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

Joy Ogugua Igwe 1, *, Harrison Odera Abone 1, Moses Chukwuemeka Ezea 1, Chika Peter Ejikeugwu 2 and Charles Okechukwu Esimone 1

1 Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Anambra State, PMB 5025, Nigeria.
2 Department of Applied Microbiology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

GSC Biological and Pharmaceutical Sciences, 2021, 16(03), 120–128

Publication history: Received on 08 August 2021; revised on 14 September 2021; accepted on 16 September 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.16.3.0270

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$_{50}$ 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

*Corresponding author: Joy Ogugua Igwe*  
Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Anambra State, PMB 5025, Nigeria.

Copyright © 2021 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0.
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, gib 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 (LD_{50}) of the extract was found lower than 5,000 mg/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

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

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

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

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)

| Groups       | Initial weight (g) | Final weight (g) | Body weight gain (g) |
|--------------|--------------------|------------------|---------------------|
| MEPG1/4      | 101±0.04           | 202±0.04         | 101                 |
| MEPGT        | 102±0.12           | 204±0.15         | 102                 |
| MEPG2        | 103±0.06           | 204±0.03         | 101                 |
| Control (D/W)| 102±0.14           | 204±0.11         | 102                 |

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).

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

| Organ      | Liver     | Kidney    | Spleen   | Lungs    | Heart   |
|------------|-----------|-----------|----------|----------|---------|
| Control (D/W) | 0.027±0.02 | 0.006±0.05 | 0.002±0.04 | 0.005±0.05 | 0.002±0.03 |
| MEPG1/4    | 0.026±0.02 | 0.006±0.02 | 0.002±0.02 | 0.005±0.03 | 0.002±0.02 |
| MEPGT      | 0.027±0.01 | 0.006±0.02 | 0.002±0.02 | 0.005±0.06 | 0.002±0.03 |
| MEPG2      | 0.026±0.03 | 0.006±0.03 | 0.002±0.02 | 0.005±0.02 | 0.002±0.01 |

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).
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)

**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)

**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

The authors wish to acknowledge Mr Yirupe Woy of Pharmacology department, College of Medicine, Uniport for assisting in handling of all the animals also, Dr Paul C. Wokpeogu for analyzing the histology results.

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).
References

[1] Tamil R Selvan, Sultan Mohideen Ak, Asrar Sheriff M, Azmathullah NMD. Phytochemical screening of Acalypha indica L. extracts. International Journal of Applied Biology and Pharmaceutical Technology. 2012; 3(2): 158-161.

[2] World health organization. Global Report on Diabetes, WHO, Geneva, Switzerland. 2016.

[3] Zeb A, Ahmad F, Ullah M, Ayaz Sadiq A. Antinociceptive activity of ethnomedicinally important analgesic plant Isodon rugosus Wall ex. Benth mechanistic study and identification of bioactive compounds. Frontiers in pharmacology. 2016; 7(200): 1-10.

[4] World Health Organization. Traditional medicine growing needs and potentials, WHO policy perspectives medicine, Geneva: World Health Organization. 2002.

[5] Biswal B, Kimberly FM, Dwayne D, Anand Y. Antimicrobial activity of leaf extracts of Guava (Psidium guajava) on two Gram positive and two Gram negative bacteria. International Journal of Microbiology. 2013; 2: 7.

[6] Morton JF. Fruits of warm climates. 2004. 425-428.

[7] Gbile ZO. Vernacular names of Nigerian plants; Yoruba forestry research institute of Nigeria Ibadan in: Okujagu T.F. (2005). Book of Abstract of Published Research finding on Nigerian Medicinal plants. 1984.

[8] Okujagu TF, Etatuvie Sam O, Ifeyinwa E, Jimoh B, Nwokeke. Book of Abstract of Published Research finding on Nigerian Medicinal Plants and Traditional Medicine Practice. 2005; 1: 90.

[9] Kumar M. A study of antibacterial activity of Psidium guajava Linn fruit extracts against Gram-positive and Gram-negative bacteria. International Journal of Institutional Pharmacy and Life Sciences. 2015; 5(2): 231-239.

[10] Begum S, Hassan SI, Ali SN, Siddiqui BS. Chemical constituents from the leaves of Psidium guajava. Natural Product Research. 2004; 18(2): 135-140.

[11] Aliyu BS. Some ethno-medicinal plants of the Savannah Regions of West Africa, description and phytochemicals. Triumph Publishing Company. 2006; 1: 135-152.

[12] Wei L, Li Z, Chen B. Clinical study on treatment of infantile rotaviral enteritis with Psidium guajava L. Chinese Journal of Intergrated Traditional and Western Medicine. 2001; 7(2): 86-89.

[13] Punta Wong S, Okonogi S, Pringproa K. In vitro antibacterial activity of Psidium guajava Linn. Leaf extracts against pathogenic bacteria in pigs. Chiang Mai Univ. J Nat Sci. 2012; 11(2): 127-34.

[14] Masud T, Inaba Y, Maekawa T, Takeda Y, Yamaguchi H, Nakamoto K, Kuninga H, Nishizato S, Nonaka A. Simple detection method of powerful antiradical compounds in the raw extract of plants and its application for the identification of antiradical plant constituents. J Agric Food Chem. 2003; 51: 1831-8.

[15] Jeong S, Cho SK, Ahn KS, Lee JH, Yang DC, Kim J. Anti-inflammatory effects of an ethanolic extract of guava (Psidium guajava L) leaves in vitro and in vivo J Med Food. 2014; 17(6): 678-85.

[16] Banu MS, Sujatha K. Antimicrobial screening of leaf extract of Psidium guajava and its isolated fraction against some pathogenic microorganisms, Drug Invent Today. 2012; 4(3): 348-50.

[17] Alabi OA, Haruna MT, Anokwuru CP, Jegede T, Abia H, Okegbue VU, Babatunde E. Esan. Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of Carica papaya (L) on clinical bacterial and fungal isolates. Advances in Applied Science Research. 2012; 3(5): 3107-3114.

[18] Lorkhe. D. A new approach to practical acute toxicity testing. 1983; 54: 275–287.

[19] OECD. Test guideline 452. Chronic Toxicity Studies. In: Draft OECD Guideline for the Testing of Chemicals (Draft Consultant Proposal Version 8 OECD TG 452) 2008.

[20] Gatsing D, Aliyu R, Kuiate JR, Garba IH, Tchouanguep FM, Toxicological evaluation of the aqueous extract of bulbs on laboratory mice and rats. Cameroon J Exp Biol. 2005; 1: 39-45.

[21] Klein B, Read PA, Babson AL. Raoid method for the quantitative determination of serum alkaline phosphatase. Clin Chem. 1960; 6: 269-275.

[22] Di Fiore. MSH. An atlas of human histology. 2nd ed. Philadelphia: Leaand Febiger. 1963.

[23] Kumar S, Paul S, Walia YK, Kumar A, Singhal P. Therapeutic potential of medicinal plants: a review. J Biol Chem Chron. 2015; 1(1): 46-54.
Onyekwe NG, Ilodigwe EE, Ajaghaku DL, Esimone CO. Acute and Subchronic Toxicities of Ethanol Root Extract of *Psidium guajava* (myrtaceae) in Experimental Animals. Journal of Pharmaceutical and Biomedical Sciences. 2011; 12(18): 1-4.

Berinyuy EB, Lawal B, Olalekan AA, Olalekan IA, Yusuf AA, Sakpe S. Hematological status and organs/body weight parameters in wister rats during chronic administration of *Cassia occidentalis*. Int Blood Res Rev. 2015; 4(3): 1-7.

Lawal B, Shittu OK, Abubakar AN, Haruna GM, Saidu S, Ossai PC. Haemapoetic effect of methanol extract of Nigerian honey bee (*Apis mellifera*) propolis in mice. J Coast Life Med. 2015; 3(8): 648-51.

Nwaka AC, Ikechi-Agba MC, Okechukwu PCU, Igwenyi IO, Agbafor KN, Orji OU. The effects of ethanol extracts of *Jatropha curcas* on some hematological parameters of chloroform intoxicated rats. Am Eurasian J Sci Res. 2015; 10(1): 45-9.

Kelly F. Veterinary Clinical Diagnosis. Baller Tindall London. 1957; 271-282.

Devaki K, Beulah U, Akila G, Gopalakrishnan VK. Effect of aqueous extract of *Passiflora edulis* on biochemical and hematological parameters of Wistar albino rats. International Journal of Toxicology. 2012; 19(1): 63-7.

Essiet GA, Takem LP, Essien AD, Akuodor GC, Udoh FV. Assessment of haematopoietic toxicity of *Salacia lehmbachii*. International Journal of Pharmacy and Pharmaceutical Research. 2016; 8(1): 326-32.

Ferreira SA, Guimaraes AG, Ferrari FC, Carneiro CM, de Paiva NC, Guimaraes DAS. Assessment of acute toxicity of the ethanolic extract of *Lychnophora pinaster* (Brazilian arnica). Revista Brasileira de Farmacognosia. 2024; 24(5): 553-60.

Shittu OK, Lawal B, Abubakar NA, Berinyuy BE, Busari MB, Ibrahim AO. Toxicological implications of methanol extract from Nigerian bee propolis on some selected rat tissues. J Pharm Biomed Sci. 2015; 5(7): 524-31.

Shittu OK, Lawal B, Haruna GM, Berinyuy EB, Yusuf AA, Ibrahim AM. Hepato-curate effects of methanol extract from Nigerian bee propolis in carbon tetrachloride (CCl4) intoxicated rat. Eur J Biotechnol Biosci. 2015; 3(7): 1-4.

Lawal B, Shittu OK, Abubakar AN, Umar MB, Ibrahim AM, Haruna GM. Biochemical evaluation in Wister rats (*Rattus noergicus*) following chronic exposure of methanol leaf extract of *Telfairia occidentalis*. J Pharm Biomed Sci 2015; 5(9): 740-4.

Rafiae AA, Mohafrash SMM, Ibrahim AW, Mossa AH. Sub-acute 28 days oral toxicity study of Deltamethrin on female rats and the protective role of Moringa Tea. Trends in Applied Sciences Research. 2017; 12(2): 10-17.

Medinat YA, Jane IE, Musa IY. Acute and chronic toxicity profiles of the Methanol leaf extracts of *Acacia ataxacantha* D.C (Leguminosae) in Wistar Rats. Bulletin of Faculty of Pharmacy, Cairo University. 2018; 56: 185-189.

Covile AC, Almeida VA, Andrade FSA, Fonseca AA, Macêdo RL, Renato LS, Santos J K GF, Colen ER, Martins NAM. Acute and Chronic Toxicity and Antimicrobial activity of the extract of *Stryphnodendron adstringens* (Mart.). Brazilian Journal of Veterinary Research. 2017; 37(8): 840-846.

Bashir L, Shittu OK, Prince CO, Asmau AN, Aisha MI. Evaluation of antioxidant activity of giant African snail (*Achachatina maginata*) haemolymph in CC14-induced hepatotoxicity in albino rats. Br J Pharm Res. 2015; 6(3): 141-54.

Ekenam JT, Yusuf OK. Some liver function indices and blood parameters in *T. brucei* infected rats treated with honey. Biokemistri. 2007; 19(2): 81-6.