthraquinones, saponins, terpenoids and flavonoids (Halilu et al., 2018; Mshelia et al., 2019).

Despite the enormous nutritional and therapeutic properties of *P. curatellifolia*, there is no study on the risk awareness which guaranteed the safety of its use. Although reports of serious health injuries or mortality due to adverse effects of traditional medicines are scanty, several researches have revealed that they could be injurious to the body (Osagie-Eweka et al., 2021). The study was aimed to ascertain safety of methanol stem bark extract of *Parinari curatellifolia* (PCME) on liver and kidney of female Wistar rats (FWR).

**MATERIALS AND METHODS**

**Plant preparation**

Fresh stem bark of *P. curatellifolia* (FSB) was obtained from Zaria town of Kaduna State, Northwest Nigeria. It was identified by Mallam Musa of Department of
Toxicity assessment of methanol extract of Parinari curatellifolia Planch ex. Benth

Biological Sciences, Faculty of Science, ABU Zaria where voucher sample (903) was kept for reference. FSB was dried under shade and powdered with wooden pestle and mortar. The powdered plant material (3kg) was macerated in 6L of methanol for 24 hours and filtered using Buckner filter. The filtrate was evaporated using a rotary evaporator. The dried extract was then weighed and the percentage yield calculated.

Experimental animals
FWR were purchased from animal centre in UDUS, Nigeria. FWR were feed with rat feed and tap water ad libitum. Housing conditions were maintained at 25±2°C at 12h day/night cycles. Ethical Committee of Department of Pharmacology and Toxicology, UDUS approved the study (PTAC/PC(OA)/OT/007-18). Protocol for care and animal handling were as established in public health guidelines.

Acute toxicity
Oral acute toxicity evaluation of PCME was conducted in two phases to determine the LD_{50} using Lorke’s method (Lorke, 1983). First phase of the study involved nine (9) FWR (n=3) which were divided into three different doses (10, 100 and 1000mg/kg). The FWR were monitored for 24h for behavioural signs of harmfulness and/or death. Next, the 2nd phase of the study involved three (3) FWR divided into three (3) doses (n=1) (1600, 2900 and 5000mg/kg). Observations were made as before.

Sub-chronic toxicity studies
The animals were allotted into 4 groups (n=6) as follows; group I, which is the normal control (5mL/kg of distilled water) and PCME groups II-IV (100, 200 and 400mg/kg/day of PCME) for 30 days. All FWR were treated orally. Twenty-four hours after last dose was given, FWR were anesthetized and blood samples drawn via cardiac puncture using a 10ml syringe.

About 2.5ml were poured into the EDTA bottles for hematological analysis while the rest of the blood was poured into the plain bottles for biochemical analysis (Yannick et al., 2022). The abdomens of animals were dissected and kidneys and livers excised and fixed in buffered 10% formal saline solution for histopathological assessment.

Hematological analysis
White blood cells (WBC), neutrophils, red blood cell (RBC), hemoglobin concentration (HBG), granulocytes (GRA), lymphocytes (LYM), MID cells (MID), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet distribution width (PDW), platelet (PLT) count, plateletcrit (PCT) and mean platelet volume (MPV) were measured using an auto-analyzer machine (Genesis™ HA6000) following protocols from the manufacturer.

Biochemical analysis
The blood collected from FWR were placed in plain bottles and centrifuged at room temperature at 3000 rpm for 5 min for serum assay. Serum albumin, total protein, total and direct bilirubin and the liver enzymes (Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were assessed using a chemistry analyzer machine (Raytochemray 120, Germany) following protocols from the manufacturer as described in the respective assay kits. Serum sodium, potassium, calcium, bicarbonate, urea and creatinine (kidney function test) were determined following standard procedures as described in the respective assay kit inserts.

Histological analysis
The fixed livers and kidneys tissues were processed in 70%, 80%, 90% and absolute alcohol following standard procedures. They were cut at 5μm (Surgecare micromtome, model 335A, USA) and dewaxed for staining with hematoxylin and eosin (H&E). Olympus microscope equipped with a digital camera for photomicrographs was used to examine the tissues at x10 and x40 objectives (Diallo et al., 2020).

Qualitative and quantitative phytochemical screenings
Qualitative phytochemical screening of PCME for the presence of saponins, alkaloids, phenolics, flavonoids, tannins, steroids/triterpenoids, cardiac glycosides, cyanogenetic glycosides and anthraquinones was carried out using the methods described by Roghini et al. (2018). The quantitative determination of phytochemicals was conducted according to the methods described by Neşe et al. (2022).

STATISTICAL ANALYSIS
Data were analyzed by SPSS 23.0 version software and presented as mean ± SEM. The differences amongst means were analysed using two way ANOVA and Dunnett’s posthoc test for comparison between treated and control groups at p≤0.05 level of significance.

RESULTS

Percentage yield of PCME
The percentage yield of PCME was 8.2 % (w/w).

LD_{50} of PCME in FWR
Treatment of FWR with PCME at doses of 10 to 5000mg/kg using Lorke’s method showed no obvious, adverse effect on the rats (table 1). PCME produced no visible toxicity/mortality signs in FWR. The LD_{50} was thus above 5000mg/kg.
Effect of PCME on hematological parameters after 30 days treatment

While RBC, PLT and MCHC levels that were increased, HBG, LYM, MID, GRA, MCV, PCT, MPV and PDW levels were not affected (table 2). WBC levels were significantly \( (p<0.05) \) reduced compared to normal control group.

Effect of PCME on biochemical parameters

The effect of PCME on serum liver function parameters is presented in figs. 1, 2 and 3. The mean activities of serum AST and ALT were not statistically different from normal control group (fig. 1). Again, serum levels of TP, ALB, ALP, TBIL and DBIL were not statistically different from control group (figs. 2 and 3).

In the kidney function test, serum potassium and chloride levels in all test groups were reduced in a dose-dependent manner (table 3) and significantly \( (p<0.05) \) at 400mg/kg with respect to normal control.

Histopathological effects of PCME on the liver and kidney

Histopathological findings from the liver treated with PCME showed normal hepatocytes in all groups (fig. 4A-D) but with mild infiltrating leukocytes and piecemeal necrosis in all the rats treated with PCME (fig. 4B-D) while that of the kidney showed normal regular glomeruli tuft, urinary space, renal tubules and interstitium in all the groups (fig. 5A-D).

Qualitative phytochemical screening

The various tests conducted revealed the presence of some classes of secondary plant metabolites with others not identified (table 4).

Quantitative phytochemical analysis

The quantity of the flavonoid and saponins were expressed in grams and as percentages in the whole plant extract (table 5).

DISCUSSION

Toxicity profile of any MP is evaluated to find and establish adverse effect, its significance and exposure level at which this effect is observed. LD\(_{50}\) is used to determine the safety or toxicity of a substance (Renata and Patrick, 2022). Our results from the acute toxicity study revealed that PCME administered via oral route to FWR at 5000mg/kg following Lorke’s method of testing acute toxicity, failed to produce any sign of toxicity and/or mortality. Therefore, the LD\(_{50}\) was greater than 5000mg/kg and thus classified as relatively safe (Renata and Patrick, 2022). Previous investigation by Halilu et al., (2020) on the LD\(_{50}\) of the methanol and ethyl acetate extracts following i.p. route were 113mg/kg and 471.17mg/kg respectively revealing toxicity. The deviation from the result of the current finding may be attributed to the differences in the route of administration. However, information from acute toxicity is usually of limited clinical application hence the necessity of sub-chronic toxicity study.

Substances that are dosed frequently in the course of disease treatment may require repeated dosing toxicological evaluation (sub-chronic study). This is because daily use may amount to drug accumulation in the body leading to impairment of tissues and organs (Bariweni et al., 2018). Therefore, sub-chronic toxicity testing is essential in assessing the impact of the substance on the target organ, haematological or biochemical parameters which are not assessed in acute toxicity study (Ugwah-Oguejiofor et al., 2019). Hence,
The sub-chronic toxicity profile of PCME was evaluated in rats using gross assessment, haematological, biochemical and histological parameters. Sub-chronic administration of PCME caused no observable marks of toxicity or mortality in the rats. Haematological parameters are frequently used to evaluate functional characteristics of blood and blood forming organs such as the liver and kidney (Olayode et al., 2019). The extent of toxicity of an agent/drug are often determined through assessment of these parameters.

In our study, PCME caused an increase in RBC and platelet counts. This suggests that PCME may possess constituents capable of causing stimulatory activities in the hematopoietic system which could lead to the rise in the production of RBC and Platelets. The reduction in white blood cell count observed suggests that PCME may possess some bioactive agents that could cause destruction or impaired production of white blood cells. Thus, administration of PCME may predispose to infection consequent of the reduction of WBC (Ononamadu et al., 2020).

Table 1: LD$_{50}$ of PCME

<table>
<thead>
<tr>
<th>Phase</th>
<th>Extract</th>
<th>Dose (mg/kg)</th>
<th>Mortality</th>
<th>Toxicity observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>PCME</td>
<td>10</td>
<td>0/3</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>0/3</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>0/3</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1600</td>
<td>0/1</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2900</td>
<td>0/1</td>
<td>Reduced activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000</td>
<td>0/1</td>
<td>sedation</td>
</tr>
</tbody>
</table>

Table 2: Effect of PCME on hematological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC ($\times 10^{12}$/L)</td>
<td>1.57±0.91</td>
<td>0.59±0.32</td>
<td>1.75±1.18</td>
<td>3.33±3.22</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>0.68±0.10</td>
<td>0.50±0.04</td>
<td>0.35±0.02</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>6.25±0.83</td>
<td>9.00±5.50</td>
<td>27.40±12.34*</td>
<td>1.45±0.86</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>4.90±0.73</td>
<td>4.58±1.29</td>
<td>9.25±2.80*</td>
<td>6.00±2.12</td>
</tr>
<tr>
<td>MCH</td>
<td>0.87±0.16</td>
<td>4.52±1.46</td>
<td>16.48±3.37*</td>
<td>0.95±0.23</td>
</tr>
<tr>
<td>MCV</td>
<td>159.05±7.45</td>
<td>154.85±5.65</td>
<td>135.25±6.81</td>
<td>99.50±8.90*</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>15.95±3.12</td>
<td>17.53±2.34</td>
<td>7.58±0.71*</td>
<td>13.73±3.71</td>
</tr>
<tr>
<td>WBC ($\times 10^{9}$/L)</td>
<td>17.73±4.91</td>
<td>10.70±1.58*</td>
<td>4.84±1.31*</td>
<td>3.19±0.28*</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>52.08±1.91</td>
<td>50.38±0.47</td>
<td>48.73±1.57</td>
<td>50.25±1.18</td>
</tr>
<tr>
<td>GRA (%)</td>
<td>26.88±1.13</td>
<td>25.73±1.17</td>
<td>28.35±0.67</td>
<td>28.65±0.58</td>
</tr>
<tr>
<td>MID (%)</td>
<td>20.90±1.03</td>
<td>22.00±0.18</td>
<td>22.93±1.44</td>
<td>21.10±0.63</td>
</tr>
<tr>
<td>PLT ($10^9$/L)</td>
<td>268.78±49.63</td>
<td>2039.80±70.68*</td>
<td>2229.50±43.91*</td>
<td>1548.80±24.85*</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>0.20±0.03</td>
<td>0.71±0.42</td>
<td>1.00±0.32</td>
<td>0.49±0.25</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>7.10±0.06</td>
<td>6.75±0.99</td>
<td>6.73±1.03</td>
<td>6.63±0.73</td>
</tr>
<tr>
<td>PDW (fL)</td>
<td>11.35±0.09</td>
<td>13.45±1.94</td>
<td>13.58±1.08</td>
<td>14.83±2.09</td>
</tr>
</tbody>
</table>

Values are mean± SEM, n = 6. *p<0.05

Table 3: Effect of PCME on serum renal function parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Na (mmol/L)</th>
<th>K (mmol/L)</th>
<th>Cl (Mmol/L)</th>
<th>HCO$_3$ (Mmol/L)</th>
<th>Urea (Mmol/L)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5 ml/kg</td>
<td>139.25±1.38</td>
<td>4.15±0.26</td>
<td>100.2±2.14</td>
<td>26.50±1.71</td>
<td>4.30±0.35</td>
<td>0.70±0.09</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>139.75±3.33</td>
<td>3.43±0.25</td>
<td>92.0±2.35</td>
<td>25.25±2.87</td>
<td>4.30±0.38</td>
<td>0.60±0.13</td>
</tr>
<tr>
<td>PCME</td>
<td>400</td>
<td>133.75±4.71</td>
<td>2.75±0.12*</td>
<td>89.2±0.63*</td>
<td>25.75±2.95</td>
<td>5.28±0.33</td>
<td>0.70±0.15</td>
</tr>
</tbody>
</table>

Values are mean± SEM, n = 6. *p<0.05
AST, ALP and ALT are classical biomarkers for evaluating liver injury (Kaid et al., 2019). The serum concentrations of these enzymes are index of damage or changes in the permeability of hepatocytes membrane (Mega et al., 2021). In our study, the levels of AST and ALT were similar to the control. However, ALP activity was significantly increased. Iso-enzymes of ALP abound in the various organs of the body including the bones, placenta, kidneys, intestines and liver. There are many clinical states (including bone and liver diseases) in which the activity of the ALP is elevated (Dana et al., 2020). With significant elevation in serum ALP activity and normal AST and ALT activities, ALP upsurge may likely emanate from other ALP iso-enzyme synthesising tissues apart from liver (Osagie-Eweka et al., 2021). Again, biliary duct obstruction may be implied (Alkali et al., 2018). It is also imperative to note that the mild infiltrating leukocytes and piecemeal necrosis observed in the liver sections treated with PCME (Fig. 4) may be indicative of mild liver injury.
the liver of rats treated with PCME might have caused hepatobiliary obstruction, resulting in increased ALP activity. Normal levels of serum total proteins and albumin imply that PCME failed to compromise the liver’s synthesizing function. Therefore, toxicity caused by the extract to the liver may be mild. These findings on liver enzymes are in accordance with previous reports on the seeds and polyherbs of Parinari curatellifolia (Dana et al., 2020). There is often preservation of the metabolic and secretory functions of the liver amidst hepatic cells damaged. Our extract induced mild histopathological effect in the liver even though the liver enzymes AST and ALT were apparently similar to the normal control. This result was similar to the necro-inflammatory and edematous changes caused polyherbal mixture of Parinari curatellifolia (Maha and Eman, 2022). These histopathological changes give credence to the deleterious effects of the stem bark extract of Parinari curatellifolia on prolonged use.

The kidneys play critical role in maintaining overall health. Estimation of urea and creatinine levels are often used to evaluate kidney function (Friedrich, 2020). In our study, urea and creatinine levels were similar to the control. The implication of the data obtained is that the ability of the kidney to carry out its functions were not compromised by PCME (Friedrich, 2020). The extract however caused a decrease in potassium and chloride levels in the animals. This is indicative of adequate renal (electrolyte regulatory) function. Increased serum potassium is indicative of renal failure (Friedrich, 2020). The extract to the liver may be mild. These findings on kidney to the liver may be mild. These findings on kidney functions were not compromised by PCME (Friedrich, 2020). The extract however caused a decrease in potassium and chloride levels in the animals. This is indicative of adequate renal (electrolyte regulatory) function. Increased serum potassium is indicative of renal failure (Friedrich, 2020). Histopathological evaluation also supported these findings.

CONCLUSION

LD₅₀ of PCME per oral was calculated to be high (>5000mg/kg) in FWR. Sub-chronic administration of PCME in Wistar rats decreased WBC and increased platelet count but did not affect serum biomarkers for liver and kidney functions except potassium and chloride ions. Histopathological effects in the liver were mild infiltrating leukocytes, vascular congestion and piece meal necrosis while the kidneys appeared normal. Thus, PCME may cause liver injury on prolonged use and therefore caution should be exercised.

REFERENCES


