

TOXICOLOGICAL AND HISTOPATHOLOGICAL EFFECTS OF CHEESE WOOD, *ALSTONIA BOONEI* DE WILD STEM BARK POWDER USED AS COWPEA PROTECTANT AGAINST COWPEA BRUCHID, *CALLOSBRUCHUS MACULATUS* (FAB.) [COLEOPTERA: CHRYSOMELIDAE] ON ALBINO RATS.

Ileke, K. D.¹, Odeyemi, O. O.², Ashamo, M. O.³ and Oboh, G.⁴

¹Department of Environmental Biology and Fisheries, Faculty of Science, Adekunle Ajasin University, PMB 001, Akungba-Akoko, Ondo State, Nigeria.

^{2&3}Department of Biology, School of Science, Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria.

⁴Department of Biochemistry, School of Science, Federal University of Technology, Akure. Tel no: +2348034318706

¹Corresponding Author: kayodeileke@yahoo.com

(Received: 30th October, 2013; Accepted: 13th January, 2014)

ABSTRACT

Toxicological and histopathological effects of *Alstonia boonei* stem bark powder on albino rats liver and kidney functions were investigated using standard methods. After two weeks of acclimatization, the rats were randomly divided into four (I - IV) groups of six animals each. Group I was fed with Basal Diet, group II - IV were for 30 days with Basal Diet containing 1%, 4% and 10% *A. boonei* stem bark powder, respectively. Blood was rapidly collected by direct heart puncture and the plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, urea and creatinine contents were determined using commercially-available kits. The levels of these biochemical functions were also determined in the liver and kidney. The *A. boonei* stem bark powder at 10% concentration caused oxidative liver and kidney damages. Histopathological results of the liver shows that the group fed with 4% *A. boonei* stem bark powder had normal hepatocytes. The group fed with 10% *A. boonei* stem bark powder had necrosis. The results of the present study showed that the supplementation of 1% and 4% *A. boonei* stem bark powder to the diets of rats for 30 days did not change the biochemical parameters of liver and kidney as well as histopathological investigation which illustrated normal architecture of liver and kidney.

Key words: *Alstonia boonei*, Albino Rats, *Callosobruchus maculatus*, Cowpea Seed, Toxicology, Histopathology.

INTRODUCTION

Alstonia boonei De Wild (Apocynaceae) is an African large evergreen deciduous crude medicinal tree that shed its leaves annually. The plant is about 45 m tall and 1.2 m in diameter. It possesses roots, stems, barks, leaves, fruits, seeds, flowers, and latex, which are claimed to have medicinal values in some cultures in African countries. The plant stem bark and its latex are applied in traditional medicine for treating many diseases. There are records on the use of alcoholic or aqueous extracts of most parts of *A. boonei* (Moronkola and Kunle, 2012). In traditional African medicine, *A. boonei* is a medicinal plant used extensively for the treatment of malaria, fever, intestinal helminths, rheumatism, hypertension (Terashima, 2003; Betti, 2004; Abel and Busia, 2005). It is used for the treatment of chronic diarrhea and dysentery, fever, pain, intestinal disorders and as an antidote for Strophanthus poison (Amole and Ilori, 2010). The extracts of the stem bark are commonly used to treat malaria and is listed in the African

Pharmacopoeia as an antimalaria drug (Olajide *et al.*, 2000). An infusion of root and stem bark is drunk as a remedy for asthma. A liquid made from the stem bark and fruit is drunk once daily to treat impotence (Majekodunmi *et al.*, 2008). The stem bark is used for the treatment of febrile illness, painful urination, rheumatic conditions and jaundice (Asuzu and Anaga, 1991), malaria, fever (Bello *et al.*, 2009; Majekodunmi and Odeku, 2009; Majekodunmi *et al.*, 2008), intestinal helminths (Weshche *et al.*, 1990), rheumatism, reversible antifertility (Raji *et al.*, 2005) and hypertension (Olajide *et al.*, 2000; Abel and Busia, 2005; Betti, 2004; Terashima, 2003) as an anti-venom against snake bite and antidote against arrow poisoning (Moronkola and Kunle, 2012). Other pharmacological uses are anti-inflammatory and as analgesic (Olajide *et al.*, 2000).

The major phytochemicals in the stem bark are saponins, alkaloids, tannins, flavonoids and cardiac glycosides (Anon, 1992, 2001; Phillipson *et al.*, 1987). In addition, it has also been suggested

that it also contains macro elements such as calcium, magnesium, sodium, potassium, phosphorus, iron, zinc, manganese, copper and cobalt to varying degrees (Akinmoladun *et al.*, 2007; Amole and Ilori, 2010). Alkaloids are medicinally useful, possessing analgesic, antispasmodic and bactericidal effects. Tannins promote healing (Oliver, 1960; Okwu and Okwu, 2004). Cardiac steroids are widely used in treating congestive heart failure (Okwu and Okwu, 2004). Flavonoids lower risk of heart diseases, saponins also promote wound healing (Okwu and Okwu, 2004).

The insecticidal activity of *A. boonei* has been reported recently (Oigiangbe *et al.*, 2007a; 2007b; Ileke and Oni 2011; Ileke *et al.*, 2012; Omoya *et al.*, 2012; Ileke *et al.*, 2013; 2014; Ojo and Ogunleye, 2013). Aqueous extracts of the leaf and stem bark of *A. boonei* was active against the pink borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), a pest of maize and some other cereals in West and Central Africa (Oigiangbe *et al.*, 2007a). Ileke and Oni (2011) reported the insecticidal potential of plants including *A. boonei* against *Sitophilus zeamais*. Ileke *et al.* (2012 and 2013) reported the insecticidal activity of *A. boonei* powder against *C. maculatus* and response of cowpea bruchid to *A. boonei* stem bark oils extracted with methanol, ethanol, acetone, petroleum ether and n-hexane. Omoya *et al.* (2012) reported the insecticidal potential of *A. boonei* leaf extract against *Anopheles* mosquito larvae in Nigeria. Ojo and Ogunleye (2013) worked on the comparative effectiveness of the powders of some underutilized botanicals including *A. boonei* stem bark powder for the control of *Sitophilus zeamais*. Ileke *et al.* (2014) reported the insecticidal activity of *A. boonei* latex against *C. maculatus*. We present the toxicological and histopathological effects of *A. boonei* stem bark powder which is scarce in literature.

MATERIALS AND METHODS

Preparation of *Alstonia boonei*

Stem bark of *Alstonia boonei* used for this study was sourced fresh from Akola farm at Igbara-Odo Ekiti, Ekiti State, Nigeria. The collected stem bark was rinsed in clean water to remove sand and other impurities, cut into smaller pieces before air-dried in the laboratory. The cleaned dried plant parts

were pulverised into very fine powder using an electric blender (Supermaster ®, Model SMB 2977, Japan). The powders were further sieved to pass through 1mm² perforations (Ileke and Oni, 2011). The powder was packed in plastic containers with tight lids and stored in a refrigerator at 4°C prior to use.

Toxicological Investigation of *A. boonei* Stem Bark Powder

Animals

Adult female albino rats weighing 160–170g were purchased from the breeding colony of the Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The rats were maintained at 25°C on a 12 hours light/dark cycle with free access to food and water. They were acclimatized under these conditions for two weeks prior to the commencement of the experiments.

Feed Formulation and Treatment Groups

The diets were freshly formulated according to the modified method of Oboh *et al.* (2005) and were kept in air tight containers and stored at 4°C until needed for use. Animals were divided into four groups:

- Group I – control rats, fed with basal diet (44% skimmed milk, 42% corn starch, 4% mineral and vitamin premix and 10% groundnut oil);
- Group II – rats fed with basal diet plus 1% *A. boonei* stem bark powder for 30 days;
- Group III – rats fed diet supplemented with 4% *A. boonei* stem bark powder for 30 days; and
- Group IV – rats fed diet supplemented with 10% *A. boonei* stem bark powder for 30 days.

Alstonia boonei Stem Bark Powder Fed Bioassay

Control and treated animals were decapitated after an overnight fast by cervical dislocation. The blood was rapidly collected by direct heart puncture and plasma was prepared using standard method. Also, the plasma AST, ALT, ALP, total protein, urea and creatinine contents were determined using commercially-available kits

(Randox Laboratories, UK).

Preparation of Tissue Homogenates

Liver and kidney of the rats were rapidly isolated and placed on ice and weighed. Tissues were rinsed in cold 0.9% normal saline (1:3, w/v), subsequently homogenized in sodium phosphate buffer (pH 6.9) and the homogenates centrifuged. The clear supernatants obtained were used for various biochemical assays (Bello *et al.*, 2004).

Histological Analysis of Liver and Kidney of Rats Fed with *A. boonei* Stem Bark Powder

The sectioning method described by Akparie (2004) was used for the histological examination. Sections of the liver and kidney 6µm thin were made and studied under the microscope. This method has the advantage of preserving the relations of cells and tissues to one another. They were dehydrated in serial concentration (50, 70, 80, and 100%) of alcohol 1½ hours each. After dehydration, they were cleared with 100% xylene and were left for 2 hours to remove any remnant alcohol, and later impregnated in liquid wax for 2 h for embedding. The embedded organs were sectioned using microtome and were stained with haematoxylin-eosin (Silva *et al.*, 1999). Excess stain

was removed with tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides. The preparations were left in the oven at 40°C and then placed under the microscope equipped with a digital camera connected to a computer system to be examined by an Histopathologist and the photographs were taken.

Data Analysis

Data were subjected to Analysis of Variance (ANOVA) and treatment means were separated using the New Duncan's Multiple Range Test. The ANOVA was performed with SPSS 16.0 software (SPSS, Inc. 2007).

RESULTS

Feeding Intake of All Experimental Animals

The average feed intake (Table 1) of all the experimental animals were calculated after the 30 days of study and it was discovered that, there exist no significant ($P>0.05$) difference in the average feed intakes of normal control rats (normal rats fed basal diets), rat fed with basal diet plus 1%, 4% and 10% *A. boonei* stem bark powder.

Table 1: Average Feed Intakes of Rats Fed Diets with Basal Diet and Basal Diet Plus *A. boonei* Stem Bark Powder

Group	Average feed intake (g/rat/day)
I	9.23±1.37 ^a
II	9.07±1.50 ^a
III	8.43±1.92 ^a
IV	8.01±1.73 ^a

Each value is a mean ± standard error of six replicates. Means followed by the same letter along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

Table 2: Change in Body Weight of Rats Fed with Basal Diet and Basal Diet Plus *A. boonei* Stem Bark Powder

Groups	Initial weight (g)	Final weight (g)	Weight gain/loss (%)
I	164.23±9.21 ^a	176.67±10.37 ^b	7.61 ^b
II	165.67±9.52 ^a	175.23±9.21 ^b	5.73 ^b
III	167.53±9.43 ^a	171.67±9.52 ^b	2.51 ^b
IV	163.33±9.19 ^a	157.13±9.79 ^a	-3.80 ^a

Each value is a mean ± standard error of six replicates. Means followed by the same letter along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

Effect of *A. boonei* Stem Bark Powder on Body Weight of Rats

Mean values for body weights of rats fed with basal diet and basal diet plus *A. boonei* stem bark powder at various doses for 30 days period is presented in Table 2. Measurement of the body weight was used to evaluate the health status of the rats during the experimental period. There was no significant difference ($p < 0.05$) in the body weights of rats from the start until the end of the experimental period in all groups apart from rat fed with basal diet plus 10% *A. boonei* stem bark powder that show weight loss compared with the normal control rats (basal diet only), rat fed with basal diet plus 1% and 4% *A. boonei* stem bark powder.

Effects of *A. boonei* Stem Bark Powder on Liver Functions of Albino Rats

Tables 3 and 4 shows the effects of *A. boonei* stem bark powder on some liver and serum biochemical indices of albino rats respectively. The liver activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total proteins (TP) of the animals fed

with basal diet (Group I), animals fed with 1% and 4% *A. boonei* stem bark powders were significantly higher than the animal fed with 10% *A. boonei* stem bark powder. However, there were significant increases in the activities of these enzymes in the serum of the animal fed with 10% *A. boonei* stem bark powder as compared with the control (Table 3). Increase in the serum enzyme activity signifies damages to the liver membrane. The serum AST activity of animal fed with 1% and 4% *A. boonei* stem bark powders were not significantly different from each other, whereas the serum AST activity of the group fed with 10% *A. boonei* stem bark powder only (78.67 IU/g) was significantly higher than that of the control (34.43 IU/g). The serum activities of ALP of the treated animals were significantly different from that of the control except group fed with 200 mg/kg, which was not significantly different. Total protein contents were reduced down the group that is in a dose-dependent manner. Generally, the activities of these enzymes in the animal fed with 1% and 4% *A. boonei* stem bark powders groups compared favourably with the animals fed with basal diet (Tables 3 and 4).

Table 3: Effects of *A. boonei* Stem Bark Powder on some Liver Biochemical Indices of Rats.

Groups	AST IU/g	ALT IU/g	ALP IU/g	Total protein min ($\times 10^{-1}$)
I	194.33 \pm 2.59 ^b	156.71 \pm 3.19 ^b	52.33 \pm 2.65 ^b	1.83 \pm 0.01 ^a
II	189.67 \pm 2.37 ^b	153.33 \pm 3.24 ^b	48.67 \pm 2.61 ^b	1.98 \pm 0.15 ^a
III	185.33 \pm 2.88 ^b	145.67 \pm 2.84 ^b	40.33 \pm 2.74 ^b	2.23 \pm 0.09 ^a
IV	113.67 \pm 2.86 ^a	88.67 \pm 2.05 ^a	17.33 \pm 1.65 ^a	4.43 \pm 0.22 ^b

Each value is a mean \pm standard error of six replicates. Means followed by the same letter along the column are not significantly different ($P > 0.05$) using New Duncan Multiple Range Test.

Table 4: Effects of *A. boonei* Stem Bark Powder on some Serum Biochemical Indices of Rats.

Groups	AST IU/g	ALT IU/g	ALP IU/g
I	35.43 \pm 2.72 ^a	33.71 \pm 2.26 ^a	44.33 \pm 2.50 ^a
II	36.33 \pm 2.08 ^a	37.33 \pm 2.50 ^a	48.67 \pm 2.89 ^a
III	40.33 \pm 2.89 ^a	42.67 \pm 2.89 ^a	53.33 \pm 2.50 ^a
IV	78.67 \pm 3.89 ^b	82.67 \pm 3.89 ^b	95.33 \pm 4.50 ^b

Each value is a mean \pm standard error of six replicates. Means followed by the same letter along the column are not significantly different ($P > 0.05$) using New Duncan Multiple Range Test.

Table 5: Effects of *A. boonei* Stem Bark Powder on some Kidney Biochemical Indices of Rats.

Groups	Urea (mmol/l)	Creatinine (mmol/l)
I	42.33±2.08 ^b	172.67±4.89 ^b
II	40.67±2.89 ^b	167.67±4.89 ^b
III	37.33±2.08 ^b	161.33±4.08 ^b
IV	15.67±2.89 ^a	99.67±2.89 ^a

Each value is a mean \pm standard error of six replicates. Means followed by the same letter along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

Table 6: Effects of *A. boonei* Stem Bark Powder on some Serum Biochemical Indices of Rats.

Groups	Urea (mmol/l)	Creatinine (mmol/l)
I	9.33±2.08 ^a	52.67±0.02 ^a
II	10.67±2.89 ^a	54.73±0.08 ^a
III	11.33±2.08 ^a	57.86±0.05 ^a
IV	29.67±2.89 ^b	107.50±0.03 ^b

Each value is a mean \pm standard error of six replicates. Means followed by the same letter along the column are not significantly different ($P>0.05$) using New Duncan Multiple Range Test.

Effects of *A. boonei* Stem Bark Powder on Some Kidney Functions of Albino Rats

Tables 5 and 6 show the effects of *A. boonei* stem bark powder on some kidney and serum biochemical indices of Albino rats respectively. The kidney activities of urea and creatinine of animals fed with basal diet (Group I), animals fed with 1% and 4% *A. boonei* stem bark powders were significantly higher than the animal fed with 10% *A. boonei* stem bark powder. Likewise, the serum activities of urea and creatinine of animals fed with basal diet (Group I), animals fed with 1% and 4% *A. boonei* stem bark powders were significantly lowered than the animal fed with 10% *A. boonei* stem bark powder. The results of kidney function tests revealed reduction in serum urea in animals fed with 1% and 4% *A. boonei* stem bark powders in comparison with animals fed with basal diet (Group I). On the other hand, there was a significant increase ($p>0.05$) in serum urea and creatinine concentration in animals fed with 10% *A. boonei* stem bark powders in comparison with the animals fed with 1% and 4% *A. boonei* stem bark powders. Increase in the serum enzyme activity signifies damages to the kidney membrane at 10% *A. boonei* stem bark powder.

Effects of *A. boonei* Stem Bark Powder on the Histopathology of Albino Rat Liver

The result of the liver histopathology show that animals fed with basal diet (control) have normal liver structure in which the muscles are in order and the sinusoids are in place as well. Sinusoids are small vessels in the liver that receive oxygen-rich blood from the hepatic artery and nutrients from intestine via the portal vein. The animals fed with 1% concentration showed depletion of sinusoids with normal hepatocytes and washing off of centre hepatocytes while the group of animals fed with 4% concentration showed dilation of sinusoids, necrosis, karyolysis and intracellular cell infiltration. Animals fed with 10% concentration showed point hepatocellular haemorrhage, depletion of sinusoids, point necrosis and karyolysis (Plate 1). Karyolysis is a break in cell or tissue.

Effects of *A. boonei* Stem Bark Powder on the Histopathology of Albino Rat Kidney

The kidney of the animals fed with basal diet showed normal kidney structure without any deformation. There is presence of hyaline cast at extreme. Hyaline is a clear glassy material produced as a result of degeneration in certain tissue e.g. connective tissue of the kidney. The animal fed with 1% concentration of *A. boonei*

stem bark powder showed presence of infiltration of cells and point necrosis of nephrones while the animal fed with 4% concentration showed presence of cell infiltration and extreme haemorrhage without necrosis. Tubular necrosis is the death of the kidney tubules interstitial cells on

cells situated within the tissue spaces. Animals fed with 10% concentration of *A. boonei* stem bark powder showed complete washing away of nephritic cells, depletion of glomerulus and focal destruction of nephritic (Plate 2).

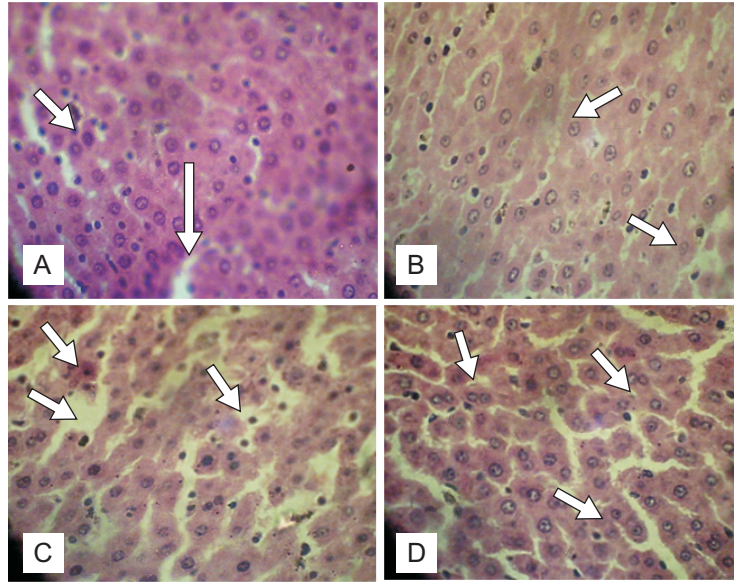


Plate 1: Effects of *Alstonia boonei* Stem Bark Powder on the Histopathology of the Albino Rat Liver

A. Basal Diet: Normal liver structure with normal muscles and sinusoids are intact with slight cell infiltration and distal karyolysis. **B.** 1%: Presence of depletion of sinusoids with normal hepatocytes and washing off of centre hepatocytes. **C.** 4%: There is slight dilation of sinusoids, necrosis, karyolysis and intracellular cell infiltration. **D.** 10%: There is point hepatocellular haemorrhage, depletion of sinusoids, point necrosis and karyolysis.

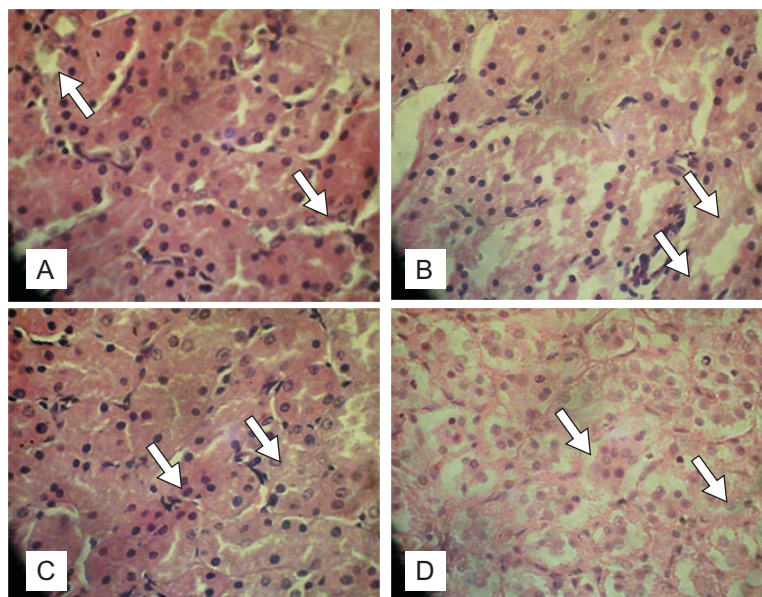


Plate 2: Effects of *Alstonia boonei* Stem Bark Powder on the Histopathology of the Albino Rat Kidney

A. Basal Diet: Presence of normal nephritic cell with cell infiltrations. There is presence of hyaline cast at extreme. **B.** 1%: Presence of infiltration of cells and point necrosis of nephrones. **C.** 4%: Presence of slight cell infiltration and extreme haemorrhage without necrosis. **D.** 10%: Complete washing away of nephritic cells, depletion of glomerulus and focal destruction of nephritic.

DISCUSSION

Previous studies by Oigiangbe *et al.* (2007a; 2007b); Ileke and Oni (2011), Ileke *et al.* (2012); Omoya *et al.* (2012); Ileke *et al.* (2013; 2014) and Ojo and Ogunleye (2013) have shown insecticidal activity of *A. boonei* powders and *A. boonei* extracts on the pink borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) a pest of maize and some other cereals, *Maruca vitrata* (Lepidoptera: Pyralidae), cowpea bruchid *C. maculatus* (Coleoptera: Chrysomelidae), mosquito larvae and *S. zeamais* (Coleoptera: Curculionidae) respectively. The insecticidal potential of this plant parts on the cowpea bruchid could be as a result of the presence of some chemical compounds of the triterpenoids, indole and alkaloid group such as alstonine, astondine, and porphine that have been identified from *A. boonei* (Phillipson *et al.*, 1987; Anonymous 1992; 2001; Oigiangbe *et al.*, 2007a; Moronkola and Kunle, 2012).

The measurement of the activities of various enzymes in tissues and body fluids play a significant and well known aid in disease investigation and diagnosis (Molomo, 2000; Yakubu *et al.*, 2003; Bamisaye *et al.*, 2013).

Alkaline Phosphate (ALP) is a marker enzyme for the plasma membrane and endoplasmic reticulum (Shahjahan *et al.*, 2004) and often employed to assess the integrity of the plasma membrane (Akanji *et al.*, 1993). The observed reduction in alkaline phosphate (ALP) activities in the liver and kidney following the administration of the 10% *A. boonei* stem bark powder may limit or hinder the adequate transportation of the required ions on the molecules across the plasma membrane (Akanji *et al.*, 1993). It may also lead to the less availability of the phosphate groups for the phosphorylation of ethanolamine and choline needed for the synthesis of major phospholipids like phosphatidylethanolamine and phosphatidylcholine (Bamisaye *et al.*, 2013). Furthermore, it can also hinder the synthesis of nuclear acids (Ramalingam and Vimaladevi, 2002).

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are also useful marker enzymes in assessing damage to organs. They are normally localised within the cells of the liver, kidney, and some other organs. These enzymes are

released into the serum especially when there is damage to the hepatic membrane as a result of chemical assaults. Serum levels of these enzymes therefore are significant diagnostic tools in assessing the level of hepatic damage (Oluba *et al.*, 2008; Odotuga *et al.*, 2010; Bamisaye *et al.*, 2013). Therefore, their presence in the serum of the powders-treated animals may be suggesting information on the tissue injury or dysfunction. The study of animals fed with 10% *A. boonei* stem bark powder revealed that the powder is toxic at relatively high concentrations to liver.

The histopathology of the liver shows that the control (basal diet) and animals fed with 1% *A. boonei* powder had normal liver structure with their sinusoids intact with little distal karyolysis. This might be due to penetration of the stain as well as sectioning of the organ. The fact that the liver sinusoids are intact shows that the basal diet and animals fed with 1% *A. boonei* powder does not have any deleterious effect on the organ (Akparie, 2004). The group fed with 4% *A. boonei* stem bark powder shows slight depletion of sinusoids with normal hepatocytes and slight washing off centre hepatocytes. This shows that the *A. boonei* stem bark powder has slight effect on the liver without any hepatocellular damage since necrosis was not noticed at this concentration for animals. Momoh *et al.* (2012) stated that at low concentration, any substance which have effect such as the one noticed above is generally safe for animal and will not cause any negative effect when used even over a long period of time. The group fed with 10% *A. boonei* stem bark powder had necrosis which is an indication that this concentrations is not safe for animals. Akparie (2004) reported that high concentrations of any plant substances is cidal to animals generally.

The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. One of the objectives of this study was designed to investigate the toxicity effect of *A. boonei* stem bark powder on renal function by evaluation of the creatinine, blood urea, and histopathological changes of kidney. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney (Walmsley *et al.*, 2010). Meanwhile, the creatinine is a waste product

from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function (Treasure, 2003). The normal range of serum creatinine is 0.2–0.8 mg/dl for rats (Weber *et al.*, 2002). It is excreted exclusively through the kidney. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of blood urea and creatinine will indicate kidney damage.

In this study, there was no significant reduction ($p > 0.05$) in serum creatinine in the rats treated with 1% and 4% of *A.boonei* stem bark powder. However, there was significant reduction ($p < 0.05$) in serum creatinine in the rats treated with high dose 10% of *A.boonei* stem bark powder compared with the rats fed with basal diet. Urea is the major nitrogen-containing metabolic product of protein catabolism. The significant increase in the serum urea concentration following the feeding of rats with 10% *A. boonei* stem bark powder may be attributed to impairment in the urea cycle leading to reduced production of the metabolic product (Yakubu *et al.*, 2003). This is an indication of abnormality in the physiological excretion of urea caused by a non-renal factor which is the plant powder in this study. The results of the present study showed that the supplementation of 1% and 4% of *A.boonei* stem bark powder to the diets of rats for 30 days did not change the biochemical parameters of kidney function as well as histopathological investigations which illustrated normal architecture of kidney. It is proved by the presence of no significant change of serum urea of all treatment groups and creatinine level of animals fed with 1% and 4% groups compared with the control (basal diet) group. Absence of pathological condition of kidney tissue in histological evaluation confirmed this claim. This study also found that body weight of the rats in all groups did not decline during the experimental period but the weight reduction of rats fed with 10% of *A.boonei* stem bark powder was correlated to the toxicity of *A.boonei* stem bark powder at this concentration, since no physical or behavioural signs of toxicity like lethargy, restlessness, respiratory distress, or convulsions could be revealed. The same observations have been made by Le *et al.* (2004) and Dollah *et al.* (2012) in normal

rats treated with the petroleum ether extract and powder of *Nigella sativa* for four and five weeks respectively.

The kidney structure of the group fed with basal diet was normal with few cell infiltration and presence of hyaline cast at extreme. This is an indication that the kidney is actively performing its function of ultrafiltration and selective reabsorption. However the group fed with 1% *A. boonei* stem bark powder had point necrosis which could be a normal death of nephrones. This is normal activity performed by an organ or tissues to remove old cells and bring in new ones. Therefore at 1% concentration, it has no deleterious effect. The group fed with 4% *A. boonei* stem bark powder also has no effect on the kidney since no necrosis was observed. The group fed with 10% *A. boonei* stem bark powder had complete washing away of nephritic cells which is an indication of toxicity of *A. boonei* stem bark powder at this concentration. It is therefore not safe for kidney nephrones.

With the evidence of normal urea and creatinine level in blood and normal kidney tissue in histology examination for rat fed with 1% and 4% of *A.boonei* stem bark powder, it is suggested that there are no toxic effect on kidney functions of rats fed with 1% and 4% of *A.boonei* stem bark powder for 30 days period.

The results of the present study showed the absence of toxic effect of *A.boonei* stem bark powder at low concentration on rat kidney and suggest that popular consumption of *A.boonei* stem bark powder by human being to treat some diseases traditionally at low concentration will not cause toxicity effect on the liver and kidney functions. It is also advocated that the *A. boonei* powders should not be used at high concentration to protect cowpea seeds meant for consumption.

CONCLUSION

The presence of high values of ALP, AST, LP and ALT in the serum of the powder-treated animals may give information on the tissue injury or dysfunction. The study of animals fed with 10% *A. boonei* stem bark powder revealed that the powder is very toxic at relatively high

concentration to liver. With the evidence of normal urea and creatinine level in serum and normal kidney tissue in histology examination for rats fed with 1% and 4% of *A.boonei* stem bark powder, it is suggested that there is no toxic effect on kidney function of 1% and 4% of *A.boonei* stem bark powder after feeding for a period of 30 days. The results of the present study also showed the absence of toxic effect of *A.boonei* stem bark powder on rat kidney and suggest that popular consumption of *A.boonei* stem bark powder by human at low concentration will not cause toxicity effect on the kidney functions. Therefore, it is advocated that the powder should not be used at high concentration to protect cowpea seeds meant for consumption as a result of possible toxicological and histopathological effect as demonstrated on albino rats fed with 10% *A. boonei* stem bark powder. Finally, medicinal plants have been used in seeds protection based on their efficacies but further toxicological evaluation should be investigated.

REFERENCE

- Abel, C. and Busia, K. 2005. An Exploratory Ethnobotanical Study of the Practice of Herbal Medicine by the Akan Peoples of Ghana (Ethnobotanical Study: Herbal Medicine in Ghana) *Alternat. Med. Rev.* 42.
- Akinmoladun, A. C., Ibukun, E. O., Afor, E., Akinrinlola, B. L., Onibon, T. R., Akinboboye, A. O., Obuotor, E. M. and Farombi, E. O. 2007. Chemical constituents and antioxidant activity of *Alstonia boonei*. *Afr. J. Biotech.* 6(10): 1197 – 1201
- Akanji, M. A., Olagoke, O. A. and Oloyede, O. B. 1993. Effect of chronic consumption of metabisulphite on the integrity of rat cellular system. *Toxicol.* 81: 173-179.
- Akparie, S. O. 2004. *General Veterinary Pathology*, 1st edition Stirling – Horden Publishers (Nig.) Ltd. 136 p.
- Amole, O. O. and Ilori, O. O. 2010. Antimicrobial activity of the aqueous and ethanolic extracts of the stem bark of *Alstonia boonei*. *International Journal of Phytopharmacology* 1(2): 119 - 123
- Anonymous. 1992. Ghana Herbal Pharmacopoeia. *Monograph of Alstonia bark*. The advent Press p. 10 - 12
- Anonymous. 2001. *Earthnotes Herb Library*. Esnestinar Parziale CH.
- Asuzu, U. and Anyanga, A. O. 1991. Pharmacological Screening of the Aqueous Extract of *Alstonia boonei* stem bark. *Fitoterap.* 63:411-417.
- Bamisaye, F. A., Oduuga, A. A., Minari, J. B., Dairo, J. O., Oluba, O. M. and Babatola, L. J. 2013. Evaluation of hypoglycemic and toxicological effects of leaf extracts of *Morinda lucida* in hyperglycemic albino rats. *Internat. Res. J. Biochemist. Bioinformat.* 3(2): 37 - 43.
- Bello, I. S., Oduola, T., Adeosun, O. G., Omisore, N. O. A., Raheem, G. O. and Ademosun, A. A. 2009. Evaluation of Antimalarial Activity of Various Fractions of *Morinda lucida* Leaf Extract and *Alstonia boonei* Stem Bark. *Global J. Pharmacol.* 3(3): 163-165.
- Betti, J. L. 2004. An ethnobotanical study of medicinal plants among the Baka Pygmies in the dja biosphere reserve, Cameroon. *African Study Monographs* 25(1): 1-27.
- Dollah, M. A., Saadat Parhizkar, S. and Izwan, M. 2012. Effect of *Nigella sativa* on the kidney function in rats. *Avicenna J. Phytomed.* 1 - 7
- Ileke, K. D. and Oni, M. O. 2011. Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored wheat grains. *Afr. J. Agricul. Res.*, 6(13): 3043 - 3048.
- Ileke, K. D., Odeyemi, O. O. and Ashamo, M. O. 2012. Insecticidal activity of *Alstonia boonei* De Wild powder against cowpea bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera : Chrysomelidae] in stored cowpea seeds. *Internat. J. Biol.* 4(2): 125 – 131
- Ileke, K. D., Odeyemi, O.O. and Ashamo, M. O. 2013. Response of Cowpea Bruchid, *Callosobruchus maculatus* (Fabr.) [Coleoptera: Chrysomelidae] to Cheese Wood, *Alstonia boonei* De Wild Stem Bark oil extracted with different solvents. *Archives Phytopathol. Plant Protect.* 46(11): 1357 – 1370
- Ileke, K. D., Oni, M. O. and Adelegan, O. A. 2014. Laboratory Evaluation of Some Plants Latex as Biopesticide against Cowpea Bruchid, *Callosobruchus maculatus* (Fab.) [Coleoptera: Chrysomelidae]. *J. Agricult.*

- Sci.* 6(1): In press.
- Le, P. M., Benhaddou, A. A., Settaf, A., Cherrah, Y. and Haddad, P. S. 2004. The petroleum ether extract of *Nigella sativa* exerts lipid lowering action in the rats. *J. Ethnopharmacol.* 94: 251 - 259.
- Majekodunmi, S. O., Adegoke, O. A. and Odeku, O. A. 2008. Formulation of the extract of the stem bark of *Alstonia boonei* as tablet dosage form. *Trop. J. Pharmaceut. Res.* 7(2): 987-994.
- Majekodunmi, S. O. and Odeku, O. A. 2009. Effects of interacting variables on the formulation of *Alstonia boonei* De Wild (Apocynaceae) tablets. *Acta Pharmaceut. Sci.* 51: 141-148.
- Molomo, S. O. 2000. Toxicological Implication of Ceftriaxone administration in rats. *N.J.B.M.B.* 15(1): 33 - 38.
- Momoh, A. O., Oladunmoye, M. K. and Adebolu, T. T. 2012. Haematological and histopathological effects of oil from castor seeds (*Ricinus communis* Linn.) on albino-rats. *J. Pharmacog. Phytother.* 4(4): 40 - 43
- Moronkola, D. O. and Kunle, O. F. 2012. Essential Oil Compositions of Leaf, Stem Bark and Root of *Alstonia boonei* De Wild (Apocyanaceae). *Internat. J. Biological Pharmaceut. Res.* 3(1): 51 - 60.
- Omoya, F., Oladipupo, K., Abe, A. and Udensi, O. 2012. Bioactivity, Qualitative and Quantitative Components of *Alstonia boonei* Leaf Extracts on *Anopheles* Mosquito Larvae in Nigeria. *J. Medical and Bioengineer.* 1(1): 39 - 41
- Oboh, G. 2005. Effect of blanching on the antioxidant property of some tropical green leafy vegetables. *LWT-Food Sci. Technol.* 38: 513 - 517.
- Odutuga, A. A., Dairo, J. O., Minari, J. B. and Bamisaye, F. A. 2010. Anti-Diabetic Effect of *Morinda lucida* Stem Bark Extracts on Alloxan-induced Diabetic Rats. *Res. J. Pharmacol.* 4(3): 78-82.
- Oigiangbe, O. N., Igbinsola, I. B. and Tamo, M. 2007a. Bioactivity of extracts of *Alstonia boonei* De Wild stem bark against *Maruca vitrata* (Lepidoptera: Pyralidae). *Adv Sci Technol.* 1(1): 67 - 70
- Oigiangbe, O. N., Igbinsola, I. B. and Tamo, M. 2007b. Insecticidal activity of the medicinal plant, *Alstonia boonei* De Wild, against *Sesamia calamistis* Hampson. *J. Zhejiang University Sci. B*, 8(10): 752-755.
- Olajide, A. O., Awe, S. O., Makinde, J. M., Ekhelar, A. I., Olusola, A., Morebise, O. and Okpako, D. T. 2000. Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stem bark. *J. Ethnopharmacol.* 71 (1-2): 179 - 186.
- Ojo, D. O. and Ogunleye, R. F. 2013. Comparative Effectiveness of the Powders of Some Underutilized Botanicals for the Control of *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). *Int. J. Pure Appl. Sci. Technol.* 16(2): 55-62
- Okwu, D. E. and Okwu, M. E. 2004. Chemical composition of *Spondias mombin* plant parts. *J. Sustain Agric Environ.* 6: 140 - 147.
- Olever-Beaver, D. 1986. Propagation and management, functional uses of Medicinal plants In: *Medicinal Plants in Tropical West Africa*. Cambridge University Press.
- Oliver, D. 1960. *Medicinal Plants in Nigeria* 1st edition. Nigerian College of Arts, Science and Technology, Ibadan. pp 43-53.
- Oluba, O. M., Adeyemi, O., Ojeih, G. C., Adebisi, K. E., Isiosio, I. O. and Aboluwoye, C. O. 2008. Effect of dietary cholesterol on some serum enzymes. *J. Med. Sci.* 8: 390 - 394.
- Omoya, F., Oladipupo, K., Abe, A. and Udensi, O. 2012. Bioactivity, Qualitative and Quantitative Components of *Alstonia boonei* Leaf Extracts on *Anopheles* Mosquito Larvae in Nigeria. *J. Med Bioengin.* 1(1): 39 - 41
- Phillipson, J. D., O'Neill, M. J., Wright, C. W., Bray, D. H. and Warhurst, D. C. 1987. Plants as a sources of Antimalaria and Amoebicidal Compound; Medicinal and poisonous plants of the Tropics: Proceedings of symposium 5 - 35 of the 14th *Internat. Bot. Cong. Berlin*. pp 70 - 78.
- Raji, Y. I., Salmon, T. M. and Akinsomsoye, O. S. 2005. Reproductive functions in male rats treated with methanol extract of *Alstonia boonei* stem bark. *Afr. J. Biomedical Res.*, 8(2): 105-111.

- Ramalingam, V. and Vimaladevi, V. 2002. Effect of mercuric chloride on membrane bound enzymes in rats testis. *Asian J. Androl.* 4: 309 - 311.
- Shahjahan, M., Sabitha, K. E., Mallika, J. and Shyamala-Devi, C. S. 2004. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. *Indian J. Medicinal Res.* 120: 194 - 198.
- Silva, A. M., Bambirra, E. A., Oliveira, A. I, Souza, P. P., Gomes, D. A., Viera, E. C. and Nicoli, J. R. 1999. Protective effect of bifidus milk on the experimental infection with *Salmonella enteritidis* subsp. *Typhimurium* in conventional and gnotobiotic mice. *J. Appl. Microbiol.*, 86:331-336.
- Terashima, H. 2003. Names, use and attributes of plants and animals among the Ituri forest foragers: a comparative ethnobotanical and ethnozoological study. *Afr. Stud. Monogr.* 28(Suppl.): 7-24.
- Treasure, J. 2003. Urtica semen reduces serum creatinine levels. *J. American Herb. Guild* 4: 22 - 25.
- Walmsley, S. J., Broeckling, C., Hess, A., Prenni, J. and Curthoys, N. P. 2010. Proteomic analysis of brush-border membrane vesicles isolated from purified proximal convoluted tubules. *American J. Physiol. Renal Physiol* 298: 1323 - 1331.
- Weber, D. K., Danielson, K., Wright, S. and Foley, J. E. 2002. Haematology and serum biochemistry values of dusky-footed wood rat (*Neotoma fuscipes*). *J. Wildlife Dis.* 38: 576 - 582.
- Weshche, D., Black, D. and Millious, W. K. 1990. *Book of Abstracts*, 39th Annual meeting of the American Society of Tropical Medicine and Hygiene, New Orleans. Poster No 138.
- Yakubu, M. T., Bilbis, L. S., Lawal, M. and Akanji, M. A. 2003. Effect of repeated administration of Sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats. *Nigerian J. Pure Appl. Sci.* 18: 1395 - 1400.