Post-Treatment Evaluation of the Side Effects of Methanol Leaf Extract from *Paullinia pinnata* (Linn.), an Antityphoid Plant

1,3Paul K. Lunga, 2Joseph M.M. Nkodo, 1Jean D. Tamokou, 1Jules-Roger Kuiate, 1Donatien Gatsing and 4Joseph Tchoumboue

1Laboratory of Microbiology and Antimicrobial Substances, Faculty of Science, University of Dschang, Dschang, Cameroon
2Laboratory of Anatomy and Pathological Cytology, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Yaoundé, Cameroon
3Laboratory of Phytobiocemistry and Medicinal Plants Study, Faculty of Science, University of Yaoundé 1, Yaoundé, Cameroon
4Laboratory of Animal Physiology and Health, FASA, University of Dschang, Dschang, Cameroon

**ABSTRACT**

**Background and Objective:** *Paullinia pinnata* is an African woody vine whose leaf decoction has been used in Cameroon for the treatment of bacterial infections like typhoid fever, syphilis, gonorrhea, diarrhoea and symptoms such as stomach-ache and waist pain. The present study was designed to evaluate the adverse side effects resulting from the use of *P. pinnata* methanol leaf extract in the treatment of *Salmonella typhimurium*-induced typhoid in Wistar rats. **Methodology:** After the establishment of infection by oral administration of a *S. typhimurium* suspension, animals were treated by the daily administration of *P. pinnata* methanol leaf extract at various doses (55.75, 111.50, 223 and 446 mg kg\(^{-1}\) b.wt.). The effect of the extract on body weight evolution was monitored daily. **Results:** Also, the effect of the extract on relative organ weight, biochemical parameters as well as liver histology was assessed. Irrespective of sex, typhoid fever induced an abnormal increase in the relative weight of most vital organs of toxicological importance. However, extract treatment normalized the excessive increase in relative organ weights; while inducing a significant (p<0.05) body weight gain in a dose-dependent manner. The level of liver enzymes (ALT and AST) were significantly (p<0.05) reduced after the treatment of the animals with *P. pinnata* leaf extract. The histopathological analysis of the liver revealed that extract treatment greatly reduced the degree of liver affections in a dose-dependent manner. However, at high doses (dose ≥223 mg kg\(^{-1}\) b.wt.) the extract was capable of stimulating hepatic necrosis. **Conclusion:** The overall results of this study indicate that the methanolic extract of *P. pinnata* leaves has hepatoprotective effects especially in males. It has adverse side effects at high doses and even at low doses in female rats and thus, should be used with caution in male and should probably be eliminated from the treatment of female subjects.

**Key words:** Antibacterial properties, *Paullinia pinnata*, adverse side effects

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**INTRODUCTION**

*Paullinia pinnata* (Sapindaceae) is a liana, used in west region of Cameroon for the treatment of bacterial infections like typhoid fever, syphilis, gonorrhea, diarrhoea as well as stomach-ache and waist pain. In East Africa, the leaves of *P. pinnata* are used in the treatment of gonorrhea, wounds and microbial infections\(^1\). The antioxidant properties of the methanol extract of the leaves and antibacterial activity of fatty acids from the roots of *P. pinnata* have been investigated\(^2\). In the ongoing studies, potential antityphoid properties of the methanol extract from the leaves of *P. pinnata*, both *in vitro* on four species of *Salmonella* (*S. typhi*, *S. paratyphi* A, *S. paratyphi* B and *S. typhimurium*) and *in vivo* on a *S. typhimurium*
induced typhoid model in Wistar rats was demonstrated. In vitro, the Minimum Inhibitory Concentrations (MICs) ranged from 48-3125 μg mL\(^{-1}\), while in vivo, the extract could eradicate the disease condition after 4 and 5 days, respectively at a daily oral dose of 446 and 223 mg kg\(^{-1}\) b.wt. However, no scientific knowledge exists pertaining to the toxicological studies of this plant. Due to the indiscriminate use of this plant by the local population irrespective of the dose, the present work was designed on the basis of its traditional use and was aimed at investigating the post treatment side effects that could accompany the use of methanol leaf extract of *P. pinnata* in the treatment of common bacterial infections.

**MATERIALS AND METHODS**

**Drugs, chemicals and kits:** Ciprofloxacin (Sigma-Aldrich), oxytetracycline (Sigma-Aldrich) and cyclophosphamide (Sigma-Aldrich) were purchased from Sigma-Aldrich (Chemie Gmbh, Steinhein, Germany). Tween 80 (Fisher Scientific, Loughborough, United Kingdom), SS agar (Liofilchem), Mueller Hinton Broth (Sigma-Aldrich), Nutrient agar (Sigma-Aldrich) were purchased from Sigma Aldrich, South Africa and stored, where necessary at 4°C. Kits for the quantification of serum creatinine, triglycerides, cholesterol, transaminases were obtained from Hospitex diagnostic, Roma, Italia.

**Plant materials:** *Paullinia pinnata* leaves were collected from Dschang, West Region of Cameroon in January 2009. The identification of plant specimens was done at the Cameroon National Herbarium in Yaounde, where a voucher specimen was deposited under the reference number 10702/SRFCam.

**Extract preparation:** Air-dried powder of *Paullinia pinnata* leaves was completely submerged in methanol in a mass to volume ratio of 50 g: 250 mL and then covered with aluminium foil. Extraction was allowed to proceed for 48 h. The mixture was then filtered using Whatman filter paper No. 1 and the filtrate was concentrated under vacuum using a rotavapor (Buchi) to obtain the crude extract. The process was repeated twice on the residue in order to maximize yield. The powdered extracts was stored at 4°C in the refrigerator till usage.

**Phytochemical screening:** The major classes of phytochemicals: Alkaloids, anthraquinone, flavonoids, polyphenols, triterpenes, steroids, saponins, tannins and anthocyanins were screened for in the crude extract using standard methods. Antityphoid assays

**Microorganism:** The *Salmonella* species used in this study was *Salmonella typhimurium*, a clinical isolate obtained from “Centre Pasteur” of Yaounde-Cameroon.

**Preparation of bacterial inoculum:** The preparation of bacterial inoculum was done by using 18 h old overnight bacterial cultures prepared in nutrient agar. A few colonies of bacteria were collected aseptically with a sterile loop and introduced into 10 mL of sterile 0.90% saline distilled water. The concentration of the suspension was then standardized by adjusting the optical density to 0.10 at 600 nm, corresponding to bacterial cell suspension of 10\(^8\) CFU mL\(^{-1}\).

**Immunosuppression of animals:** Animals were immunosuppressed two day before infection by the oral administration of 30 mg kg\(^{-1}\) b.wt. of cyclophosphamide as previously described with slight modification.

**Typhoid induction:** For infection, 1 mL of *Salmonella typhimurium* suspension prepared above was orally administered to each animal. Only infected animals were selected on the basis of their fecal bacterial load on petri dishes and used.

**Grouping of animals and treatment:** Animals were arranged into sixteen groups of four animals each. Groups 1, 2, 3, 4, 5, 6, 7 and 8 were males while groups 1b, 2b, 3b, 4b, 5b, 6b, 7b and 8b were females selected from the infected stock. The animals were treated as follows:

- Group one was not infected and received distilled water during the treatment period (reference groups).
- Group two received distilled water during the treatment period (negative control groups).
- Groups three and four received ciprofloxacin (7.14 mg kg\(^{-1}\) b.wt.) and oxytetracycline (5 mg kg\(^{-1}\) b.wt.) during treatment (positive control groups).
- Group five, six, seven and eight received the *P. pinnata* leaf extract at concentrations of 55.75, 111.50 and 223.00 and 446.00 mg kg\(^{-1}\) b.wt., corresponding to MIC, 2MIC, 4MIC (MBC) and 8MIC, respectively.

Food and water were given to the animals before and during the treatment ad libitum. Treatment was done by administering the extracts orally, every morning. Each day, the fecal matter was collected during the administration process. The fecal matter of each animal
was cultivated on SS agar to assess the number of colonies with time and this indicated how the animals were complying with treatment using the extract at various doses.

**Healing trends of the treatment groups:** Irrespective of sex, group 3, 4, 5, 6, 7 and 8 were healed after 4, 4, 8, 6, 5 and 4 days of treatment, respectively.

**Effects of crude extract of P. pinnata on the evolution of body weights:** The individual weights of the animals were taken every morning prior to treatment with the help of an electronic balance (Mettler PE 160).

**Determination of relative organ weight:** Immediately after blood collection from the rats, the heart, spleen, kidney, lungs and liver were excised from the rat with the help of surgical forceps and scissors. They were blotted dry on a tissue paper and weighed (using and electronic balance, Mettler PE 160). The relative organ weight was calculated for each rat as shown below:

\[
\text{Relative organ weight (\% b.wt.)} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100
\]

**Effects of crude extract of P. pinnata on some biochemical parameters in Salmonella typhimurium-induced typhoid in rats**

**Collection of blood:** Each time a group was completely healed, animals were anesthetized with chloroform vapour and dissected. Blood was collected by cardiac puncture into non-heparinised tubes (for serum creatinine, triglycerides, cholesterol, transaminases). These tubes were left for 3 h at room temperature for blood to coagulate, after which they were centrifuged at 3000 g for 15 min; the supernatant (serum) was collected and preserved at -30°C for biochemical analysis. The liver was stored in 10% formalin saline solution for histopathological analysis.

**Evaluation of biochemical parameters:** The level of serum creatinine, total serum cholesterol and HDL cholesterol, triglyceride and transaminases were evaluated using commercial kits (Hospitex diagnostic, Roma, Italia).

**Histological analysis:** After treatment, the animals were sacrificed and the liver tissues of each animal was stored in 10% formalin saline solution (v/v) for two weeks and then, embedded in paraffin. Sections of 5-6 mm were fixed on slides and routinely stained with haema-toxylin and eosin (H and E). Pathological observations were performed on gross and microscopic basis. Histological plates were encrypted for analysis by a histopathologist. Any alterations compared to the normal structure were registered.

**Ethics:** The experiments were conducted according to the ethical guidelines of Committee for Control and Supervision of Experiments on Animals (Registration No. 173/ CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research. Moreover, all procedures involving animals were carried out in strict compliance with the rules and regulations of local Ethics Committee.

**Statistical analysis:** Where possible, data was subjected to one-way analysis of variance and differences between samples at p<0.05 were determined by Waller–Duncan test using the Statistical Package for the Social Sciences (SPSS) program. The experimental results were expressed (where appropriate) as Mean±SD of four replicates.

**RESULTS**

**Phytochemical screening:** All the classes of phytochemicals tested (alkaloids, anthraquinone, flavonoids, polyphenols, triterpenes, steroids, saponins, tannins and anthocyanins) were detected in P. pinnata extract.

**Variation of body weight gain:** Body weight gain (Fig. 1) was calculated daily from the animal weight. It is seen that after infection, the animals lost weight abruptly until the third day when treatment started. Weight loss was more pronounced in the female rats especially on the third day, when infection was completely established. Irrespective of the sex, weight gain was directly proportional to the extract dose. The extend to which the animals complied with treatment was indirectly revealed by the weight gain. The more the improvement of the state of healing, the higher the weight gain. However, the negative control group (untreated group) lost weight continuously through out the experimental period, whereas the normal group (uninfected group) gained weight significantly (p<0.05) during the experiment.
**Relative organ weight:** The organ weight of major organs involved in toxicity study: Liver, heart, lungs, spleen and kidney were express as a percentage of the body weight (Table 1). Extract-treatment significantly (p<0.05) reduced, the relative liver and kidney weights in male rats in a non dose-dependent manner, but instead increased their weights in female rats. The relative weight of the lungs did not show significant variation irrespective of the sex of the animal. The relative weights of the heart and spleen significantly (p<0.05) reduced to beyond the normal values in male animals at a dose of 446 mg kg\(^{-1}\). In general, extract treatment shifted the relative weights of the organs of the infected animals towards the normal in a dose dependent manner.

**Variation of biochemical parameters:** Biochemical parameters, indicators of the toxicity state of the extract to the animal was equally evaluated post to the treatment process on serum obtained after the sacrifice (Table 2). The results show that infection by typhoid fever leads to the release of aminotransferases in blood. Irrespective of the sex, the level of these enzymes were significantly (p≤0.05) reduced after the treatment of the animals with the *P. pinnata* leaf extract. Total cholesterol did not vary significantly (p>0.05) in males, though there was a significant reduction in female rats. The extract at the high doses had negative effects on the HDL-cholesterol level especially on the female animals. At the highest dose (446 mg kg\(^{-1}\) b.wt.), serum creatinine was significantly (p≤0.05) high in both sexes compared to the other extract doses. In males, triglyceride did not vary significantly with dose whereas in females, a significantly (p≤0.05) dose-dependent increase was observed at doses less than or equal to 223 mg kg\(^{-1}\) b.wt. Like the male triglyceride level, the female LDL-cholesterol level showed a dose-dependent reduction up to the dose of 223 mg kg\(^{-1}\) b.wt. and then increased at 446 mg kg\(^{-1}\) b.wt. However, male LDL-cholesterol level did not show any dose-dependent variation. The risk factor of developing coronary heart diseases after treatment with the extract was significantly high in female animals especially at 223 mg kg\(^{-1}\) b.wt.

Judging from LDL-cholesterol, triglyceride and creatinine variation, it implies that the extract should be used with caution especially in the female sex and the maximum dose should be avoided.
Along each column and same sex values, with the same letter superscripts are not significantly different. Waller-Duncan (p<0.05).

Cipro: Ciprofloxacin, Oxy: Oxytetracycline, Ref: Reference values from uninfected, untreated animals. Only groups of the same sex are compared among themselves.

Table 2: Variation of biochemical parameters of the animals as a function of dose of *Paulinia pinnata* methanol leaves extract and sex after treatment of *Salmonella typhimurium* induced typhoid in rats

<table>
<thead>
<tr>
<th>Dose (mg kg⁻¹ b.wt.)</th>
<th>ALAT (U L⁻¹)</th>
<th>ASAT (U L⁻¹)</th>
<th>CHO total (mmol L⁻¹)</th>
<th>LDL CHO (mmol L⁻¹)</th>
<th>Triglyceride (mmol L⁻¹)</th>
<th>LDL CHO (mmol L⁻¹)</th>
<th>Risk factor of CHD</th>
<th>Creatinine (μmol L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>153.19±15.69a</td>
<td>60.23±1.51b</td>
<td>8.47±0.22b</td>
<td>5.35±0.92b</td>
<td>1.91±0.33b</td>
<td>0.24±0.11d</td>
<td>0.05±0.04d</td>
<td>447.91±8.09d</td>
</tr>
<tr>
<td>55.75</td>
<td>108.83±20.08b</td>
<td>34.92±3.49b</td>
<td>8.17±0.78b</td>
<td>4.66±0.54b</td>
<td>2.58±0.42b</td>
<td>0.18±0.01cd</td>
<td>0.04±0.02b</td>
<td>339.25±7.51b</td>
</tr>
<tr>
<td>111.5</td>
<td>85.84±9.29b</td>
<td>22.98±4.12b</td>
<td>8.98±0.52b</td>
<td>5.80±0.58b</td>
<td>2.42±0.70b</td>
<td>0.15±0.03bc</td>
<td>0.03±0.00b</td>
<td>334.33±10.92b</td>
</tr>
<tr>
<td>223</td>
<td>62.56±9.88b</td>
<td>33.17±3.14b</td>
<td>8.17±0.29b</td>
<td>4.93±0.32b</td>
<td>2.87±0.20b</td>
<td>0.07±0.05c</td>
<td>0.02±0.03c</td>
<td>295.00±8.50b</td>
</tr>
<tr>
<td>Cipro (7.14)</td>
<td>54.70±6.05b</td>
<td>25.89±5.68b</td>
<td>7.79±0.92b</td>
<td>4.69±0.86b</td>
<td>2.50±0.43b</td>
<td>0.11±0.00ab</td>
<td>0.02±0.01b</td>
<td>781.75±28.63b</td>
</tr>
<tr>
<td>Oxy (5.00)</td>
<td>85.26±10.80b</td>
<td>23.86±4.80b</td>
<td>11.56±0.90b</td>
<td>5.61±0.10b</td>
<td>2.35±0.48b</td>
<td>0.78±0.02a</td>
<td>0.14±0.06</td>
<td>511.33±20.56b</td>
</tr>
<tr>
<td>Ref</td>
<td>51.79±3.97b</td>
<td>18.62±5.33b</td>
<td>8.67±0.60b</td>
<td>5.46±0.28b</td>
<td>2.84±0.59b</td>
<td>0.07±0.01b</td>
<td>0.01±0.00b</td>
<td>388.41±16.91b</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>92.98±10.03d</td>
<td>59.07±4.29b</td>
<td>6.70±1.10b</td>
<td>4.49±0.86cd</td>
<td>1.86±0.42b</td>
<td>0.06±0.01b</td>
<td>0.01±0.00b</td>
<td>354.00±36.90b</td>
</tr>
<tr>
<td>55.75</td>
<td>92.53±8.99b</td>
<td>48.64±1.20a</td>
<td>7.12±0.61b</td>
<td>3.75±0.59b</td>
<td>2.55±0.51b</td>
<td>0.16±0.01c</td>
<td>0.04±0.031</td>
<td>405.62±7.37</td>
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<tr>
<td>111.5</td>
<td>38.41±2.17a</td>
<td>46.85±3.25a</td>
<td>9.38±0.36a</td>
<td>5.34±0.47b</td>
<td>3.46±0.34d</td>
<td>0.11±0.01ab</td>
<td>0.02±0.00b</td>
<td>457.25±16.45d</td>
</tr>
<tr>
<td>223</td>
<td>67.72±5.50a</td>
<td>35.21±3.21a</td>
<td>7.58±0.22a</td>
<td>2.53±0.57b</td>
<td>4.53±0.25b</td>
<td>0.10±0.08ab</td>
<td>0.05±0.01</td>
<td>580.16±31.15</td>
</tr>
<tr>
<td>Cipro (7.14)</td>
<td>59.36±7.14a</td>
<td>46.85±3.24a</td>
<td>9.81±0.95a</td>
<td>5.87±0.84b</td>
<td>3.77±0.34b</td>
<td>0.03±0.061</td>
<td>0.01±0.00</td>
<td>727.66±20.41d</td>
</tr>
<tr>
<td>Oxy (5.00)</td>
<td>59.94±6.44a</td>
<td>45.10±3.39a</td>
<td>8.64±0.50a</td>
<td>4.82±0.67de</td>
<td>3.50±0.27d</td>
<td>0.06±0.04</td>
<td>0.01±0.01b</td>
<td>649.00±14.25g</td>
</tr>
<tr>
<td>Ref</td>
<td>22.98±2.43</td>
<td>27.35±5.22</td>
<td>6.52±0.53</td>
<td>3.27±0.86b</td>
<td>2.90±0.38e</td>
<td>0.07±0.02a</td>
<td>0.02±0.00</td>
<td>324.50±11.50</td>
</tr>
</tbody>
</table>

Along each column and considering same sex values, with the same letter superscripts are not significantly different. Waller-Duncan (p<0.05).

Cipro: Ciprofloxacin, Oxy: Oxytetracycline, Ref: Reference values from uninfected, untreated animals. Only groups of the same sex are compared among themselves.

**Histopathological analysis:** The histopathological analysis of the liver of animals treated at various doses of the *P. pinnata* methanol leaf extract (Fig. 2) reveals that typhoid fever provokes some liver damages (dilation of the Centrolobular vein, constriction of the hepatocytes, dilation of the hepatic portal vein). Extract treatment greatly reduced the degree of liver affections in a dose-dependent manner. However, at high doses (dose ≥ 223 mg kg⁻¹ b.wt.) the extract was capable of stimulating hepatic necrosis especially in female rats.

**DISCUSSION**

Phytochemical analysis of the methanol leaf extract of *P. pinnata* revealed the presence of alkaloids, polyphenols, triterpenes, steroids, saponins and tannins. These substances were present in the stem bark extract.
<table>
<thead>
<tr>
<th>Dose (mg kg⁻¹ b.wt.)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><img src="image1" alt="Histopathological images" /></td>
<td><img src="image2" alt="Histopathological images" /></td>
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<tr>
<td>55.75</td>
<td><img src="image3" alt="Histopathological images" /></td>
<td><img src="image4" alt="Histopathological images" /></td>
</tr>
<tr>
<td>111.5</td>
<td><img src="image5" alt="Histopathological images" /></td>
<td><img src="image6" alt="Histopathological images" /></td>
</tr>
<tr>
<td>223</td>
<td><img src="image7" alt="Histopathological images" /></td>
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</tr>
<tr>
<td>446</td>
<td><img src="image9" alt="Histopathological images" /></td>
<td><img src="image10" alt="Histopathological images" /></td>
</tr>
</tbody>
</table>

Ciprofloxacin (7.14)

Oxytetracycline (5.00)

Reference

Fig. 2: Histopathological analysis of the liver after the treatment of typhoid fever with various doses of *Paullinia pinnata* methanol leaf extract, Magnification: 250X, C: Dilation of the Centro-lobular vein, S: Dilation of the Sinusoid (Constriction of the hepatocytes, increasing the spaces in between them) H: Dilation of the hepatic portal vein, St: Development of steatosis, P: Modification of the hepatic parenchyma cells, N: Necrosis
of *Erythrina klinei* and induced weight gain in experimental animals. The presence of these groups of compounds in the methanol extract of *P. pinnata* could explain the dose-dependent periodic eradication of typhoid fever and thus the weight gain observed in this study. Besides, many compounds from this extract were isolated; including oleanane-type triterpenoid saponins and a steroidal saponin with remarkable anti-salmonella activities *in vitro*. This phenomenon was further enhanced by the eradication of the disease condition since it was found that typhoid fever induces weight loss. In addition, these phytochemical classes of compounds in *Crassocephalum bauchiense* extract revealed that relative weights of different vital organs (spleen, kidney, liver, lung and heart) of treated groups were not significantly different from that of control group in both sexes. This corroborates the results of the present work, in which the *P. pinnata* extract fights against the excessive organ weight gain induced by typhoid fever; normalizing them towards the reference values.

According to Tiez [19], creatinine levels increase in the serum or in the urine when the cortex and/or the glomeruli are damaged. The increase in creatinine levels in both sexes of the none treated groups implies that typhoid condition leads to a distabilization of the integrity of these organs. The significant extract-induced reduction of serum creatinine levels in this study reveals a possible repair of the liver following the administration of *P. pinnata* extract. However, the situation was critical at the maximum dose, where instead of reducing the creatinine levels, the extract increased the levels to above those of the untreated groups in both sexes. The significant increase in the liver enzymes, alanine aminotransferase and aspartate amino-transferase, in serum of untreated group shows that typhoid fever affects the liver. *P. pinnata* extract plays a vital role in the re-establishment of the liver integrity, leading to a reduction in the levels of these enzymes in the treated groups of both sexes.

These observations corroborate liver damages as revealed by the histopathological examination of the cross sections of this organ. The absence of vascular congestions on the liver sections could be due to the vasodilatation action of the *P. pinnata* extract on the walls of blood vessels [21]. This extract could contain some substances capable of acting like none steroidal anti-inflammatory drugs by provoking a hypersensitivity reaction that led to inflammation (necrosis) [22] observed at high doses especially in female rats. Tubular edema could result from an inflammation or an increase of the level of carbohydrate plasma expanders (such as dextrose, mannitol and dextran) in blood whose renal excretion could lead to osmotic nephritis. During osmotic nephritis, these carbohydrates infiltrate into the cells of the proximal tubules, increasing the osmotic gradient across the plasma membrane, thus facilitating the entry of water into the cells which then swell. The presence of empty vacuole-like spaces in-between the hepatocytes (sinusoids) could be due to abnormal infiltration of intracellular substances from the hepatocytes or to a malfunctioning of the latter. This prevents the hepatocytes from ensuring the normal metabolism of the nutrients carried by blood, leading to their accumulation in the cytoplasm of hepatic cells [23].

The results reveal an increase in total cholesterol and LDL cholesterol in females. This may suggest that the *P. pinnata* extract favors the accumulation of cholesterol in blood capillaries and predisposes the female animals to possible risk of atherosclerosis and cardiovascular diseases [24]. This was further confirmed by the high value of risk factor of coronary heart disease in females. However, the situation was inversed in the male animals suggesting that this extract could be a good candidate for the treatment of microbial infections in male patients, especially those predisposed to cardiovascular diseases. To the best of our knowledge, this study is the first to show that *P. pinnata*, which is claimed to be a cure for infectious diseases, is a medicinal plant with some adverse biological properties. It is equally rare to find scientific reports outlining the side effects of locally used medicinal plants; most of which focus on acute and sub-chronic toxicities. Thus, the fact that these toxicological parameters are from extract-healed animals give the present work its uniqueness. If an extrapolation of the above results is to be made to humans, then it may be said that, precaution is necessary during the use of *P. pinnata* in the treatment of microbial infections, especially at doses > 446 mg kg\(^{-1}\) b.wt.

This study provides valuable data on undesirable side effects that accompanies the use *P. pinnata* in the treatment of bacterial infections that should be very useful for any future *in vivo* and clinical study of this medicinal plant. The methanolic extract of the leaves of *P. pinnata*, though very effective in the treatment of typhoid fever is slightly toxic especially at high doses and in females. Moreover, daily administration of the methanolic extract at doses ranging from 55.75 to 446 mg kg\(^{-1}\) b.wt. could restore typhoid fever-induced
alteration of liver histology in a dose-dependent manner. However, complementary parameters need to be measured to confirm this.

**CONCLUSION**

The overall results of this study indicate that the methanolic extract of *P. pinnata* leaves has hepatoprotective effects at doses $\geq 223 \text{ mg kg}^{-1} \text{ b.wt.}$ At higher doses, it has adverse side effects, leading to the alteration of the liver in females. It negatively affects the levels of toxicity biomarkers in female rats. This plant should thus be used with caution in male and should probably be eliminated in the treatment of female subjects.

**REFERENCES**