Dietary effect of Alstonia boonei stem bark extract on hematological profiles of Wistar albino rats after inducing oxidative stress with CCl₄

Robert I. Uroko¹, Victor E. Okpashi²*, Bayim P.R. Bayim³, Anthony U. Onwuekwe⁴, Kate M. Ucho⁵ and Igori Wallace⁶

¹Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. E-mail: uroko.robert@unn.edu.ng
²Department of Biochemistry, Cross River University of Technology, Nigeria. E-mail: vic2reshu@gmail.com
³Department of Biochemistry, Cross River University of Technology, Nigeria. E-mail: bayim22@yahoo.co.uk
⁴Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. E-mail: dmakpo@yahoo.com
⁵Department of Biochemistry, Cross River University of Technology, Nigeria. E-mail: kateucho@gmail.com
⁶Department of Chemistry, College of Education, Oju, Benue State, Nigeria. E-mail: igoriwallace@gmail.com

Abstract

This study examined the dietary outcome of Alstonia boonei stem bark. A. boonei stem bark was extracted with ethanol after air-drying and grinding. Hematological indices of Wistar albino rats were impacted with carbon tetrachloride (CCl₄). Eighteen male albino mice were used to check acute toxicity of the extract while 30 albino rats were used to examine the hematological functions – packed cell volume (PCV), hemoglobin (Hb) concentration, total white blood cell (WBC) count, total red blood cell (RBC) count and platelet count. The rats were separated into six sets of five rats each. Set-1 stands for normal control, and 2 ml/kg body weight of distilled water was given to them. Set-2 to 6 rats were induced with CCl₄, though set-2 was untreated, it represents negative control while set-3 was treated with Silymarin drug and represents positive control. Set-4 to 6 rats were given 100, 200 and 500 mg/kg body weight of A. boonei extract for 14 days. The acute toxicity study of A. boonei extract showed greater safeties as no death was recorded after 24 h. After giving classified doses of A. boonei extract, a non-significant (p > 0.05) increase in albumin concentration was observed which proposes no toxic effect, an indication that the extract may have protective properties for hepatocytes. It was warned that the extract may be toxic to bile function since there was a significant (p < 0.05) increase in total bilirubin for all the sets that were given A. boonei stem bark extract. The no significant (p > 0.05) decrease in PCV count, also suggests a marked loss of red blood cells. The no significant (p > 0.05) increase in hemoglobin concentration recommended that a low concentration of the extract was not toxic to hemoglobin concentration. The non-significant (p > 0.05) increase in WBC and RBC count in rats — given a low dose of A. boonei extract show that at low concentration, the extract may exercise antibody effect. The non-significant (p > 0.05) decrease in platelet concentration in all the sets given A. boonei extract compared to the normal control (set-1) might offer homeostatic properties or decreased platelet production due to A. boonei stem bark extract. The findings advocate that A. boonei stem bark if consumed, may protect hepatic cells due to its potentials against assault from intoxicants.

Keywords: A. boonei, Hematology, CCL4, Oxidative stress, Nutrition and antioxidant

© 2020 African Journal of Biological Sciences. This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.
1. Introduction

During the earliest eras, people have witnessed nature as a resource and liberation from countless sicknesses. The early stages of the curative plants were natural, as applicable to animals (Olajide et al., 2000). Despite no sufficient evidence to confirm the causation of particular illnesses or the link between plants and how it could be utilized to cure a particular sickness, everything was based on involvement and trials. Currently, the motives for using precise curative plants for the treatment of certain diseases are discovered. Thus, the utilization of medicinal plants has gained the attention of many scientists all over the world for the synthesis of drugs and nutritional supplements that can cure and manage chronic health conditions (Raji et al., 2005). More so, the declining efficacy of synthetic drugs coupled with increasing contraindications and associated side effects gave the need for natural drugs with no or fewer side effects. Studies by scientists have suggested that herbal-founded drugs and extracts are healthier and safer (Cragg and Newman, 2005).

![Figure 1: Leaves of A. boonei (Siepel et al., 2004)](image)

To ensure that human health complications are treated with plant properties, *Alstonia boonei* stem bark was extracted with ethanol and use to check for its hematological effect. *Alstonia* is a genus of the family Apocynaceae where other therapeutic vital floras fit in (Nweze et al., 2004) (Figure 1). *Alstonia* species are tropical plants that grow in several parts of Africa and South Asia. *A. boonei* is known as “ahun” in Yoruba language Nigeria, “ogbu-ora” by the Igbo tribe of Nigeria and “ukhu” by Hausa people (Elisabetsky and Costa-Campos, 2006). The plant fragments are rich in numerous bioactive combinations such as echitamidine, Nα-formylechitamidine, boonein, loganin, lupeol, ursolic acid, and b-amyrin amid alkaloids and triterpenoids which form the main lot (Croquelois et al., 1992). It is used as therapeutics for dysentery, typhoid, gonorrhea and asthma, ulcers, toothache, snakebites, rheumatic pain and as a galactagogue (Koduru et al., 2006). To check its potency against oxidative stress, several parameters were investigated including total protein which measures the concentration of proteins in the serum exception of clotting factors. Albumin with antibodies made to fight infections was determined (AACC, 2001). Albumin is a soluble, single polypeptide protein with 585 amino acids chain length and molecular weight of 66,000 Daltons. It is a useful indicator of hepatic function (De-cercq et al., 2010; and Marshall, 2012). Total albumin pool measures about 3.5-5.0 g/body weight (Nicholson et al., 2000). Albumin acts non-specifically as a transport protein for various constituents plus free fatty acids, certain ions (Ca²⁺, Zn²⁺), bilirubin and some drugs (Rochling, 2001; and Marshall, 2012). Total bilirubin was selected to measure conjugated and unconjugated bilirubin, the normal range is 0.1-1.0 mg/dl (2-15 µmol/L) (Thapa and Walia, 2007). Elevated total bilirubin may reason jaundice (McCacchey, 2002). Total bilirubin usually indicates both prehepatic – increased bilirubin production due to hemolytic anemia, or post-hepatic – impairment of the bile ducts to excrete bilirubin (Lee, 2009). Furthermore, other hematological functions such as packed cell volume (PCV), hemoglobin (Hb) concentration, total white blood cell (WBC) count, total red blood cell (RBC) and
platelet count were likewise examined after administering the plant extract to the rats. Despite the interesting functions of hematological indices, they are prone to suffer damage caused by oxidative stress (Farrugia, 2010; and John-Prosper et al., 2012). Thus, the need to manage the oxidative stress effect is critical to sustaining the health of humans. This study was intended to investigate the hematological indices of Wistar albino rats after induction of carbon tetrachloride (CCl₄) and subsequent treatment with ethanol extract of A. boonei stem bark to evaluate its efficacy at ameliorating oxidative stress.

2. Materials and methods

2.1. Collection of A. boonei stem bark

The bark of A. boonei was gotten from plant orchard in the Michael Okpara University of Agriculture, Umudike, Nigeria and was authenticated by Dr. K. N. Ibe of the Department of Forestry, College of Natural Resources and Environmental Management (CNREM), Michael Okpara University Nigeria.

2.2. Preparation of A. boonei stem bark

The fresh bark of A. boonei was hand-picked from their bole and dried at room temperature thereafter, the dried sample was ground into a fine powder, weighed and stored in a sterile container for subsequent extraction.

2.3. Extraction of coarse A. boonei stem bark with ethanol

A quantity 500 g of the coarse sample of A. boonei stem bark was soaked in 1.5 L of ethanol for 72 h in a sterile container. It was first filtered with a mesh cloth and later with a Whatman No. 1 filter paper. The filtrate was placed in a water bath at 60 °C and allowed to evaporate. The residues were weighed and the yield calculated.

2.4. Collection of Wistar albino rats

Eighteen male Wistar albino mice and 30 male Wistar albino rats were purchased from the Animal House, Department of Zoology, University of Nigeria. The animals were acclimatized for seven days under 12 h dark and light with permitted access to standard animal feed and water.

2.5. Investigational plan

The rats were divided into six sets of five rats each for this investigation, presented in Table 1.

| Sets               | Categories of extract and standard drug administration                                      |
|--------------------|------------------------------------------------------------------------------------------------|
| SET 1 (Normal control) | Received 2 ml/kg body weight of distilled water for 14 days.                                  |
| SET 2 (Negative control) | Received 2 ml/kg body weight of CCl₄, standard animal feed, and water for 14 days.             |
| SET 3 (Positive control) | Received 2 ml/kg body weight of CCl₄ was treated with 25 mg/kg body weight of silymarin orally for 14 days. |
| SET 4               | Received 2 ml/kg body weight of CCl₄ and was treated with 100 mg/kg body weight of A. boonei stem bark extract orally for 14 days. |
| SET 5               | Received 2 ml/kg body weight of CCl₄ and was treated with 200 mg/kg body weight of A. boonei stem bark extract orally for 14 days. |
| SET 6               | Received 2 ml/kg body weight of CCl₄ and was treated with 500 mg/kg body weight of A. boonei stem bark extract orally for 14 days. |

2.6. Instruments/Reagent

All instruments and reagents used for this study were of analytical criteria and originate from the Department of Biochemistry Laboratory, Michael Okpara University of Agriculture, Umudike and Cross River University of Technology Laboratory, Calabar, Nigeria.
2.7. Acute toxicity studies (LD₅₀)

The acute toxicity test of the concentrated A. boonei stem bark extract was determined using Lorke’s method (1983). The test was applied in two phases, where phase I and II were further set into three sets each. Phase I received the extract doses – 10, 100 and 500 mg/kg body weight, respectively, while phase II received extract doses – 1,000; 2,900 and 5,000 mg/kg body weight. Both phases – I and II were observed for 24 h while checking for unconcealed signs of toxicity, drowsiness, nervousness, in-coordination or death.

2.8. Test for albumin concentration

Albumin concentration was determined using the Randox laboratory kit described by Rodkey (1965). A volume 0.01 ml serum, 3 ml of BCG reagent was added, mixed and incubated for 5 min at 20-25 °C. The absorbance of the sample and standard were measured against the reagent blank at 578 nm. Albumin concentration was calculated by applying equation 1:

\[
\frac{\text{Albumin (g/dl)}}{} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} \tag{1}
\]

2.9. Test for total protein concentration

Total protein was determined using the method described by Weichselbaum (1946). Three test tubes; blank, standard and sample were labeled and to each tube, 0.02 ml of serum was added, 0.02 ml of protein standard was added and 0.02 ml of water added to the blank test tube. A volume 1 ml of protein reagent was added to the test tubes each. This was mixed and left to stand for 25 min at 20-25 °C. The absorbance was read at 540 nm. Total protein concentration was calculated using equation 2:

\[
\frac{\text{Total serum protein (g/dl)}}{} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5 \tag{2}
\]

2.10. Test for total bilirubin concentration

Total bilirubin concentration was determined using Randox assay kits described by Jendrassik and Grof (1938). Two test tubes were set-up for sample and blank. A volume, 200 µl of R1 was dispensed into both test tubes. One drop of R2 was dispensed into the sample test tube, R3 - 1000 µl was pipetted into both tubes and mixed. The mixtures were incubated at room temperature for 10 min. R4 - 1000 µl was then added into both test tubes and incubated additional for 5 min, thereafter, absorbance was read at 540 nm using a spectrophotometer. The concentration of bilirubin in mg/dl was interpolated by multiplying absorbance by a factor of 10.8.

2.11. Test for packed cell volume (PCV)

Packed cell volume was described by Ochei and Kolhatkar (2008), using equation 3.

\[
\text{PCV} \% = \frac{\text{Packed RBC column height}}{\text{Total blood volume height}} \times 100 \tag{3}
\]

2.12. Test for hemoglobin (Hb) concentration

Hemoglobin concentration was determined in line with the method described by Ochei and Kolhatkar (2008). Whole blood - 0.02 ml was collected using a heparinized capillary tube and added to 5 ml of Drabkin’s solution in a test tube (1:250 dilution). This was mixed and allowed to stand for 10 min. Absorbance was read at 540 nm with Drabkin’s solution as a blank using colorimeter. Hemoglobin concentration was calculated as grams of hemoglobin per 100 ml of blood.

2.13. Test of total white blood cell

The total white blood cell count was determined by haemocytometry described by Ochei and Kolhatkar (2008). An aliquot - 0.02 ml of blood was added to 0.38 ml of diluting fluid – (Acetic acid, tinged with gentian violet) and mixed. The counting chamber was charged with well-mixed diluted blood (after discarding the first five drops) with the aid of a pipette. Cells were allowed to settle in a moist chamber for 3 min. The four corners of the chamber were visualized on (10X) objective and the cells counted in four marked corner squares. Thereafter, the cells were calculated using equation 4:
\[ \text{Total WBC} \times \text{cu mm} = \text{Number of cells counted} \times \text{Dilution factor} \]

\[ \text{Number of cells counted} = \text{Area counted} \times \text{depth of fluid} \] \hspace{1cm} ...(4)

Where: Dilution = 1:200; Area counted = 1/5 sq.mm; depth of fluid = 1/10 mm; Number of red cells counted = \( N \times 200 \); Total WBC = \( N \times 200 \times 50 = N \times 10,000 \)

2.14. Test for red blood cell count

Red blood cell count was determined following the method of Ochei and Kolhatkar (2008).

An aliquot - 0.02 ml of blood was added to 3.98 ml of sodium citrate and mixed. After 5 min, the first few drops were discarded and the counting chamber was charged with the fluid. It was allowed to settle for 3 min by switching to (10x) objective. The center large square with 25 small squares was adjusted to the light and high power (40x) objective. The red blood cells in the four corner squares and one central square were counted by applying equation 6.

\[ \text{Total RBC} \times \text{cu mm} = \text{Number of cells counted} \times \text{Dilution factor} \]

\[ \text{Number of cells counted} = \text{Area counted} \times \text{depth of fluid} \] \hspace{1cm} ...(5)

Where: Dilution 1:200; Area counted 1/5 sq.mm; Depth of fluid 1/10 mm; Number of red cells counted \( N \times 200 \); Total RBC = \( N \times 200 \times 50 = N \times 10,000 \)

2.15. Test for platelet count

Platelet count was determined using the method of Ochei and Kolhatkar (2008). Whole blood was diluted with 1% ammonium oxalate solution. The dilution was mixed and incubated to lysis the erythrocytes. The dilution was mounted on a hemacytometer where the cells were allowed to settle and counted in a specified area of the hemacytometer chamber with a microscope. The number of platelets calculated per \( \mu \text{L} \) \((\times 10^9/\text{L}) \) of blood was calculated with equation 7.

\[ \text{Total number of cells counted} \times \text{Dilution factor} \times \frac{1}{\text{Volume factor}} = \frac{\text{Cells}}{\text{mm}^3} \]

\[ \frac{\text{cells}}{\text{mm}^3} = \frac{\text{cells}}{\mu \text{L}} = \frac{\text{cells}}{\mu \text{L} \times 10^3} = \frac{\text{cells}}{10^9 / \text{L}} \] \hspace{1cm} ...(6)

2.16. Statistical analysis

Results are presented as mean ± standard deviation, Duncan’s multiple comparison post hoc test (LSD) was used to compare significant differences in mean, while significance was \( p < 0.05 \) degree of confidence.

3. Results

3.1. The yield of A. boonei stem bark extract

After extraction and concentration of 500 g of ground A. boonei stem bark sample, the extract gave a 23% yield.

3.2. Acute toxicity outcome of A. boonei stem bark extract (LD\(_{50}\))

Acute toxicity of A. boonei stem bark in Table 2 showed that the extract caused no death after giving the rats 5,000 mg/kg body weight of the extract.

| Table 2: Phase I and II acute toxicity A. boonei stem bark extract |
|---------------------------------------------------------------|
| Dosage (mg/kg body weight) | Mortality | Dosage (mg/kg body weight) | Mortality |
|---------------------------|-----------|---------------------------|-----------|
| **Phase I**               |           | **Phase II**              |           |
| Set 1                     | 10        | 0/3                       | Set 4     | 1,000      | 0/3        |
| Set 2                     | 100       | 0/3                       | Set 5     | 2,900      | 0/3        |
| Set 3                     | 500       | 0/3                       | Set 6     | 5,000      | 0/3        |
3.3. Effects of A. boonei stem bark on Albumin (ALB) concentration

Figure 2 is the albumin (ALB) concentration of carbon tetrachloride (CCl\(_4\)) induced male Wistar albino rats treated with ethanol extract of A. boonei stem bark. The result indicates that only the ALB concentration of set-2 rats induced with CCl\(_4\) untreated showed a significant decrease in albumin level. There was an increase in ALB concentration in set 3 rats that were induced with CCl\(_4\) and treated with Silymarin (standard drug). Set 5 and 6 that were CCl\(_4\) induced but treated with 200 and 500 mg/kg body weight of A. boonei stem bark extract, respectively while rats in set 4 which were CCl\(_4\) induced but treated with low dose of A. boonei stem bark showed no decrease in ALB concentration compared to the normal control (set 1) that was not CCl\(_4\) induced.

![Figure 2: Albumin (ALB) concentration of male Wistar albino rats administered A. boonei stem bark extract]

| Set 1 (Normal control) | Received only distilled water |
|------------------------|------------------------------|
| Set 2 | CCl\(_4\) induced untreated |
| Set 3 | CCl\(_4\) induced but treated with the standard drug (Silymarin) |
| Set 4 | CCl\(_4\) induced but treated with 100 mg/kg body weight of A. boonei stem bark |
| Set 5 | CCl\(_4\) induced but treated with 200 mg/kg body weight of A. boonei stem bark |
| Set 6 | CCl\(_4\) induced but treated with 500 mg/kg body weight of A. boonei stem bark |

3.4. Effects of A. boonei stem bark on the total protein concentration of male Wistar albino rats induced with CCl\(_4\)

Figure 3 shows the total protein concentration of CCl\(_4\) induced male Wistar albino rats treated with A. boonei stem bark extract with ethanol. Results revealed that relative to the total protein of normal control (set 1) that was not CCl\(_4\) induced, only the total protein concentration of set 2 rats that were CCl\(_4\) induced but untreated and set 4 CCl\(_4\) induced but treated with low dose of A. boonei stem bark showed significant (p < 0.05) decrease, as there was significant (p < 0.05) increase in total protein of set 3 that were CCl\(_4\) induced but treated with standard drug (Silymarin), while set 5 and 6 that were CCl\(_4\) induced but treated with 200 and 500 mg/kg body weight of A. boonei stem bark showed no significant (p > 0.05) decrease in total protein concentrations.

3.5. Effects of Alstonia boonei stem bark extract on total Bilirubin concentration of male Wistar albino rats induced CCl\(_4\)

It was observed that only rats in sets 4-6 induced with CCl\(_4\) and treated with categorized doses of A. boonei stem bark showed significantly (p < 0.05) increase in total bilirubin concentrations (TBil), as rats in set 2 that were CCl\(_4\) induced but untreated showed no significant (p > 0.05) increase in total bilirubin concentration (Figure 4).

3.6. Effects of A. boonei stem bark on Packed Cell Volume (PCV) of male Wistar albino rats induced with CCl\(_4\)

Packed cell volume (PCV) of carbon tetrachloride (CCl\(_4\)) induced male Wistar albino rats treated with ethanol extract of A. boonei stem bark indicates that rats in set 3 that were CCl\(_4\) induced but treated with standard drug
(Silymarin) and set 4 CCl₄ induced but treated with low dose of A. boonei stem bark extract had no significant (p > 0.05) increase in PCV, same to set 4 the lowest, compared to normal control (set 1) (Figure 5).
3.7. Effects of *A. boonei* stem bark extract on hemoglobin (Hb) concentration of CCl$_4$ induced male Wistar albino rats

Data in Figure 6 shows that only rats in set 3 that were CCl$_4$ induced but treated with the standard drug (Silymarin) and set 4 CCl$_4$ induced but treated with low dose of ethanol extract of *A. boonei* stem bark showed no significant ($p > 0.05$) increase in Hb concentrations, as no significant ($p > 0.05$) decrease in Hb concentrations when compared with the normal control (set 1) rats that were CCl$_4$ induced but untreated, see Figure 6 below.

![Figure 5: PCV count of carbon tetrachloride (CCl$_4$) induced male Wistar albino rats administered *A. boonei* stem bark extract](image1)

![Figure 6: Hb concentration of male Wistar albino rats administered with *A. boonei* stem bark extract](image2)
3.8. Effects of A. boonei stem bark extract on White Blood Cell of male Wistar albino rats

Figure 7 shows that rats in set 2 that were CCl\textsubscript{4} induced but untreated and set 3 that were CCl\textsubscript{4} induced but treated with Silymarin, showed significantly ($p < 0.05$) decrease in WBC count, but set 4 CCl\textsubscript{4} induced but treated with low dose of A. boonei stem bark extract had no significant ($p > 0.05$) increase in WBC count.

![Graph showing WBC count of male Wistar albino rats](image)

3.9. Effects of A. boonei stem bark extract on RBC of male Wistar albino rats

In Figure 8, rats in set 3 that were CCl\textsubscript{4} induced but treated with standard drug (Silymarin) and set 4 CCl\textsubscript{4} induced but treated with low dose of ethanol extract of A. boonei stem bark showed no significant ($p > 0.05$) increase in RBC count compared with normal control (set 1). There was no significant ($p > 0.05$) decrease in the RBC count of rats in set 2 that were CCl\textsubscript{4} induced untreated.

![Graph showing RBC count of male Wistar albino rats](image)
3.10. Effects of A. boonei stem bark extract on platelet count of male Wistar albino rats

Figure 9, indicated that set 2 that were CCl₄ induced but untreated, set 3 CCl₄ induced but treated with standard drug (Silymarin), together with sets 4, 5 and 6 CCl₄ induced but treated with graded doses of A. boonei stem bark extract had significant (p < 0.05) decrease in platelet count compared to the normal control (set 1).

| Set | Treatment |
|-----|-----------|
| 1   | Normal control (received only distilled water) |
| 2   | CCl₄ induced untreated |
| 3   | CCl₄ induced but treated with the standard drug (Silymarin) |
| 4   | CCl₄ induced but treated with 100 mg/kg body weight of ethanol extract of A. boonei stem bark |
| 5   | CCl₄ induced but treated with 200 mg/kg body weight of ethanol extract of A. boonei stem bark |
| 6   | CCl₄ induced but treated with 500 mg/kg body weight of ethanol extract of A. boonei stem bark |

Figure 9: Platelet counts of male Wistar albino rats administered with A. boonei stem bark extract

4. Discussion

Albumin is the principal plasma protein produced by the liver, it is a valuable pointer of hepatic function (Mengel and Schwiebert, 2005). There was no significant (p > 0.05) increase in albumin concentration in rats induced with CCl₄ and treated with Silymarin. Also, set 5 and 6 that were CCl₄ induced but treated with graded doses of A. boonei stem bark extract suggest no toxicity. This followed that set 4 that were CCl₄ induced but administered with low dose of the extract show no significant (p > 0.05) decrease in albumin concentration, which proposes a changed in the synthesis of albumin by the liver that alter cellular roles such as the control of colloidal osmotic pressure of the blood (Figure 2) (Masaki et al., 2006).

Figure 2 is an illustration of the total protein concentration of male Wistar albino rats treated with A. boonei stem bark extract, after CCl₄ induction. The result indicates that the total protein concentration of set 2 rats that were CCl₄ induced but untreated and set 4 CCl₄ induced and treated with a low dose of A. boonei stem bark extract showed significant (p < 0.05) decrease, compared to the total protein of normal control (set 1) not CCl₄ induced. There was significant (p < 0.05) increase in total protein of set 3 that was CCl₄ induced and treated with Silymarin, while set 5 and 6 CCl₄ induced and treated with 200 and 500 mg/kg body weight of the A. boonei stem bark extract showed no significant (p > 0.05) decrease in total protein concentrations. Furthermore, total protein concentrations of set 3 CCl₄ induced and treated with Silymarin, and set 4 – 6 CCl₄ induced and treated with 100, 200 and 500 mg/kg body weight of A. boonei stem bark extract exhibited significant (p < 0.05) increase compared to set 2 CCl₄ induced but untreated.

Elevated total bilirubin has been implicated in jaundice or hemolytic anemias (McClatchey, 2002). Bilirubin is sorted out by the liver to permit its removal from the body. Result disclosed significant (p < 0.05) increase in total bilirubin concentration in all the sets administered with graded doses of A. boonei stem bark extract which put forward that the extract was toxic to the bile function and could cause irregularity in processing and eliminating bilirubin from the body. This was attributed to the disruption of normal blood activities and poor breakdown of RBCs as presented in (Figure 4) (Farrugia, 2010).

Increased total bilirubin has been implicated in jaundice or hemolytic anemias (McClatchey, 2002). Bilirubin is sorted out by the liver to permit its removal from the body. Result disclosed significant (p < 0.05) increase in total bilirubin concentration in all the sets administered with graded doses of A. boonei stem bark extract which put forward that the extract was toxic to the bile function and could cause irregularity in processing and eliminating bilirubin from the body. This was attributed to the disruption of normal blood activities and poor breakdown of RBCs as presented in (Figure 4) (Farrugia, 2010).

The packed cell volume is used for checkmating the percentage circulating red blood cells and a major index of anemia, polycythemia, and dehydration (Dino et al., 2008). The non-significant (p > 0.05) increase in PCV in CCl₄ induced rats but treated with Silymarin, and the set administered with low dose of A. boonei stem
bark extract signify that at low concentration of the extract, the rats suffered dehydration in their lymph or the extract possess no toxic effect on the PCV of the circulating blood. The no significant ($p > 0.05$) decrease in PCV in CCl$_4$ induced rats but untreated, in combination with graded doses of extract proffer marked the loss of red blood cell (Figure 5). This is because high graded doses of the extract did not have a positive acute effect on the rat’s PCV even at 500 mg/kg body weight. This was associated with failure in the production of bone marrow (Tschoep et al., 2000).

The no significant ($p > 0.05$) increase in hemoglobin concentration in CCI4 induced rats but treated with Silymarin, together with rats administered with a low dose of A. boonei stem bark extract suggests that at low concentration, there was no impacted on hemoglobin concentration. The no significant ($p > 0.05$) decrease in hemoglobin concentration in CCI$_4$ induced rats but untreated and the sets administered with high doses of the extract submit that at high concentration - 500 mg/kg body weight, the extract pose threat to hemoglobin concentration. The decrease in hemoglobin concentration was linked to abnormal secretion and binding of hemoglobin molecule to RBCs as a response to cellular hypoxia mediated by hypoxia-inducible transcription factors (Figure 6) (Harrison et al., 2011).

White blood cells are produced in the marrow and stored in the blood cells and lymphatic tissue, its prime role is to defend the body against infection and diseases. Rats in set 2 and 3 induced with CCl$_4$ but untreated, and rats induced with CCI$_4$ and treated with Silymarin respectively, revealed significant ($p < 0.05$) decrease in WBC count which may suggest the incidence of malnutrition or anaphylaxis (Parguina et al., 2012). More so, CCI$_4$ induced rats and administered with a low dose of extract had no significant ($p > 0.05$) increase in WBC count. This portrays that at a low concentration of extract, the extract may retain antibody activity (Figure 7). An increase in WBC represents a response of the body to intense bodily action and severe emotional responses Lin et al. (2000).

The no significant ($p > 0.05$) increase in red blood cell count of CCI$_4$ induced rats and treated with Silymarin, rats administered with a low dose of A. boonei stem bark extract submits that at low concentration, the extract no efficacy in upgrading the RBC count. The increase RBC might be polycythemia vera, - marrow makes too many red cells, which causes the blood to become dense. The no significant ($p < 0.05$) decrease in RBC count in CCI$_4$ induced rats, but untreated, as well as those administered with high doses of extract, depict a loss of red blood cells, which may be linked to shortness of inhalation, low oxygen levels, increased heart rate or kidney disease. Thus, at a high dose of the extract, it may pose a toxic effect on the RBC count (Figure 8).

Platelets are small enucleates cell fragments that play a crucial role in managing vascular integrity and regulating hemostasis. The no significant ($p > 0.05$) decrease in platelet concentration observed in all the sets relative to the normal control (set 1) suggests that the extract has homeostatic properties, and decreased platelet production due to the administration of A. boonei stem bark extract as shown in Figure 9.

5. Conclusion

Research to investigate the herbal benefit of A. boonei stem bark was designed. The stem bark was cut to sizes and air-dried at room temperature. Ethanol was used as a polar solvent to get the extract. Male Wistar albino rats were used to check the plant - A. boonei efficacy. The rats were induced with CCI$_4$ to exert oxidative stress and subsequently treated with ethanol extract of A. boonei stem bark. Some proteins and hematological indices were monitored. The findings of this study suggest that the ethanol extract of A. boonei stem bark has hematological and hepatoprotective capabilities against assault from intoxicants.

References

AACC (2001). Aspartate aminotransferase. Labtestsonline. 56–71.

Cragg, G. and Newman, D. J. (2005). Biodiversity: a continuing source of novel drug leads. Pure and Applied Chemistry. 77 (1), 7-24.

Croquelois, G., Kunesch, N., Debray, M. and Poisson, J. (1992). Alstonia boonei alkaloids. Medicinal Plants and Phytotherapy. 6 (2), 122-127.

De-Cercq, E., Ferr, G., Kaptein, S. and Neyts, J. (2010). Antiviral treatment of chronic hepatitis B virus (HBV) infections. Viruses. 2 (6), 1279-1305.

Dino, D. C., Jon, F. E., Daniel, I., Ronald, G. T., and Mehmet, T. (2008). Equilibrium separation and filtration of particles using differential inertial focusing. Analytical Chemistry. 80 (6), 2204-2211. doi: 10.1021/ac702283m.
Elisabetsky, E. and Costa-Campos, L. (2006). The alkaloid alstonine: a review of its pharmacological properties. Evidence-Based Complementary and Alternative Medicine. 3 (1), 39-48.

Farrugia, A. (2010). Albumin usage in clinical medicine. Transfusion Medicine Reviews. 24, 53-63.

Harrison, P., Mackie, I. and Mumford, A. (2011). Guidelines for the laboratory investigation of heritable disorders of hemoglobin function. British Journal of Haematology. 155 (1), 30-44.

Jendrassik, L. and Grof, P. (1938). Determination of total bilirubin. Biochemistry. 16 (2), 79-81.

John-Prosper, K. A., Genevieve, E. A., Yaw, O. B. and Frederick, A. A. (2012). A Review of the Ethnobotany and Pharmacological Importance of Alstonia boonei DeWild (Apocynaceae). International Scholarly Research Network (ISRN) Pharmacology, Article ID 587160, 9 pages doi:10.5402/2012/587160.

Koduru, S., Grierson, D. S. and Afolayan, A. J. (2006). Antimicrobial activity of Solanum aculeastrum (Solanaceae). Pharmacological Biology. 44, 284-286.

Lee, M. (2009). Basic skills in interpreting laboratory data. American Society of Health-System Pharmacists. 259-261.

Lin, Y., Weisdorf, D. J., Solovey, A. and Hebbel, R. P. (2000). Origins of circulating endothelial cells and endothelial outgrowth from blood. Journal of Clinical Investigation. 105 (1), 71-77.

Lorke, D. (1983). A new approach to practical acute toxicity testing. Archives of Toxicology. 54 (2), 75-87.

Marshall, W. (2012). Albumin (serum, plasma). Association for Clinical Biochemistry, 1-2.

Masaki, T., Ohkusu, K., Hata, H., Fujiwara, N., Hayashi, M. and Asano, Y. (2006). Mycobacterium sp. recovered from a clinical specimen and the first isolation report of Mycobacterium arupense in Japan. Journal of Microbiology and Immunology. 50, 889-897.

McClatchey, K. D. (2002). Clinical laboratory medicine. Lippincott Williams and Wilkins. 288-293.

Mengel, M. B. and Schwiebert, L. P. (2005). Family medicine: Ambulatory care and prevention (5th ed., pp. 49-55).

Nicholson, J. P., Wolmarans, M. R. and Park, G. R. (2000). The role of albumin in critical illness. British Journal of Anaesthesia. 85 (4), 599-610.

Nweze, E. T., Okafor, J. I. and Njoku, O. (2004). Antimicrobial activities of methanol extract of Trumeguineesis (schumm and thorn) and Morinda lucinda benth used in Nigerian herb. Journal of Biological Research and Biotechnology. 2, 34-46.

Ochei, J. and Kolhatkar, A. (2008). Medical laboratory sciences; theory and practice (pp. 321-324). New Delhi: Tata McGraw-Hill Publishing Co. Ltd.

Olajide, O. A., Awe, S. O. and Makinde, J. M. (2000). Studies on the anti-inflammatory, antipyretic and analgesic properties of Alstonia boonei stem bark. Journal of Ethnopharmacology. 71 (2), 179-186.

Parguina, A. F., Rosa, I. and Garcia, A. (2012). Proteomics applied to the study of leucocyte-related diseases. Journal of Proteomics. 76, 275-286.

Raji, Y. I., Salmon, T. M. and Akinsomsoye, O. S. (2005). Reproductive functions in male rats treated with mechanicol extract of Alstonia boonei stem bark. African Journal of Biomedical Research. 8, 105-111.

Rochling, F. A. (2001). Evaluation of abnormal liver tests. Clinical Cornerstone. 3 (6), 1-12.

Rodkey, F. L. (1965). Direct spectrophotometric determination of albumin in human serum. Clinical Chemistry. 2 (4), 478-487.

Siepel, A., Poorter, L. and Hawthorne, W. D. (2004). Ecological profiles of large timber species: Biodiversity of West African forests (An ecological atlas of woody plant species). Centre for Agriculture and Bioscience International. 22, 391-445.

Thapa, B. R. and Walia, A. (2007). Liver function tests and their interpretation. Indian Journal of Pediatrics. 74 (7), 663-671.

Tschoepe, D., Roesen, P. and Kaufmann, L. (2000). Evidence for abnormal hematocrit expression in diabetes mellitus. European Journal of Clinical Investigation. 20 (2), 166-170.

Weichselbaum, T. E. (1946). Determination of total protein. American Journal of Clinical Pathology. 16, 40.