Acute and chronic toxicity evaluation of methanol leaf extract of *Psidium guajava* (Myrtaceae)

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Abstract

The medicinal value of herbal plants have been widely described in a number of studies, this has resulted in their increased usage. This study aimed to evaluate the safety of methanolic leaf extract of *Psidium guajava* extract in albino rats using biochemical, hematological and histological parameters. In acute toxicity, the extract was administered orally up to 5,000 mg/kg body weight once to male albino mice. While in chronic toxicity, twenty four adult male albino rats were randomly divided into four groups of six rats for each group. The control group received 10 ml/kg body weight distilled water daily. The other groups received 50, 200 and 400 mg/kg body weight of extract daily for 90 days. All the rats were observed daily for signs of toxicity and mortality. At the end of the treatment period, biochemical and hematological tests were carried out on prepared sera. Histology of vital organs was evaluated. Acute toxicology showed the LD<sub>50</sub> of the extract to be less than 5000 mg/kg. Chronic toxicological study revealed that at 200 mg/kg, there was no significant (P > 0.05) differences in hematological and biochemical parameters, and there was no alterations in the histology of the organs. However, at 400 mg/kg body weight, the concentrations of the liver biomarkers were increased, with distorted liver. Since no alterations was observed at 200 mg/kg, the extract may be considered to be relatively safe at this dose and could be used for long term treatment of infections.

Keywords: Acute toxicity; Chronic toxicity; *Psidium guajava*; Hematological parameters; Biochemical parameters; Safety

1. Introduction

Microbial infectious diseases are considered as a leading cause of infections and deaths in humans and animals worldwide. Many of these causative microorganisms are becoming increasingly resistant to antibiotic treatment threatening public health and calls for urgent search for antimicrobial agents from natural products [1]. The World Health Organization (WHO) estimates that 4 billion people, which is about 80% of the world’s population, particularly the developing countries use herbal medicines for various aspects of primary health care where new drugs are often beyond the reach of the poor [2, 3]. In support of the use of herbal medicines, the World Health Organization has recommended the use of these natural products but has emphasized the need to ascertain their safety/toxicity before consumption [4].

*Psidium guajava* belongs to the family Myrtaceae, it is considered to have originated from tropical South America. *Psidium guajava* tree grows in tropical and sub-tropical areas of the world such as Asia, Africa and Hawaii [5]. The plant
is also called Guava in English, commonly known as goyave and goyavier in French, guyabaorgoeajaab in Dutch, goiaba and goaibeira in Portuguese, jambubatu in Malaya, guayabo in Spanish, in Mexico and America it is known as pichi, posh and enandi [6]. In Nigeria, the common names include, guaba in Yoruba, giba in Hausa, gova in Igbo and ugwaba in Efik [7, 8].

Various parts of Psidium guajava plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, and inflamed gums [9]. Guava plant has also been used for the control of conditions such as diabetes, hypertension, obesity [10], rheumatism [11] and infantile diarrhea [12]. The plant is known to have antibacterial [13], antioxidant [14], anti-inflammatory [15], and antiviral [16] properties.

In view of the enormous medicinal properties of the plant as seen above, and based on the fact that the plant is readily available and within the reach of the indigenous people. There is need to ascertain the efficacy and safety of the plant when used daily for a long period.

2. Material

2.1. Collection and preparation of methanol leaf extract of Psidium guajava

The leaves of Psidium guajava were identified by Dr Suleiman Mikailu of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, (Uniport) and authenticated with the voucher number, Psidium guajava Myrtaceae (UPHM0453) then deposited in the Herbarium of the Department. The crude extract was prepared by the method described by [17] with slight modification.

2.2. Experimental Rats used

Male Albino rats (100-150) g, male albino mice (10-30) g were used for this study. All the animals were obtained from the animal house of the department of Pharmacology, Uniport. They were housed in wooden cages, allowed free access to food and water ad libitum throughout the stabilization period of two weeks. Ethical approval was obtained from Abia State University Research, Ethics and Intellectual Development Committee. All ethical guidelines on animal studies of the institute was adhered to.

2.3. Determination of LD50

The oral median lethal dose (LD50) of the extract was determined in mice using the method described by [18]. Twenty seven (27) mice were divided into nine groups of three mice per group for the extract and control. After eleven hours of fasting the different groups were given graded oral doses of the plant extract at (250, 500, 1000, 2000, 3000, 4000, 5000, 10000 mg/kg) once. While the control group received 10 ml/kg Distilled water. All the mice were allowed free access to food and water and were observed for signs of toxicity, behavioral changes or death within 24 hours.

2.4. Experimental design for chronic toxicity

A total of 24 albino rats assigned into four (II-IV) groups of six rats in each group was used for this study. Group I served as the control and was administered 10ml/kg distilled water. Groups II-IV received oral doses of 50, 200 and 400 mg/kg body weight of methanol leaf extract of Psidium guajava. All treatments were administered daily for 90 days [19].

2.5. Collection of blood, serum and organs

At the end of the 90 days treatment period, the rats were denied of feeds but were given water ad libitum for 24 h before being sacrificed under chloroform vapor. The blood, serum and organs were collected by the method described by [20]. The whole blood was collected in sample bottles containing ethylene diamine tetraacetic acid (EDTA) for hematological analyses. Another was collected in clean plain tubes which was allowed to stand for 10 min at room temperature before being centrifuged at 1000 rpm for 15 min to get the serum which was used for biochemical analyses. Thereafter, the rats were dissected and the organs (liver, kidneys spleen lungs, and heart) were removed, cleaned, weighed and were used for histopathological analysis.
2.6. Determination of body weight and relative organ weight
The body weights of rats were determined every two weeks till the end of the experiment. The weight gain within this period was calculated as shown below (a).

Relative organ weights were computed by expressing the absolute weight of the organs to the body weight of rats as described below (b).

a. Weight gain = Final weight of rat (g) – Initial weight of rat (g)

b. Relative organ weight = organ weight (g)/body weight (g) × 100

2.7. Hematological assays
The following hematological parameters: white blood cell (WBC), Red blood cell (RBC), Platelet count (PLT), and Hemoglobin concentration (HB) were analyzed using the automated blood analyzer (QBC Autoread Plus, UK). Briefly, the blood samples in EDTA bottles were pipetted into QBC capillary tubes, spun in a parafuge centrifuge (Becton Dickson, UK) for 5 minutes then read by the use of auroread analyzer.

2.8. Biochemical assays
Assays for liver function tests such as, serum alanine amino transferase (ALT), aspartate amino transferase (AST) were analyzed using Randox kits (Randox laboratories, UK). Serum alkaline phosphatase (ALP) was determined by the method described by [21], kidney function tests such as urea and creatinine were estimated, using Fortress kits (Fortress Diagnostics, UK).

2.9. Histopathological assays
The following stages were used to prepare the harvested tissues of Liver, Kidney, lungs, spleen and heart: tissues were fixed in 10% formalin, processed by preparation of thin slices called microtomy, dehydrated through ascending grades of alcohol 70-100% to remove water, then cleared in xylene, embedded in paraffin wax and sectioned into five micrometers thickness with the rotary microtome, and stained with hematoxylin and eosin. The sections were examined with digital microscope x400 for histopathological analysis of any organ changes [22].

2.10. Statistical analysis
Data were analyzed using SPSS version 17 and presented as means ± SEM. Comparisons between different groups were done using ANOVA. Values of P < 0.05 were considered as statistically significant. All results were obtained in triplicates.

3. Results

3.1. Acute toxicity
The groups of mice dosed 250-2000 mg/kg methanol leaf extract of Psidium guajava (ii-iv) in acute toxicity study did not exhibit any signs of toxicity after 24 hours of treatment. Group (v) dosed with 3000 mg/kg of the methanol extract showed signs of weakness of body, restlessness, and dullness. Mice in group VI dosed 4000 mg/kg showed symptoms of toxicity such as lack of appetite scratching of body, calmness, dullness, sluggishness and weakness of body within 3 hrs of administration of the extract. These symptoms disappeared after 24 hrs. However, at 5000 mg/kg all the mice were very weak, highly dehydrated as shown on their skin, with loss of appetite, two deaths were recorded in this group. The oral median lethal dose (LD₅₀) of the extract was found lower than 5,000mg/kg.

3.2. Hematological parameters
The administration of methanol leaf extract of Psidium guajava at the doses of 50, 200, and 400 mg/kg body weight for 90 days did not cause any significant increase (P < 0.05) in hematological parameters of the red blood cells (RBC), hemoglobin (HB), platelets (PLT), packed cell volume (PCV), and white blood cells (WBC) as they were within normal range when compared with control rats. (Table 1).
Table 1 Effects of methanol leaf extract of *Psidium guajava* on the hematological parameters after 90 days of oral administration on albino rats

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>WBC (10^9/L)</th>
<th>RBC (10^9/L)</th>
<th>HB (g/dl)</th>
<th>PLT (10^9/L)</th>
<th>PCV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D/W)</td>
<td>7.10 ± 0.02</td>
<td>7.57 ± 0.03</td>
<td>14.83 ± 0.03</td>
<td>233.73 ± 1.58</td>
<td>50.32 ± 1.23</td>
</tr>
<tr>
<td>MEPG1/4</td>
<td>7.50 ± 0.27</td>
<td>6.42 ± 0.03</td>
<td>14.20 ± 1.18</td>
<td>208.17 ± 3.99</td>
<td>51.47 ± 1.42</td>
</tr>
<tr>
<td>MEPGT</td>
<td>7.97 ± 0.07</td>
<td>6.44 ± 0.06</td>
<td>14.24 ± 0.20</td>
<td>210.81 ± 3.86</td>
<td>52.25 ± 1.00</td>
</tr>
<tr>
<td>MEPG2</td>
<td>7.47 ± 0.37</td>
<td>6.51 ± 0.59</td>
<td>14.10 ± 0.66</td>
<td>213.95 ± 5.46</td>
<td>52.57 ± 1.03</td>
</tr>
</tbody>
</table>

Key: MEPG1/4 = Methanol leaf extract of *Psidium guajava* at (50 mg/kg), MEPGT = Methanol leaf extract of *Psidium guajava* at (200 mg/kg), MEPG2 = Methanol leaf extract of *Psidium guajava* at (400 mg/kg), n=6.

3.3. Biochemical parameters

Biochemical studies showed a dose dependent significant elevation of serum AST, ALT, ALP at 400 mg/kg administration of the extract. A decrease of ALT at 50 mg/kg was observed. Group of rats administered 200 mg/kg (MEPGT) showed normal values when compared with control. Administration of the extracts did not affect the values of kidney biomarkers, urea and creatinine (Table 2).

Table 2 Effects of methanol leaf extract of *Psidium guajava* on the biochemical parameters after 90 days of oral administration on albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Urea mg/dl</th>
<th>Creatinine (mg/dl)</th>
<th>ALP (iu/l)</th>
<th>AST (iu/l)</th>
<th>ALT (iu/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D/W)</td>
<td>3.69 ± 0.21</td>
<td>36.44 ± 0.08</td>
<td>45.57 ± 1.28</td>
<td>34.53 ± 2.26</td>
<td>131.08 ± 0.06</td>
</tr>
<tr>
<td>MEPG1/4</td>
<td>3.74 ± 0.15</td>
<td>36.16 ± 0.98</td>
<td>43.54 ± 2.68</td>
<td>34.49 ± 2.13</td>
<td>105.05 ± 1.31</td>
</tr>
<tr>
<td>MEPGT</td>
<td>3.42 ± 0.06</td>
<td>38.42 ± 0.83</td>
<td>44.69 ± 2.76</td>
<td>41.67 ± 7.57</td>
<td>132.55 ± 1.02</td>
</tr>
<tr>
<td>MEPG2</td>
<td>3.76 ± 0.04</td>
<td>38.68 ± 1.38</td>
<td>62.63 ± 3.95</td>
<td>67.64 ± 2.87</td>
<td>152.63 ± 2.45</td>
</tr>
</tbody>
</table>

Key: MEPG1/4 = Methanol leaf extract of *Psidium guajava* at (50 mg/kg), MEPGT = Methanol leaf extract of *Psidium guajava* at (200 mg/kg), MEPG2 = Methanol leaf extract of *Psidium guajava* at (400 mg/kg), n=6.

3.4. Body weight and relative organ weight

Body weight gain in groups of rats administered MEPG for 90 days were normal when compared with the control rats (Table 3). The relative organ/body weight ratios indicated that the liver, spleen, kidney, and heart body weight ratios of the rats were not significantly (P > 0.05) different from those of the control rats (Table 4).

Table 3 Effect of methanol leaf extract of *Psidium guajava* on body weight (g)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Body weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEPG1/4</td>
<td>101 ± 0.04</td>
<td>202 ± 0.04</td>
<td>101</td>
</tr>
<tr>
<td>MEPGT</td>
<td>102 ± 0.12</td>
<td>204 ± 0.15</td>
<td>102</td>
</tr>
<tr>
<td>MEPG2</td>
<td>103 ± 0.06</td>
<td>204 ± 0.03</td>
<td>101</td>
</tr>
<tr>
<td>Control (D/W)</td>
<td>102 ± 0.14</td>
<td>204 ± 0.11</td>
<td>102</td>
</tr>
</tbody>
</table>

Key: MEPG1/4 = Methanol leaf extract of *Psidium guajava* at (50 mg/kg), MEPGT = Methanol leaf extract of *Psidium guajava* at (200 mg/kg), MEPG2 = Methanol leaf extract of *Psidium guajava* at (400 mg/kg), n=6.

Table 4 Effect of methanol leaf extract of *Psidium guajava* on relative organ weight (g)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Lungs</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (D/W)</td>
<td>0.027 ± 0.02</td>
<td>0.006 ± 0.05</td>
<td>0.002 ± 0.04</td>
<td>0.005 ± 0.05</td>
<td>0.002 ± 0.03</td>
</tr>
<tr>
<td>MEPG1/4</td>
<td>0.026 ± 0.02</td>
<td>0.006 ± 0.02</td>
<td>0.002 ± 0.02</td>
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3.5. Histopathological analysis

The heart, kidney, lung and spleen architectures did not show any changes on evaluation at any of the test doses. At (50, 200 mg/kg) the liver tissue showed normal architecture (figures 2 and 3). However, the liver architecture at 400 mg/kg showed distorted liver with microvesicular steatosis of the hepatocytes (Figure 4). Figure 1 shows the photomicrograph of the control liver.

![Figure 1](image1.png)

**Figure 1** Photomicrograph of liver tissue of albino rats administered with distilled water orally for 90 days showing histologically normal liver with i) patent central vein, ii) cords of normal hepatocytes, iii) sinusoids

![Figure 2](image2.png)

**Figure 2** Photomicrograph of the liver section obtained from albino rat dosed with 50mg/kg of methanol leaf extract of *Psidium guajava* orally for 90 days showing normal hepatocytes
Figure 3 Photomicrograph of the liver section obtained from albino rat dosed with 200mg/kg of methanol leaf extract of *Psidium guajava* orally for 90 days showing normal hepatocytes

Figure 4 Photomicrograph of liver tissue of albino rats treated orally with 400mg/kg of methanol leaf extract of *Psidium guajava* orally for 90 days showing mildly distorted liver with microvesicular steatosis of the hepatocytes (Starred)

4. Discussion

Plants have been used in traditional medicine for treatment of several diseases for many years, they still remain a major part of routine use in different parts of the world [23]. There is need therefore for safety assessment of these plants. The present study was undertaken to evaluate the acute and chronic toxicity of methanol leaf extract of *Psidium guajava*. Our findings revealed that in the acute toxicity evaluation, two rats died at 5000 mg/kg indicating that the LD$_{50}$ was less than 5000 mg/kg. The LD$_{50}$ of 1.352 mg/kg was reported by [24].

Evaluation of hematological parameters HB, WBC, PLT, RBC provide valuable information on the adverse effects of foreign bodies on the blood which can be in the form of chemical compounds, medicinal plants or drugs [25, 26, 27]. In
the present study, administration of methanol leaf extract of *Psidium guajava* at various doses of 50, 200 and 400 mg/kg for 90 days did not cause any significant increase \( (P < 0.05) \) in WBC, RBC, PLT and HB and compared well with the control rats. This finding also indicates the non hematotoxic nature of the extract and the improbability of the extract to induce anaemia after use for extended period of 90 days [28]. Similar findings have been previously reported by the following authors [29, 30, 31].

Evaluation of serum biochemical indices in rats has become the most valuable way for assessing the integrity and functionality of the organs as well as risk assessment, pathological condition and general health status of the body [32, 33, 34]. The liver enzymes, Alanine aminotransferase (ALT), aspartate aminotransaminase (AST), and alkaline phosphatase (ALP) are biomarkers of hepatic integrity and can be used to assess and evaluate the liver function [35]. Consequently, in the present work, the results of the enzyme biomarkers was dose dependent indicating elevation of ALT, ALP and AST in the albino rats dosed with the extract at 400mg/kg for 90 days. [36, 37] reported dose dependent effect of the extracts causing liver and kidney toxicity. The serum ALT was significantly lowered in rats administered 50 mg/kg of the extract for 90 days when compared to the control rats. This could be as a result of the inhibition of the enzyme activities probably due to phytochemical constituents of the extract [38]. This result may affect amino acid and carbohydrate metabolism thereby affecting the production of ATP [39]. Creatinine and urea are kidney biomarkers. The values for creatinine and urea at all doses was significantly normal when compared to control.

The daily clinical evaluation showed no observable changes in the rats body weight, no significant \( P < 0.05 \) differences was revealed in water and food consumption. Organ body weight ratios are normally investigated to determine whether the size of the organ has changed relative to the weight of the whole animal. The absence of an effect on the computed organs/body weight ratios suggests that the extract did not cause any form of swelling or changes on the organs [32]. These are some of the parameters used in the study of the safety of a product with therapeutic aim.

Histological examination did not indicate any significant microscopic changes in heart, kidney, lungs and spleen. However, in the liver there was a significant microscopic alteration in the group treated with 400 mg/kg for 90 days compared to the control group, such injuries could be associated with toxic activity of tannin present in the extract.

Therefore, since only mild alteration was observed at dose 200 mg/kg, methanol leaf extract of *Psidium guajava* may be considered to be relatively safe for consumption especially for a prolonged period of time and could be explored as oral remedy at 200 mg/kg.

5. Conclusion

The use of medicinal plants as alternative to a couple of antibiotics that are now resistant to many infections is in the increase. However, the toxic side effects of these medicinal plants are limitation to their potential usefulness. The current study has shown that low dose of methanol leaf extract of *Psidium guajava* (200 mg/kg body weight/daily) have no adverse effects on the organs of the albino rats while higher dose (400 mg/kg body weight/day) posed severe threat to the liver organ. This study therefore, highlights the potential ability of *Psidium guajava* extract to induce morphological changes in the liver of humans consuming high dose of the extract for medicinal purposes. This indicates that the plant extract is not harmful at 200 mg/kg and can be safely used as an antibacterial agent for a prolonged period.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors report no conflict of interest.

Statement of ethical approval

Ethical approval for use and handling of animals was obtained from Abia State University Research, Ethics and Intellectual Development Committee with reference number (ABSU/REC/BMR/017).
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