EVALUATION OF SUB-ACUTE TOXICITY OF THE HYDRO-METHANOL STEM BARK EXTRACT OF BURKEA AFRICANA IN ALBINO RATS

Terhemen Festus Swem1, Patrick Emeka Aba2, Samuel Chukwuneke Udem2, Victor Masekaven Ahur1, Fidelis Aondonder Gberindyer3

1Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue State, Nigeria.
2Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.
3Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

ABSTRACT

Objective: This study was designed to investigate the sub-acute toxicity profile of hydro-methanol extract of Burkea africana (BA) stem bark in rats.

Methods: The stem bark of BA was extracted by cold maceration using 80% methanol. Twenty female albino rats were randomly assigned into four groups of five rats each. Group 1 (only distilled water). Groups 2-4 received the extract (100, 200, and 400 mg/kg) orally, once daily for 28 days. The rats were observed for signs of toxicity and the bodyweight (b.wt) of rats taken weekly. Blood samples were collected on day 28 for hematology and serum chemistry. Visceral organs were harvested for organ-somatic index and histopathology.

Results: There were no toxicity signs observed and no significant (P<0.05) change in body weight but the pulmo-somatic index was significantly (P<0.05) higher at 400 mg/kg compared with the control and other treated groups. Significant (P<0.05) increase in PCV, RBC, and MCV and significant (P<0.05) decrease in MCHC, Total WBC count, and lymphocytes were observed. Also, there were significant (P<0.05) decreases in ALT, total protein, globulin, total bilirubin of test groups when compared with the control group. Urea concentration of test groups significantly (P<0.05) increased when compared with that of the control group.

Conclusions: BA stem bark extract can be said to have no deleterious effect on erythrocyte, but rather serve to improve erythropoiesis and also has no overt toxic effect on the visceral organs. Also the extract may have immunosuppressive and oxidative tendencies on prolong use.

Keywords: Biochemical changes, gas chromatography, immunosuppression, medicinal plants, mass spectrometry, oxidative stress.

Article Info: Received 12 January 2021; Revised 3 February; Accepted 25 February, Available online 15 March 2021

Cite this article: Swem TF, Aba PE, Udem SC, Ahur VM, Gberindyer FA. Evaluation of sub-acute toxicity of the hydro-methanol stem bark extract of Burkea africana in albino rats. Universal Journal of Pharmaceutical Research 2021; 6(1):16-24. DOI: https://doi.org/10.22270/ujpr.v6i1.534

Address for Correspondence: Dr. Terhemen Festus Swem, Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue State, Nigeria. Tel: +2348165743539; E-mail: swemfestus422@gmail.com

INTRODUCTION

The act of using plants for treatment, prevention, and control of various disease conditions is an ancient phenomenon1,2. Developed countries also have experienced significant increase in the use of herbal remedies, with the belief that they are more efficacious and less harmful2,3. Nonetheless, the fact that they are natural does not make them safe, because little knowledge is available on the safety to validate the claim by manufacturers or traditional healers2,3. Many herbal products or medicinal plants have been demonstrated by researchers to be toxic, mutagenic, and carcinogenic2. Research has also shown that many medical plants used as herbal remedies contain phytochemical constituents with ability to cause deleterious effect to the body. Such toxic principles include pyrrolizidine alkaloids, benzophenanthrine alkaloids, lectins, saponins, diterpenes, cyanogenic-glycosides, and furanocoumarins2. Evaluation of
medicinal plant and herbal products to determine the level of toxicity in order to establish consequences of long term use is therefore imperative. Toxicity may be seen physically and clinically. Animals or humans may display signs such as restlessness, ataxia, circling, anorexia and subsequently death. The effect of the toxic agent is often seen on the organs especially the liver, kidney, lungs, and heart. This often displays as changes in some biochemical parameters which gives a picture of which organ is mostly affected. *Burkea africana* (Caesalpiniaeae), a medium size deciduous tree with a wide spread top common in Nigeria is widely used as a remedy for a wide range of ailments in traditional medicine. It has been used often as an anti-venomous agent, cutaneous and subcutaneous parasitic infections, anticonvulsant, hepatic disorders, analgesic, anti-inflammation, anti diarrheal, wound healing, and toothache.\(^6\)\(^7\). Empirical evidences exist on its antibacterial, antifungal, larvicidal, molluscicidal, and antioxidant activities\(^8\)\(^9\)\(^10\). Also, claims for its anti diarrheal, anticonvulsant, analgesic, and anti-inflammatory properties have been reported\(^11\)\(^11\)\(^12\). In this study, the GC-MS analysis and the sub-acute toxicity of methanol stem bark extract of BA were investigated.

**MATERIALS AND METHODS**

**Plant material**

Fresh stem bark of *Burkea africana* (BA) were obtained from Ajaba village, a sub-urb of Makurdi metropolis in Benue State and identified by Plant Taxonomists Mr. Yeke Titus of the Department of Forestry, Federal University of Agriculture Makurdi and a voucher specimen number; UAM/FH/0326 assigned and kept in the Departmental Herbarium.

**Preparation of plant extract**

The BA stem bark was dried in an open shade at room temperature and pounded into smaller piece using a mortar and pestle. This was further made into powdered form using a grinding machine. The powdered material (1000 g) was soaked in 4 L of 80% methanol for 48 h with periodic shaking. The extract was then filtered with a Whatman (No. 1) filter paper. The filtrate was concentrated in a vacuum using a hot air oven at 37\(^\circ\)C into a semi-solid form, yielding in a ratio of 1:10 w/v crude to extract and stored at 4\(^\circ\)C in the refrigerator for future use.

**Experimental animals**

Female rats weighing 110-120 g were purchased from a commercial animal farm in Nsukka, Enugu State. The animals were kept for seven days in Aluminum cages to acclimatize at the animal house of the Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria Nsukka. During this period, they were provided with potable drinking water and fed adequately with commercially prepared poultry feed pellets (Topfeeds®). The Ethical Committee of the Department of Veterinary Physiology and Pharmacology, University of Nigeria Nsukka gave approval for this research to be conducted with the approval reference number: FVM-VPP-UNN-IACUC-2018-039. The handling and management of animals during this period was in line with good laboratory animal practice regulations as well as the principles of laboratory animal use and care as enshrined by the Natural Research Council guidelines of 2011\(^13\).

**Phytochemical Screening of extracts**

The phytochemical screening of the 80% methanol extract of BA stem bark was carried out using standard procedures as described by Trease and Evans\(^14\) and Sofowora\(^16\)\(^17\). The powdered hydro-methanol extract of BA stem bark was reconstituted to obtain the test aliquot by dissolving 1 g in 500 ml of distilled water. The aliquot was thereafter screened for the presence of alkaloids, flavonoids, tannins, phlobatannins, saponins, glycosides, phenols, terpenoids, steroids, reducing sugar, resins, and volatile oils.

**Gas chromatography mass spectroscopy**

One gram (1g) of the methanol stem bark extract of BA was sent to Ahmadu Bello University, Zaria for Gas Chromatography Mass Spectroscopy (GC-MS) analysis (Perkin Elmer Auto sampler XLGC coupled with Turbo Mass Spectrophotometer, Norwalk CT06859, USA) using analytical conditions described by Adeyemi et al.\(^17\). The setup had an electron ionization of 70v and the source of the ion had a temperature of 250\(^\circ\)C. Helium gas (99.9% purity) was the carrier gas used, while HP-5ms (30nm X 0.25mm X 0.320µm) was the stationary phase. The oven had a temperature of 80\(^\circ\)C and was kept at that for 5 minutes and then increased to 250\(^\circ\)C. The retention time was 16 minutes, running at the speed of 4 degrees/minute, 1μl was automatically injected to finalize the running time of 50 minutes. Mass Hunter Data Analysis Software was used to analyze and interpret the GC-MS result.

**Sub-acute toxicity experiment**

Twenty female albino rats randomly were assigned into four groups. Groups 2-4 were administered the extract at the dose of 100, 200, and 400 mg/kg b.wt respectively, orally for 28 consecutive days. Whereas group 1 served as a negative control and were administered distilled water at 10 ml/kg body weight for the period. The body weights of rats in each group were obtained weekly and recorded accordingly. Blood samples for hematology and serum biochemistry were collected at day 28 post treatments using standard methods. All rats in each group were sacrificed humanely at day 28 and visceral organs (liver, kidney, heart, spleen and lung) were collected, weighed and relative organ versus body weight calculated. Liver and kidneys were preserved using 10% formalin to be used for histopathology examination.

**Hematological and serum biochemical analyses**

Hematological parameters were evaluated using standard methods\(^18\). Also, alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were assayed as described\(^19\)\(^20\). Total serum protein and albumin were evaluated using a clinical refractometer as described by Johnson *et al.*\(^21\). Serum globulin concentrations were derived from the difference between total serum protein and albumin. Total and direct bilirubin assay was by the method of Tietz\(^22\) while Urea and creatinine were
assayed by the method of Burtis and Ashwood\textsuperscript{23}. Also, malondialdehyde, catalase and glutathione were assayed by the methods of Stocks and Dormandy\textsuperscript{24} modified by Sicinska \textit{et al.},\textsuperscript{25} Góth\textsuperscript{26}, and Moron \textit{et al.}.\textsuperscript{27}

**Histopathological Examination**

Tissue samples from the liver and kidney were histologically examined using the conventional staining technique of Hematoxylin and Eosin as described by Drury \textit{et al.}.\textsuperscript{28}

### Table 1: GC-MS profile of methanol extract of \textit{Burkea africana} stem bark.

| S.N. | Suggested compound                          | Molecular Formula | Molecular weight (g/mol) | Retention Time (Min.) | Chemical Group       |
|------|--------------------------------------------|-------------------|--------------------------|-----------------------|----------------------|
| 1    | (2H) pyrrole-2-carbonitrile, 5-amino-3,4-dihydro- | C\textsubscript{6}H\textsubscript{7}N\textsubscript{3} | 109                      | 15.712                | Alkaloid             |
| 2    | 1-Butanamine, N-nitroso-N-propyl            | C\textsubscript{7}H\textsubscript{16}N\textsubscript{2}O   | 144                      | 18.386                | Amine                |
| 3    | Resorcinol                                 | C\textsubscript{10}H\textsubscript{12}O\textsubscript{2}  | 110                      | 28.424                | Phenol               |
| 4    | Oleic acid                                 | C\textsubscript{18}H\textsubscript{32}O\textsubscript{2}  | 282                      | 55.937                | Fatty acid           |
| 5    | 9, 17-octadecadienal, (Z)-                 | C\textsubscript{36}H\textsubscript{52}O                  | 265                      | 60.553                | Unsaturated Aldehyde |

### RESULTS

**Phytochemical screening**

Qualitative phytochemical screening of the extract showed that the extract contained alkaloids, glycosides, resins, reducing sugars, volatile oil and phlobotanins, flavonoids, saponins, sterols, terpenes tannins, terpenoids, and phenols.

**Gas chromatography mass spectroscopy of methanol extract of \textit{Burkea africana} stem bark**

Results of the GC-MS analysis of the plant extract are presented in Table 1. Results suggested that the extract contains (2H) pyrrole-2-carbonitrile, 5-amino-3,4-dihydro-, 1-Butanamine, N-nitroso-N-propyl, Resorcinol, Methyl 11-oxo-9-undecenate, Oleic acid, and 9, 17-octadecadienal, (Z).

### Sub-acute effects of the extract on organ-somatic index and body weight

Results showed no significant different in the organ-somatic index between the control and treated groups for all the organs. However, the pulmo-somatic index was higher in animals treated at the dose rate of 400 mg/kg of the extract as compared with the control and other treated groups (Figure 1). Also, No significant (\(P>0.05\)) difference was observed in the body weights of animals in all the treated groups when compared with the control group (Figure 2).

### Effects on some hematological parameters

Result in Table 2 showed significantly (\(P<0.05\)) higher values of packed cell volume (PCV) and red blood cells (RBC) in animals treated with the extract at the dose rate of 200 mg/kg and 400 mg/kg as compared to those administered 100 mg/kg dose of the extract as well as the control group.

![Figure 1: Organ-somatic index of rats treated for 28 days with methanol extract of \textit{Burkea africana} stem bark\textsuperscript{10\textsuperscript{3}g})](image-url)

Bars with the same alphabet for each organ (liver, kidney, heart, spleen and lungs) are not significantly (\(p < 0.05\)) different for each. MSBEBA- Methanol stem bark extract of \textit{Burkea africana}.  

**Statistical analysis**

All results of this study were expressed descriptively as mean\(±\)standard error of mean (S.E.M) and group means were compared using one-way analysis of variance (ANOVA) at significance level of 5\% (\(P<0.05\)). Significant differences between means were separated using Duncan multiple range post hoc test. Data was analyzed using SPSS version 21. Bar charts and tables were used to present the data generated in the study.
No significant (P> 0.05) difference in the hemoglobin (Hb) and mean corpuscular hemoglobin (MCH) values between all the treated and the control groups. Also, the mean corpuscular volumes (MCV) were observed to be significantly (P<0.05) higher in animals administered the extract at doses 100 mg/kg and 400 mg/kg compared to the control and the group treated at the dose of 200 mg/kg. Only animals treated with the extract at the dose rate of 100 mg/kg were observed with a significantly (P<0.05) lower MCHC value as compared to both the other two treated and the control groups. Furthermore, result revealed a significantly (P< 0.05) higher total white blood cell count (TWBC) in those animals treated with 200 and 400 mg/kg dose of the extract as compared with the control group as well as those given a 100 mg/kg dose of the extract. For the neutrophils count, a significantly (P <0.05) lower value was observed in the animals that were treated with the extract at a dose rate of 100 mg/kg when compared with the control and those treated with the higher doses. Again, a significantly (P<0.05) lower lymphocyte count was observed in those animals that were treated with the extract at a dose rate of 400 mg/kg when compared to the control group and the other two groups on lower doses. Furthermore, the result showed no significant (P>0.05) difference between the extract treated and control groups in the observed values of monocytes, eosinophil, and basophils.

**Effect on some serum biochemical parameters**

In Table 3 results of biochemical assay showed significantly (P<0.05) decreased in rats treated with the extract at the dose rate of 100 and 200 mg/kg when compared with those treated at the dose rate of 400 mg/kg and the control group. Total proteins were significantly (P<0.05) lower at all the doses of extract administered, when compared with the control group. Albumin showed significantly (P< 0.05) higher values in animals treated with the extract at the dose rate of 100 and 200 mg/kg when compared with the group administered 400 mg/kg of the extract and the control group. Globulin decreased significantly (P<0.05) in animals treated with the extract at the dose rate of 100, 200 and 400 mg/kg. Also total bilirubin was significantly (P<0.05) lowered in animals administered 100mg/kg of the extract when compared with those administered 200 and 400 mg/kg and control group. Urea significantly (P<0.05) increased in animals treated with the extract at the dose rate of 200 and 400 mg/kg when compared with those administered the extract at the dose rate of 100 mg/kg and control group.

---

**Table 2: Haematological parameters of rats treated with methanol extract of *Burkea africana* stem bark for 28 days.**

| Parameters             | Control    | Extract Treated Groups |
|------------------------|------------|------------------------|
|                        | 100mg/kg   | 200mg/kg               | 400mg/kg               |
| PCV (%)                | 42.00±1.18\(^a\) | 44.60±2.20\(^a\) | 51.67±0.76\(^b\) | 48.20±1.69\(^c\) |
| Hb (g/dL)              | 15.00±0.27\(^a\) | 15.75±0.26\(^a\) | 15.52±0.33\(^a\) | 15.28±0.46\(^a\) |
| RBC (X10\(^12\)/L)     | 7.48±0.03\(^a\) | 7.55±0.02\(^a\) | 7.64±0.03\(^b\) | 7.72±0.04\(^b\) |
| MCV (FL)               | 56.11±1.45\(^a\) | 68.43±0.91\(^a\) | 58.39±2.92\(^b\) | 62.40±1.92\(^c\) |
| MCH (Pg)               | 20.04±0.30\(^a\) | 20.87±0.37\(^a\) | 20.32±0.45\(^a\) | 19.79±0.60\(^a\) |
| MCHC (g/dL)            | 35.79±0.72\(^a\) | 30.55±0.89\(^a\) | 35.02±1.22\(^b\) | 31.87±1.58\(^b\) |
| TWBC (X10\(^12\)/L)    | 6.77±1.89\(^b\) | 6.60±0.19\(^a\) | 5.84±0.07\(^b\) | 5.48±0.10\(^b\) |
| Neutrophils (%)        | 65.67±1.87\(^a\) | 61.67±1.20\(^a\) | 68.00±0.89\(^b\) | 69.60±0.40\(^b\) |
| Lymphocytes (%)        | 31.67±2.09\(^b\) | 36.00±1.46\(^b\) | 32.40±2.48\(^b\) | 26.00±1.26\(^b\) |
| Monocytes              | 2.33±0.33\(^a\) | 1.67±0.61\(^a\) | 2.00±0.63\(^a\) | 3.20±0.49\(^a\) |
| Eosinophils (%)        | 0.00±0.00\(^a\) | 1.00±0.45\(^a\) | 0.40±0.40\(^a\) | 0.80±0.49\(^a\) |
| Basophils              | 0.33±0.33\(^a\) | 0.00±0.00\(^a\) | 0.00±0.00\(^a\) | 0.40±0.40\(^a\) |

Values are Mean±S.E.M, n = 5. Values with different superscripts on the same row are significantly different at P<0.05. PCV-Packed cell volume, Hb-Hemoglobin, RBC-Red blood cell count, MCV-Mean corpuscular volume, MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration, and TWBC-Total white blood cell.

**Figure 2: Weekly Mean body weight of rat treated with methanol extract of *Burkea africana* stem bark in grams (g)±SEM**

Bars with asterisks for various days are not significantly (p < 0.05) MSBEBA-Methanol stem bark extract of *Burkea africana*.
Table 3: Some serum biochemical parameters of rats treated with methanol extract of *Burkea africana* stem bark for 28 days.

| Groups       | Control          | Extract Treated Groups          |
|--------------|------------------|---------------------------------|
|              | 100mg/kg         | 200mg/kg                        | 400mg/kg                        |
| AST (IU/L)   | 61.67±2.64<sup>a</sup> | 58.50±1.43<sup>a</sup>          | 56.60±3.30<sup>a</sup>          | 62.00±2.70<sup>a</sup> |
| ALT (IU/L)   | 38.57±1.70<sup>b</sup> | 34.18±0.58<sup>b</sup>          | 33.28±0.88<sup>b</sup>          | 35.51±1.15<sup>b</sup> |
| ALP (IU/L)   | 94.77±3.31<sup>c</sup> | 96.18±1.24<sup>c</sup>          | 95.12±1.87<sup>c</sup>          | 96.44±1.17<sup>c</sup> |
| T.P (g/dL)   | 5.59±0.13<sup>c</sup> | 4.83±0.21<sup>c</sup>          | 4.47±0.11<sup>c</sup>          | 4.67±0.23<sup>c</sup> |
| ALB (g/dL)   | 2.10±0.06<sup>c</sup> | 2.40±0.10<sup>c</sup>          | 2.98±0.09<sup>c</sup>          | 2.64±0.07<sup>c</sup> |
| GLB (g/dL)   | 3.49±6.13<sup>c</sup> | 2.43±1.11<sup>c</sup>          | 1.49±0.17<sup>c</sup>          | 2.41±0.20<sup>c</sup> |
| TBLI (mg/dL) | 2.12±0.02<sup>c</sup> | 1.65±0.14<sup>c</sup>          | 1.74±0.19<sup>c</sup>          | 1.93±0.19<sup>c</sup> |
| DBIL (mg/dL) | 1.11±29<sup>d</sup> | 0.81±0.12<sup>d</sup>          | 0.60±0.07<sup>d</sup>          | 1.16±0.28<sup>d</sup> |
| InDBIL (mg/dL) | 1.01±30<sup>d</sup> | 0.83±0.16<sup>d</sup>          | 1.14±0.23<sup>d</sup>          | 0.77±0.44<sup>d</sup> |
| Urea (mg/dL) | 25.23±1.01<sup>d</sup> | 24.43±1.44<sup>d</sup>         | 27.02±2.74<sup>d</sup>         | 32.70±2.77<sup>d</sup> |
| Creat.(mg/dL) | 0.88±0.03<sup>d</sup> | 0.88±0.04<sup>d</sup>          | 0.88±0.02<sup>d</sup>          | 0.94±0.01<sup>d</sup> |

Values are Mean±S.E.M, n=5. Values with different superscripts on the same row are significantly different (p < 0.05). AST – Aspartate amino transferase, ALT–Alanine aminotransferase, ALP-Alkaline phosphatase, T.P-Total protein, ALB–Albumin, GLB–Globulin, TBLI–Total bilirubin, DBIL–Direct bilirubin, InDBIL–Indirect bilirubin.

The result showed no significant (*P* > 0.05) difference between the extract treated and control groups in the values of creatinine observed.

**Effect of methanol extract of *Burkea africana* stem bark administration on oxidative stress markers of rats**

At day 28, Malondialdehyde (MDA) concentration significantly (*P* < 0.05) increased in the extract treated groups when compared with the control group. Catalase activity also increased significantly (*P* < 0.05) in all extract treated groups when compared with the control group. Glutathione (GSH) on the other hand was observed to be significantly (*P* <0.05) lowered in animals treated 100 and 200 mg/kg of the extract, while those that were administered the extract at the dose rate of 400 mg/kg showed significantly (*P* <0.05) increased GSH when compared with the control group (Table 4).

**Histopathological changes in some visceral organs**

Histopathological examination of the liver of rats treated with methanol extract of *Burkea africana* stem bark for 28 days, revealed normal morphology of the hepatocytes at all doses (Green arrows), with moderate infiltration of inflammatory cells at the sinusoids and periportal area. The hepatocytes of rats treated with the extract at the dose of 400 mg/kg body weight appeared to have hypochromic nuclei (Green arrow on Plate 4).
enal cortex also showed normal glomeruli, normal renal tubules, and normal interstitial spaces (Black arrow) (H & E X400).

The liver of control rats showed normal central venules with the characteristic morphology of the hepatocytes and sinusoids (Plate 1). The kidney tissues of the treated rats were almost same with those of the control. The normal architecture kidney tissue was seen at all doses. The renal cortex also showed normal glomeruli with normal mesangial cells and capular spaces (Plate 1). The renal tubules, including distal convoluted and proximal convoluted tubules appeared normal with normal interstitial spaces. At the doses 100mg/kg and 200mg/kg, the interstitial spaces showed areas of mild infiltration of inflammatory cells (Black arrow) (H & E X400), proximal convoluted tubules appeared normal with normal mesengial cells and capsular spaces (Plate 1). The renal cortex shows normal glomeruli, normal mesengial cells and capular spaces (White arrow), normal renal tubules, and convoluted tubules (Green arrow), the interstitial Spaces mildly infiltrated with inflammatory cells (Black arrow) (H & E X400).

Table 4: Oxidative stress markers of rats treated with methanol stem bark extract of Burkea africana for 28 days.

| Groups    | MDA (mg/mL) | Catalase (IU/L) | GSH (mg/dL) |
|-----------|-------------|----------------|-------------|
| Control   | 2.24±0.06a  | 8.30±0.19a     | 2.27±0.05a  |
| 100mg/kg  | 3.58±0.08b  | 12.03±0.29b    | 1.66±0.11b  |
| 200mg/kg  | 3.60±0.12c  | 20.61±0.41c    | 1.75±0.09c  |
| 400mg/kg  | 3.77±0.13d  | 25.62±0.73d    | 2.58±0.05d  |

Values are Mean±S.E.M, n = 5. Values with different superscripts on the same column are significantly different (P < 0.05). MDA- Malondialdehyde, GSH-Glutathione.

The presence of antioxidants such as phenols and flavonoids, saponins, tannins and terpenoids in the methanol stem bark extract of Burkea africana suggests its anti-oxidative stress potential. There are many reports on the antioxidant, antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and properties of these phytochemicals suggesting wide range of biological activities. The GC-MS result revealed the presence of (2H) pyrrole-2-carbonitrile, 5-amino-3, 4-dihydro-, an alkaloid and 9, 17-octadecadienal, (Z)-, an unsaturated aldehyde which has been found to have antimicrobial and anti-inflammatory activities. This probably explains the findings of Tor-anjiin and Anyam, and Musa et al., 7. Resorcinol, a phenolic compound is a known antioxidant with hepatoprotective activity. This corroborates a report by Cordier et al., 4 that the plant is rich in phenol, making it a potent antioxidant. Oleic acid which is also a fatty acid has been proven to be a potent antihypertensive and is found to be in abundance in olive oil. Wei et al., 31 also discovered that oleic acid present in Michelia champaca flower may also be responsible for the antimicrobial properties of the plant. This further agrees with the antibacterial, many reports on the antioxidant, antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and properties of these phytochemicals suggesting wide range of biological activities. The GC-MS result revealed the presence of (2H) pyrrole-2-carbonitrile, 5-amino-3, 4-dihydro-, an alkaloid and 9, 17-octadecadienal, (Z)-, an unsaturated aldehyde which has been found to have antimicrobial and anti-inflammatory activities. This probably explains the findings of Tor-anjiin and Anyam, and Musa et al., 7. Resorcinol, a phenolic compound is a known antioxidant with hepatoprotective activity. This corroborates a report by Cordier et al., 4 that the plant is rich in phenol, making it a potent antioxidant. Oleic acid which is also a fatty acid has been proven to be a potent antihypertensive and is found to be in abundance in olive oil. Wei et al., 31 also discovered that oleic acid present in Michelia champaca flower may also be responsible for the antimicrobial properties of the plant. This further agrees with the antibacterial, many reports on the antioxidant, antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and properties of these phytochemicals suggesting wide range of biological activities. The GC-MS result revealed the presence of (2H) pyrrole-2-carbonitrile, 5-amino-3, 4-dihydro-, an alkaloid and 9, 17-octadecadienal, (Z)-, an unsaturated aldehyde which has been found to have antimicrobial and anti-inflammatory activities. This probably explains the findings of Tor-anjiin and Anyam, and Musa et al., 7. Resorcinol, a phenolic compound is a known antioxidant with hepatoprotective activity. This corroborates a report by Cordier et al., 4 that the plant is rich in phenol, making it a potent antioxidant. Oleic acid which is also a fatty acid has been proven to be a potent antihypertensive and is found to be in abundance in olive oil. Wei et al., 31 also discovered that oleic acid present in Michelia champaca flower may also be responsible for the antimicrobial properties of the plant. This further agrees with the antibacterial, many reports on the antioxidant, antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and properties of these phytochemicals suggesting wide range of biological activities. The GC-MS result revealed the presence of (2H) pyrrole-2-carbonitrile, 5-amino-3, 4-dihydro-, an alkaloid and 9, 17-octadecadienal, (Z)-, an unsaturated aldehyde which has been found to have antimicrobial and anti-inflammatory activities. This probably explains the findings of Tor-anjiin and Anyam, and Musa et al., 7. Resorcinol, a phenolic compound is a known antioxidant with hepatoprotective activity. This corroborates a report by Cordier et al., 4 that the plant is rich in phenol, making it a potent antioxidant. Oleic acid which is also a fatty acid has been proven to be a potent antihypertensive and is found to be in abundance in olive oil. Wei et al., 31 also discovered that oleic acid present in Michelia champaca flower may also be responsible for the antimicrobial properties of the plant. This further agrees with the antibacterial, many reports on the antioxidant, antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and properties of these phytochemicals suggesting wide range of biological activities. The GC-MS result revealed the presence of (2H) pyrrole-2-carbonitrile, 5-amino-3, 4-dihydro-, an alkaloid and 9, 17-octadecadienal, (Z)-, an unsaturated aldehyde which has been found to have antimicrobial and anti-inflammatory activities. This probably explains the findings of Tor-anjiin and Anyam, and Musa et al., 7. Resorcinol, a phenolic compound is a known antioxidant with hepatoprotective activity. This corroborates a report by Cordier et al., 4 that the plant is rich in phenol, making it a potent antioxidant. Oleic acid which is also a fatty acid has been proven to be a potent antihypertensive and is found to be in abundance in olive oil. Wei et al., 31 also discovered that oleic acid present in Michelia champaca flower may also be responsible for the antimicrobial properties of the plant. This further agrees with the antibacterial,
antifungal, larvicidal, molluscicidal\textsuperscript{11} and anti-influenza\textsuperscript{10} activities of this plant. Knowledge of the possible toxic or adverse effects of many medicinal plants is grossly inadequate. In evaluating the safety status of medicinal plant, acute, sub acute and sometimes chronic toxicity studies are carried out in laboratory animals\textsuperscript{2}. In this study, daily oral administration of methanol extract of \textit{Burkea africana} stem bark at the doses of 100, 200 and 400mg/kg body weight for 28 consecutive days did not cause any change in behavior or mortality in treated rats, suggesting that the extract is relatively safe. Sign of toxicity such as sedation, lethargy, anorexia, drowsiness and ultimately death have been used to evaluate toxic effect of chemicals and natural medicinal plant products used in traditional medicine by scientists. The absence of these signs is used as a criterion to support that the plant extract is safe for use medicinally\textsuperscript{2}. There was no significant (\(P < 0.05\)) effect on the body weight and organ-somatic index of the treated rats compared with the normal control (Figure 1 and Figure 2). These findings indicate that the extract showed no adverse effect on the organs (liver, kidney, lungs, heart and spleen) at all doses used in this study and therefore is considered to be safe. Also, the extract may be said to have no anti-nutritive and growth inhibiting effect since it had no effect on the body weight. According to Unuofin et al.,\textsuperscript{2} weight loss of about 10\% has been related to an adverse effect. In the same vain, organ-somatic index is often used in toxicological investigations\textsuperscript{33-35}. After 28 days of a single daily oral administration of methanol extract of \textit{Burkea africana} stem bark, hematological parameters showed some significant changes (Table 2). The fact that the hematopoietic system is readily attacked by toxic substances makes it imperative to always evaluate hematological parameters in toxicity studies to monitor the physiologic and pathological state of animals and humans\textsuperscript{5}. The PCV and RBC count of treated groups increased significantly in a dose-dependent manner, with no significant effect on hemoglobin. Hemoglobin, MCH and MCHC remained unaffected, with significant (\(p < 0.05\)) increase in MCV of treated groups at all doses. Circulating blood carries oxygen, nutrients and foreign substances, making it prone to toxic attacks leading to damages in RBCs, WBCs, platelets and hemoglobin. This gives rise to various forms of anemia depending on the component of the RBC affected and nature of the effect and also immune system failure\textsuperscript{2}. The results of this study suggests that the extract probably has stimulatory effect on erythropoiesis and hence useful in the treatment of anemia. The decreases in Leucocytes at 200 and 400 mg/kg, neutrophils at 100 mg/kg, and lymphocytes at 400 mg/kg body weight observed could be due to immunosuppressive potential of the extract. These changes may also be due to inflammatory response and/or stress\textsuperscript{36}. The effect of methanol extract of \textit{Burkea africana} stem bark on the liver was assessed by evaluating serum activities of liver enzymes. The enzymes (AST and ALT) activities are often used to evaluate the functional status of the liver and the condition of the hepatocytes due to the high amount of these transaminases found in the hepatocytes\textsuperscript{37,38}. However, ALT is considered more specific to liver\textsuperscript{37,39}. Treatments with this extract significantly decreased (\(P < 0.05\)) the serum activities of ALT after 28 days of oral administration, with no effect on AST and ALP. This suggests the absence of hazardous effect of the extract on the liver. The decrease in serum total protein observed could be due to decrease in globulin. This may be thought to be from the effect of some components of the extract on lymphoid organs with possibility of liver involvement\textsuperscript{37,40}. Albumin increased significantly when rats were treated with extract at 100 and 200 mg/kg. Studies have shown that albumin concentration and function in liver cirrhosis is often reduced\textsuperscript{41}, which further corroborate with our earlier suggestion that the extract has no adverse effect on the liver. The extract at 100 mg/kg slightly increased total bilirubin, whereas direct (conjugated) and indirect (unconjugated) bilirubin remained unaffected (Table 5), suggesting that there is no problem with bilirubin conjugation in the liver. Hemoglobin metabolism which takes place in the liver, spleen and bone is the major source of bilirubin in the serum\textsuperscript{3}. Elevated serum bilirubin is due to increased destruction of erythrocytes resulting to increased release of hemoglobin as well as obstructive liver disorders\textsuperscript{34,35}. The extract may be said to have bile ducts obstructing tendencies, which is one of the major causes of increased serum bilirubin\textsuperscript{37}. This also is in doubt considering the fact that ALP was consistently unaltered throughout the period of treatment at all doses used in this study. Urea and creatinine are used to evaluate the functional status of the kidney, although serum creatinine concentration is considered a more reliable marker for evaluation of kidney function\textsuperscript{7,42}. The kidney as an excretory organ, is prone to toxic attack. This toxic effect on the kidney often result in impaired renal functions such as impaired excretion of metabolic waste, maintenance of fluid and electrolyte balance, and hormonal imbalance due to impaired synthesis of such hormones (erythropoietin). Serum urea and creatinine concentrations increase due to inability of the kidney to excrete urea and creatinine proportionately to their formation\textsuperscript{33,37}. Daily treatment with methanol stem bark extract of \textit{B. africana} showed serum urea levels to be elevated at 400mg/kg b. wt. Elevated MDA and decreases in GSH (Table 8) observed is an indication that the crude extract enhances lipid peroxidation and free radical formation when administered for a long period\textsuperscript{37}. Malondialdehyde (MDA) is end result of lipid peroxidation due to increased free radical production or decrease in antioxidant defense system\textsuperscript{33,45}. Reduced glutathione is a natural antioxidant in the liver and serves to conjugate with toxic metabolite, making them more polar and readily excreted\textsuperscript{45}. Glutathione also serves to scavenge free radicals and reduce oxidative effect in cells and eventual cell death. Therefore the ability of cells to sustain GSH concentration is useful for cell function and survival\textsuperscript{46}. Cerese et al.,\textsuperscript{46} postulated that low GSH with corresponding decrease in glutathione reductase enzyme (GR) creates an oxidative imbalance, inspiring oxidative processes and then cell death.
Reduction in GSH is marked by increase lipid peroxidation caused by free radical reaction seen as increased MDA.\textsuperscript{46} The significant (P<0.05) decrease in GSH in the treated group at the doses used in this study suggest that the extract may have inhibitory effect on the enzyme glutathione reductase which reduces oxidized glutathione (GSSH) thereby depleting reduced Glutathione (GSH). This explains the increased MDA observed in this study. Decrease in catalase activity in serum can be due to imbalance in its utilization and synthesis or as a problem with expression in the gene controlling its synthesis, resulting in oxidative stress and tissue damage induced by precursors of oxidation (pro-oxidants).\textsuperscript{47} In the subacute administration of the extract; catalase was significantly elevated compared to the normal control. This is an indication that the extract has some stimulatory effect on catalase activity and release which further explains the antioxidant properties of this plant in spite of the increased lipid peroxidation and decrease in GSH. Catalase is a very potent antioxidant enzyme in cells and a molecule of catalase can neutralize millions of peroxide molecules to water and oxygen in seconds.\textsuperscript{47} Histopathology of the liver and kidney revealed little or no pathological effect that is due to the treatment with methanol extract of \textit{Burkea africana} stem bark. Pathological changes in the parenchymal cells of the liver are often associated with changes in serum activities of liver enzymes.\textsuperscript{48} Absence of necrosis of the hepatocyte in the treated groups further agrees with the results of the enzyme assay (Table 3). The mild infiltration of inflammatory cells noticed at the perportal region and in the sinusoid is to be considered none pathologic. The kidney cellular morphology appeared normal, indicating that its functional status may not have been altered by the extract. This is further substantiated by the serum urea and creatinine levels (Table 3).

In conclusion, the methanol extract of \textit{Burkea africana} stem bark administration orally for consecutive 28 days up to a dose of 400 mg/kg body weight had no obvious deleterious effect in rats. The results in this study suggest that the plant is safe for use in treatment of the claimed ailments and therefore supports its use in traditional medicine by rural dwellers. Therefore the use of this plant in treatment is best if used for short durations or at lower doses.

**AUTHOR’S CONTRIBUTION**

TF Swem take care of the laboratory work, PE Aba was involved in designing the experiment, interpreted the data. SC Udem conceived the study, contributed in the design of the experiment, VM Ahur and FA Gberindyer interpreted the clinical chemistry and hematology results.

**ACKNOWLEDGEMENTS**

This research was fully funded by Tertiary Education Trust Fund (TETFund) academic staff sponsorship of the, Benue State, Nigeria. Special acknowledgement to the staff of Department of Veterinary Physiology and Biochemistry of the Federal University of Agriculture Makurdi and Department of Veterinary Physiology and Pharmacology of the University of Nigeria Nsukka.

**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**REFERENCES**

1. Muthulakshmi A, Jothibai MR, Mohan VR. GC-MS analysis of bioactive components of \textit{Feronia elephantuncorrea} (Rutaceae). J Appl Pharmaceut Sci 2012; 02: 69-74. https://irn.sars.co.za/wps/wcm/connect/12456789/151116
2. Unuofin JO, Onunlu GA, Afofayan AJ. Acute and subacute toxicity of aqueous extract of the tuber of \textit{Kedrostis africana} (L.) Cogn in Wistar rats. J Comp Integ Med 2018; 20170139: 1-11. https://doi.org/10.1515/jctim-2017-0139
3. Abdullah SS. Acute and sub-acute toxicity of \textit{Castraegus aronia} syn. Azarolus (L.) Whole plant aqueous extract in wistar rats. Amer J Pharmacol Toxicol 2011; 6: 37-45. https://doi.org/10.26538/tjnpr/v2i8.1
4. Agbaire PO, Emudainohwo JOT, Peretiemo-Clarke BO. Phytochemical screening and toxicity studies on the leaves of \textit{Mannophyton fulvum}. Int J of Plant Environ Sci 2013: 1–6.
5. NonyaneF, Masupa T. \textit{Burkea africana} Hook.
6. Maroyi A. \textit{Burkea africana} Hook. Lemmens RHMI, Louppe D, Oteng-Amoako AA (eds) [Internet] Plant Resource of Trop. Afr.2010 [Cited 2018 Oct. 26], Record from PROTA4U.
7. Musa AO, Habatullah KU, Irsim T, Amina BO, Abubakar BA, Hadiza B. Analgesic and anti-inflammatory studies of methanol extract of \textit{Burkea africana} stem bark Hook (Fabaceae). Trop J Nut Prod Res 2018; 2: 375-379. https://doi.org/10.26538/tjnpr/v2i8.1
8. Diallo D, Marston A, Terreaux C, Toure Y, Paulsen BS, Hostettmann K. Screening of Malian medicinal plants for antifungal, larvicidal, molluscidal, antioxidant and radical scavenging activities. Phytother 2001; 15:401-406. https://doi.org/10.1002/jpr.738
9. Cordier W, Gulumiyan M, Cronardy AD, Steenkamp V. Attenuation of oxidative stress in U937 cells by polyphenolic-rich bark fractions of \textit{Burkea africana} and \textit{Syzgium cordatum}. BMC Compl and Altern Med 2013; 116: 1-12.https://doi.org/10.1186/1472-6882-13-116
10. Malterud KE. Ethnopharmacology, chemistry and biological properties of four Malian medicinal plants. Plants 2017; 11: 1-13 https://doi.org/10.3390/plants6010011
11. Tanko Y, Iliya B, Mohammed A, Mahdi MA, Musa KY. Modulatory effect of ethanol stem bark extract of \textit{Burkea africana} on castrol oil induced diarrhoeal on experimental animals. Arch of Appl Sci Res 2011;3: 122-130.
12. Tor-Anyin AT, Anyam VJ. Phytochemical evaluation and antibacterial activity: A comparison of various extracts from some Nigerian trees. Peak J Med Plant Res 2013;1: 13–18.
13. Natural Research Council of the National Academies (US). Guide for the Care and Use of Laboratory Animals: Committee for the Update of the Guide for the Care and Use of Laboratory Animals Institute for Laboratory Animal Research Division on Earth and Life Studies. (8th Ed.). The National academies press, Washington, D.C. 2011:1-213; https://doi.org/10.17226/12910
14. Treasure GE and Evans WC. Textbook of pharmacognosy.3rd ed. Bailliere Tindal, London. 1989; 493–508.https://doi.org/10.1111/j.2042-7158.1949.tb12463.x
15. Sofowora EA. Medicinal Plants and Traditional Medicine in Africa. 2nd ed. England, John and Wiley and Sons Ltd.1993; 55-62. https://doi.org/10.5772/intechopen.80348
16. Sofowora EA. Medicinal Plant and Traditional Medicine in Africa. 1st ed. University of Ife Press, Nigeria. 1994; 1-23. https://doi.org/10.1088/acrm.1996.2.365

17. Adeyemi MA, Ekunseitan DA, Abiola SS, Dipeolu MA, Egbeye JT, Sogunle OM. Phytochemical analysis and GC-MS determination of Lagernea breviflora R. Fruit. Int J Pharmacog Phytochem Res 2017:9: 1045-1050. https://doi.org/10.25257phyto.v9i07.11178

18. Cheesbrough M. Haematological tests. In: District Laboratory practice in tropical countries Part 2, 2nd ed. Cambridge University Press, Cambridge, UK. 2006; 268-347.

19. Thomas L.Clinical Laboratory Diagnostics.1st ed. Swem Sons Limit Company, Philadelphia. 2007.

20. Moss DW and Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, (eds.) Tietz Textbook of Clinical Chemistry, 3rd ed. W.B. Saunders Company, Philadelphia. 1999; 617-620. https://doi.org/10.1097/00097455-199904000-00021

21. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA &Ashwood ER (eds.) Tietz textbook of clinical chemistry,3rd ed. W.B. Saunders Company, Philadelphia. 1999; 447-540. https://doi.org/10.1046/j.1537-2995.1999.39070794.x

22. Tietz NW. Fundamentals of Clinical Chemistry. W.B. Saunders Philadelphia. 1976; 15-16.

23. Burtis CA, Ashwood ER (eds.) Tietz Textbook of Clinical Chemistry. 3rd ed. W.B. Saunders Company, Philadelphia. 1999; 1388-1843. https://doi.org/10.1093/clinchem/45.6.913

24. Stocks J, Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. Brit J Haematol 1971; 20: 95-111. https://doi.org/10.1111/j.1365-2141.1971.tb00790.x

25. Sicinska P, Bukowska B, Pajak A, Koevea-Chyla A, Pietras T, Nizinkowski P. Decreased activity of butyryl cholinesterase in blood plasma of patients with chronic obstructive pulmonary disease. Arch of Med Sci 2017;13: 645-51. https://doi.org/10.5114/ams.2016.60760

26. Göth L. A simple method for determination of serum catalase activity and reference range. Clin Chim Acta 1991;196: 143-152. https://doi.org/10.1016/0009-8981(91)90067-n

27. Moron MS, De Pierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim et Biophys Acta 1979; 582: 67-68. https://doi.org/10.1016/0009-8681(79)90289-7

28. Drury R, Wallington A, Carleton SR. Carleton’s Histological Techniques. Oxford University Press, New York. 1967; 120-234. PMCID: PMC285117

29. Janakiramam N, Johnson M, Sahaya SS. GC-MS analysis of bioactive constituents of Peristrople bicalyculata (Retz.) Nees. (Acanthaceae). Asian Pac J Trop Biomed 2012; 11(Suppl): 46-49

30. Lopez-Huertas E. Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. Pharmaco Res 2010:61: 200–207. https://doi.org/10.1016/j.phrs.2009.10.007

31. Wei LS, Wei W, Song JFY, Syamsunir DF. Characterization of antimicrobial, antioxidant, anticancer propery and chemical composition of Michelia champaca seed and flower extracts. Stanford J Pharmac Sci 2011; 4: 19–24. https://doi.org/10.3329/sjps.v4i1.8862

32. Mohamed AEHH, El-Sayed MA, Hegazy ME, Helaly SE, Esmaiil AM, Mohamed NS. Chemical constituents and biological activities of Artemisia herba-alba. Records of Nat Prod 2010; 4: 1–25.

33. Tahraoua A, El-Hilaly J, Issaïl ZH, Lyouis B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachida province). J Ethnopharmacol 2007; 100: 105–117. https://doi.org/10.1016/j.ejep.2006.09.011

34. Arsal SS, Mohd EN, Hamzah H, Othman F. Evaluation of acute, subacute and subchronic toxicity of Rhophilophila decorata (Roxb.) Schott extract in male Sprague Dawley rats. J Med Plant Res 2013;7: 3030-3040. https://doi.org/10.5897/MPR2013.2611

35. Balogun SO, Da Silva IF, Colodell EM, De Oliveira RG, Ascenso SD, Martins DT. Toxicological evaluation of hydro-ethanolic extract of Helicteres sacarabola A J Ethnopharmacol 2013; 157: 285–91. https://doi.org/10.1016/j.jep.2014.09.013

36. Weiss DJ, Wardrop KJ (eds.). Schalm’s veterinary hematology, 6th ed. Wiley-Blackwell: A John Wiley & Sons Limited Publication, Iowa, USA, 2010; 200-250. https://doi.org/10.1111/j.1939-165X.2011.00324.x

37. Ezejie MI, Anaga AO, Asuzu IU. Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longispicata in rats. J Ethnopharmacol 2014;151: 1155-1164. https://doi.org/10.1016/j.jep.2013.12.034

38. Uddin N, Hasan MR, Hasan MM, Hossain MM, Alam MR. Assessment of toxic effects of the methanol extract of Citrus macropera Montr. Fruit via biochemical and hematological evaluation in female sprague-dawley rats. Flora One 2014; 652. https://doi.org/10.1371/journal.pone.0111101

39. Ramiaah SK. Preclinical safety assessment. Current gaps, challenges and approaches in identifying transplantable biomarkers of drug- induced liver damage. Clin Lab Med 2011; 31: 161-172.https://doi.org/10.1016/j.cll.2010.10.004

40. Donga S, Shukia VJ, Ravishankar B, Ashok BK, Mishry IU. Chf bolic toxicity study of Butea monosperma (Linn) Kunze seeds in albino rats. Ayur 2011; 32: 120-125. https://doi.org/10.4103/0974-8520.85743

41. Garcia-Martinez R, Caraceni P, Bernardi M, Gines P, Arroyo V. Albumin: pathophysiological basis of its role in the treatment of cirrhosis and its complications. Hepatol 2013; 58: 1836–1846. https://doi.org/10.1002/hep.25383

42. Mukinda JT, Eagles PK. Acute and sub-chronic oral toxicity profile of the aqueous extract of Pohangula fruticosa in female mice and rats. J Ethnopharmacol 2010; 128: 236–240.https://doi.org/10.1016/j.jep.2010.01.022

43. Kandhare AD, Raghude KS, Ghosh P, Ghule AE, Bodhankar SL. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia 2012; 83: 650-659.https://doi.org/10.1016/j.jep.2012.01.010

44. Visnagri A, Kandhare AD, Shiva Kumar V. Elucidation of ameliorative effect of Co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomed Aging Path 2012; 2: 157–172. https://doi.org/10.1016/j.biomag.2012.10.006

45. Adil M, Kandhare AD, Ghosh P, Venkata S, Raghude KS, Bodhankar SL. Ameliorative effect of naringin in acetaminophen- induced hepatic and renal toxicity in laboratory rats: role of FXR and KIM-1. Renal Fail 2016; 6049: 2-15.https://doi.org/10.3109/0886022x.2016.1163998

46. Cerese S, Sophie B, Parviz P, Andre’ R. Thiram-induced cytotoxicity is accompanied by a rapid and drastic oxidation of reduced glutathione with consecutive lipid peroxidation and cell death. Toxicol 2001; 163: 153–162. https://doi.org/10.1016/j.tox.2001.05.009

47. Sen S, Chakraborty R. The role of antioxidants in human health. Oxidative Stress: Diagnosis, Prev Ther 2011; 1083: 1–37. https://doi.org/10.1016/j.bmk.2011-1083.ch001

48. Okoye TC, Akah PA, Eziike AC, et al. Evaluation of the acute and subacute toxicity of Annona senegalensis root bark extracts. Asian Pac J Trop Med 2012; 5: 277-282. https://doi.org/10.1016/S1995-7645(12)6009-X