LIVER FUNCTION AND TOXICITY OF AQUEOUS LEAF EXTRACT OF *Alstonia boonei* (DE WILD) ADMINISTERED ON MICE AND WISTAR RATS

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Abstract: This research evaluates the acute and sub-acute toxicity of aqueous extract of *Alstonia boonei* leaves on mice and Wistar rats and its effect on liver parameters like (ALT), (AST), and (ALP), (TP), (TB) and (DB). Mice weighing between 15 – 25 g, Twenty-four (24) male and female Wistar rats weighing between 120 – 230g were randomly assigned into eight (8) groups (A – H) of three (3) rats per group. Groups (A and E) were used as male and female controls respectively while male groups (B, C, D) and female groups (F, G, H) served as the test groups and received 500, 1000 and 2500 mg/kg of the extract respectively. All animals were sacrificed after 28 days of extract administration via oral gavage. Livers were isolated for histopathological analysis and blood was collected by cardiac puncture for biochemical analysis. In the acute toxicity study, *A. boonei* was found to be non-toxic at a single dose of 10,000 mg/kg body weight (b.wt) on mice. While in sub-acute toxicity study, a significant difference ($p > 0.05$) or increase was observed in the serum levels of ALT and AST for male and female rats at all the doses tested except serum level of ALT in males which was significantly different ($p < 0.05$) at all the doses tested. There was a significant ($p < 0.05$) decrease in serum level of ALP at the dose of 1000 mg/kg (54.42±5.27 µ/L) in male animals. There was a significant ($p < 0.05$) decrease in serum ALB in male animal at the lowest dose of 500 mg/kg (2.22±0.26 g/dL). A significant ($p < 0.05$) difference or increase in serum total bilirubin (TB) was observed in female animals treated with higher doses of 1000 and 2500 mg/kg b. wt. (1.03±0.03, 0.86±0.03 mg/dL). There was a significant ($p < 0.05$) decrease in serum direct bilirubin (DB) at the lowest dose of 500 mg/kg (0.13±0.04 mg/dL) in female animals when compared with the control (0.25±0.03 mg/dL). Histopathological studies revealed that both male and female animals showed necrotic liver cells and extensive necrosis of liver cells at the doses tested. Thus, prolonged oral use of low and higher doses of *A. boonei* aqueous leaf extract should be discouraged.

Keywords: Liver function, *Alstonia boonei*, lethal dose, toxicity

Introduction

Therapeutic potential of plants voluminous pharmacological studies have been conducted to ascertain the subjective traditional uses of various medicinal plants, there is a need for further investigation of herbal remedies and phytochemicals to incorporate the observations of short and long-term toxicity manifestations (Bello *et al.*, 2016).In contrast to general old age myth that herbal drugs are safe and do not have toxic effects, these drugs may cause some moderate to severe side effects due to complex nature of their chemical compositions, hence, there is a need to establish safety to herbal drugs through validated scientific toxicity studies or protocols (Bhushan *et al.*, 2014).

Acute toxicity test (single dose) and Sub-acute toxicity test (daily dose) are performed on laboratory animals for detection of toxicity of a compound (Bhardwaj *et al.*, 2012; Enegide *et al.*, 2013).

The adverse effect(s) should occur within 14 days of the administration of the substance. The assessment of the median lethal dose (LD_{50}) (the dose that kills 50% of test animals population) has now been used as a major parameter in measuring acute toxicity and also as an initial procedure for general screening of chemical and pharmacological agents for toxicity (Enegide *et al.*, 2013). Apart from mortality and gross behaviour, other biological effects and the time of onset, duration and degree of recovery of survived animals, are also important in acute toxicity evaluation. Acute toxicity study solely gives information about (LD_{50}), therapeutic index (i.e. ratio between the lethal dose and the pharmacologically effective dose in the same strain and species (LD_{50}:ED_{50}), and the degree of safety of a pharmacological agent (Bhardwaj *et al.*, 2012). The aim of acute toxicity test isto determine the therapeutic index, i.e. ratio between the lethal dose (LD) and the pharmacologically effective dose (ED) in the same strain and species (LD_{50}:ED_{50}). The greater the index, the safer is the compound,

Sub-acute toxicity is the adverse effect (s)observed following repeated daily exposure to a chemical, or exposure for a significant part of an organism's lifespan (usually not exceeding 10%) or specific target organ/systemic toxicity arising from a repeated exposure (Haque and Haque, 2011; Bhardwaj *et al.*, 2012). The objectives of sub-acute toxicity studies are; the identification of the hazardous properties of a chemical, the identification of target organs, characterisation of the dose response relationship, identification of a no-observed-adverse-effect level (NOAEL) or point of departure for establishment of a Benchmark Dose (BMD), the prediction of chronic toxicity effects at human exposure levels, and provision of data to test hypothesis regarding mode of action (Bhardwaj *et al.*, 2012).

Alstonia boonei is a large evergreen tree belonging to the dogbane family Apocynaceae and genus Alstonia consisting of about 40 – 60 species (Ngueguim *et al.*, 2017; Ojo *et al.*, 2015).It is a native of tropical and subtropical (rain forests) of West and Central Africa, South east Asia, Central America and Australia where the roots, leaves, stem bark, latex, flowers and fruits are used extensively for medicinal purposes, the plant grows up to 45 m tall and 1.2 m in diameter, the bole (stem) branchless for up to 25 m, and the inner bark creamy or pale yellow with copious latex (Ojo *et al.*, 2015; Anyalogbu *et al.*, 2013; Kumar *et al.*, 2011; Owolabi *et al.*, 2014). The plant parts are rich in various bioactive compounds such as echitamidine, Nα-formylechitamidine, boonein, loganin, lupeol, ursolic acid, b-amyrin, saponin, alkaloids, tannins and steroids among which the alkaloids and triterpenoids form a major portion (Opoku and Akoto, 2015; Owolabi *et al.*, 2014).

Alstonia boonei plant is used in traditional medicine for the treatment of several diseases including diabetes, malaria, jaundice and typhoid fever (Ngueguim *et al.*, 2017). Therapeutically, the stem bark and leaves have been found to possess anti-rheumatic, anti-inflammatory, analgesic, anti-malaria, antipyretic, anti-diabetic (hypoglycemic), anti-helminthic, antimicrobial, antifungal, antiviral, antithrombosis, anti-tumour, antioxidant, antifertility and antibiotic properties (Iniaghe *et al.*, 2012; Opoku and Akoto,

2015). *Alstonia boonei* especially the stem bark has medicinal uses for treating febrile illness, micturation, jaundice, painful micturation (Awodele *et al.*, 2010).

Source: Field survey (2018)
Fig. 1: (A) Whole plant and (B) leaves of *A. boonei*

Alstonia boonei is known by different names in different countries. These include; Alstonia, cheese wood, Pattern-wood in English; Eghu-ora, akpi, eghu in Igbo; Awun in Yoruba; Ukhu in Benin, Edo State; Okhugbo in Itsekiri; ukpukuhu in Urhobo; ndondo in Ijaw; (John-Prosper *et al.*, 2012; Kumar *et al.*, 2011; Ngueguim *et al.*, 2017; Owolabi *et al.*, 2014).

There is lack of information on the effect of aqueous extract of *Astonia boonei* leaves on liver function; hence this research study to investigate the acute and sub-acute toxicity of aqueous extract of *Astonia boonei* leaves on mice and Wistar rats with emphasis on liver function, due to the roles the liver plays in filtration of blood from the digestive tract before passing it to the rest of the body, detoxification of chemicals and drugs metabolism, secretion of bile, and production of proteins for blood clotting and other functions (Hayfaa, 2016).

Materials and Methods

Laboratory experimental mice wistar rats

Healthy albino mice weighing between 15 – 25 g of both sexes and Wistar rats weighing between 120 – 230 g of both sexes were used for this study. The Mice and Rats were obtained from Faculty of Pharmaceutical Sciences, University of Benin, Benin City, Edo State, Nigeria and were housed in the experimental animal house of Faculty of life sciences, University of Benin, Benin City, Edo state, Nigeria. The animals were acclimatized for 14 days and observed for general condition every day and weighed on the next day of arrival and on the last day of acclimatization. They were fed with formulated feeds and water was administered *ad libitum*. The mice and rats were harboured in cages made of wooden frames and metal netting (gauze) under standard laboratory condition and room temperature (25±2^{0}C) of 12 h light/dark cycle. The guide for care and use of laboratory Animals (1996) of the Institute of Laboratory Animal Research (ILAR) Commission on life Science, National Research Council was duly followed (Ebiloma *et al.*, 2012).

Collection of plant materials and authentication

Fresh matured leaves of *Alstonia boonei* were collected from the matured tree in the forest of Obayantor village in Ikpoba-Okha LGA, Benin City, Edo State, Nigeria, in January 2017. The plant was identified by Dr. H. A. Akinnibosun (Plant Biologist and Biotechnologist/Taxonomist), Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.

Preparation of plant extract

Fresh matured leaves of *A. boonei* were picked, washed with distilled water to remove any form of contaminant and air dried. The dried leaves were ground to fine powder using an electric grinder. Exactly 600 g of the fine powder obtained was soaked in 6000 ml of sterile distilled water for 72 h after which it was filtered. The filtrate was collected in stainless steel pots and was evaporated to dryness using a temperature-regulated water bath pre-set to yield the crude extract concentrate. The extract was stored in a refrigerator at 4^{0}C prior to use.

Determination of extract yield

The percentage yield of the aqueous leaf extract of *A. boonei* was determined by weighing the ground leaf powder before extraction and the concentrated extract which was obtained after extraction and then calculated using the formula:

$$\text{Percentage Yield} = \frac{Weightofextract}{Weightofleafpowder} \times 100\ \%$$

$$= \frac{140\text{g}}{600\text{g}} \times 100$$

Percentage yield of extract = 23.33%.

Toxicological studies

Acute oral toxicity and lethality (LD_{50}) test (Lorke's method)

The oral acute toxicity(LD_{50}) study of aqueous extract of *Alstonia boonei* leaves was carried out in mice using modified Lorke's method (Enegide *et al.*, 2013) which has two phases.

Animal grouping for acute toxicity test

Phase One (1)

Group A (3 Control mice): Received proportionate volume of 0.5 ml sterile distilled water.
Group B (3 mice): Received *A. boonei* extract at a dose of 10 mg kg^{-1} b.wt
Group C (3 mice): Received *A. boonei* extract at a dose of 100 mg kg^{-1} b.wt.
Group D (3 mice): Received *A. boonei* extract at a dose of 1000 mg kg^{-1} b.wt.

Phase Two (2) (increment in dose)

Group A (3 mice): Received *A. boonei* extract at a dose of 5000 mg kg^{-1} b.wt.
Group B (3 mice): Received *A. boonei* extract at a dose of 7500 mg kg^{-1} b.wt.
Group C (3 mice): Received *A. boonei* extract at a dose of 10000 mg kg^{-1} b.wt.

Observation: There was no death recorded in any group within 24 h and after fourteen (14 days) of observation and monitoring. If death had occurred in any group, the LD_{50} would have been calculated by using the formula:

$$LD_{50} = \sqrt{(D_0\ x\ D_{100})}$$

Where: D_0 = Highest dose that gave no mortality; D_{100} = Lowest dose that produced mortality; **LD_{50}** = √ Maximum tolerated dose x Minimum toxic dose

Sub-acute toxicity test

Administration of the aqueous extract of *A. boonei* leaves was done by oral intubations using orogastric tube which was attached to a 1 ml syringe and lasted for 28 days at a single dose per day according to the following groupings:

Group A (Male Control Rats): Received proportionate volume of 0.5 ml of sterile dist. water.
Group B (Male rats): Received 500 mg/kg body weight of aqueous extract of *A. boonei* leaves.
Group C (Male rats): Received 1000 mg/kg body weight of aqueous extract of *A. boone* leaves.
Group D (Male rats): Received 2500 mg/kg body weight of aqueous extract of *A. boonei* leaves.
Group E (Female Control Rats): Received proportionate volume of 0.5 ml of sterile dist. water.
Group F (Female rats): Received 500 mg/kg body weight of aqueous extract of *A. boonei* leaves.
Group G (Female rats): Received 1000 mg/kg body wt. of aqueous extract of *A. boonei* leaves.

Group H (Female rats): Received 2500 mg/kg body weight of aqueous extract of *A. boonei* leaves.

Observations: Toxic manifestations and mortality were monitored daily and body weight changes were recorded every three (3) days till the end of the study.

At the end of 28 days, the rats were sacrificed by oral gavage, blood was collected by cardiac puncture in lithium heparin bottles for biochemical analysis and the organs were also collected in universal bottle containing 10% normal saline for histological analysis.

Determination of relative organ weights

After blood collection, the livers were carefully removed and weighed.

The relative weight of internal organ was calculated as follows:

$$\text{Relative organ weight } (\%) = \frac{Organweight}{Bodyweight} \times 100$$

Sample collection for biochemical analysis

At the end of the experimental period, the rats were reweighed, starved for 24 h and sacrificed by oral gavage under chloroform anaesthesia. 5 ml of arteriovenous blood was collected from the respective groups into heparinized sample bottles. Collection of blood was done by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and centrifuge at 4000 rev/min for 10 min to separate the serum from the blood which was used for serum biochemical estimations.

Measurement of liver enzyme parameters

The activity of Liver enzymes (transaminases) such as(Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and alkaline phosphatase (ALP), including total protein, albumin, total bilirubin, direct bilirubin, were measured using commercially available Diagnostic kits (Randox Laboratories Kits, St Louis, MO, USA) purchased from Pyrex Ltd, Benin, City.

Histological studies and immune histochemical staining

The rats were first anaesthetized by placing them each in a properly covered transparent bowl containing cotton wool soaked with chloroform, thereafter the rats were dissected from the stomach diligently to avoid bursting the abdominal aorta using a dissecting scissor. The liver of the rats was carefully located, harvested, weighed and fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. After deparaffinization and dehydration, the paraffin blocks were stained with hematoxylin and eosin for microscopic examination. Histopathological examinations were performed on the livers to assess whether or not organs ortissues had been damaged using Leica light microscope (X 100) and were photographed.

Results and Discussion

Percentage yield of the extract

The (Percentage %) yield of the aqueousextract of *A. boonei* leaves was found to be 23.33%.

Acute toxicity effects of aqueous extract of Alstonia boonei leaves on mice

The acute toxicity studies (**LD_{50}**) of the aqueous extract of *A. boonei* leaves showed that there was no death recorded in any group within 24 h and after fourteen (14days) of observation and monitoringeven after the mice received increased doses between (10 and 10000 $mgkg^{-1}$ b.wt.**)**of the aqueous extract of *A.boonei* leaves. Therefore the (LD_{50}**)** of the aqueous extract was considered greater than 10,000 mg/kg body weight.

Sub-acute toxicity effects of aqueous extract of A. boonei

The aqueous extract of *Alstonia boonei* leaves at doses of 500, 1000 and 2500 mg/kg administered orally at a single dose each day for 28 days did not produce any mortality in tested animals. No sign of observable toxicity was detected during the experimental period.

The results of acute toxicity effects of aqueous extract of *Alstonia boonei* leaves on mice is presented in (Table 1). The results of sub-acute toxicity effects of aqueous extract of *Alstonia boonei* leaves on serum level of ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin; TB = Total Bilirubin; DB = Direct Bilirubin in male wistar rats is presented in (Table 2).

Table 1: Acute toxicity effects of aqueous extract of *Alstonia boonei* leaves on mice

S/N	Single Dosage (mg/kg)	Behavioural Changes Observed in Mice
1	10	Calmness, wobbling, increase heart rate, shivering, grooming, and hyperactivity
2	100	Hyperactivity, itching, wobbling, grooming, pilo erection and sleeping
3	1000	Shivering, calmness, wobbling, and hyperactivity
4	5000	Wobbling, hyperactivity, grooming, rest ing, sleeping, pilo erection and calmness
5	7500	Calmness
6	10000	increase heart rate, calmness, sleeping, hyperactivity, grooming and wobbling

Table 2: Effects of aqueous extract of *Alstonia boonei* leaves on serum level of various parameters of male wistar rats

Parameters	Daily Dosage (mg/kg)			Control
	500	1000	2500	
ALT (µ/L)	46.33±1.33^{b}	40.00±1.15^{b}	44.00±2.31^{b}	30.67±2.40^{a}
AST (µ/L)	101.00±9.07^{b}	39.00±1.73^{a}	74.00±20.21^{ab}	59.00±17.04^{ab}
ALP (µ/L)	60.50±5.25^{ab}	54.42±5.27^{a}	77.53±0.00^{c}	70.72±6.02^{bc}
TP (g/dL)	7.09±2.38^{a}	8.44±0.12^{a}	8.85±0.22^{a}	8.47±0.71^{a}
ALB (g/dL)	2.22±0.26^{a}	2.93±0.31^{ab}	2.83±0.05^{ab}	3.34±0.29^{b}
TB (mg/dL)	0.83±0.13^{a}	1.04±0.12^{a}	1.15±0.19^{a}	1.30±0.34^{a}
DB (mg/dL)	0.24±0.13^{a}	0.10±0.00^{a}	0.25±0.03^{a}	0.17±0.07^{a}

Values are Means ± Standard Error of Mean (SEM) of the three Replicate determinations. Similar letters indicate means that are not significantly different from each other, but different letters are significantly different from each other; p< 0.05 - Significant; p>0.05 - Not Significant; mg/kg - Milligram per kilogram; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin; TB = Total Bilirubin; DB = Direct Bilirubin

Table 3: Effects of aqueous extract of *Alstoni aboonei* leaves on serum level of various parameters of female wistar rats

Parameters	Daily Dosage (mg/kg)			Control
	500	1000	2500	
ALT (µ/L)	43.33±6.06^{a}	51.00±0.58^{a}	49.00±0.58^{a}	48.00±2.40^{a}
AST (µ/L)	108.00±0.0^{a}	126.00±12.70^{a}	110.50±4.33^{a}	59.00±17.04^{a}
ALP (µ/L)	58.38±1.75^{a}	57.46±3.03^{a}	58.51±1.21^{a}	70.72±6.02^{a}
TP (g/dL)	8.57±1.00^{a}	8.30±0.24^{a}	8.05±0.05^{a}	8.47±0.71^{a}
ALB (g/dL)	4.00±0.34^{a}	3.81±0.38^{a}	3.95±0.08^{a}	3.34±0.29^{a}
TB (mg/dL)	0.62±0.04^{a}	1.03±0.03^{c}	1.86±0.03^{b}	1.30±0.34^{a}
DB (mg/dL)	0.13±0.04^{a}	0.15±0.03^{ab}	0.25±0.03^{b}	0.17±0.07^{b}

Values are Means ± Standard Error of Mean (SEM) of the three Replicate determinations. Similar letters indicate means that are not significantly different from each other, but different letters are significantly different from each other; p< 0.05 - Significant; p> 0.05 - Not Significant; mg/kg - Milligram per kilogram; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin; TB = Total Bilirubin; DB = Direct Bilirubin

The results of sub-acute toxicity effects of aqueous extract of *Alstonia boonei* Leaves on serum level of ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline Phosphatase; TP = Total Protein; ALB = Albumin; TB = Total Bilirubin; DB = Direct Bilirubinin Female Wistar Rats is presented in (Table 3).

The results of the effects of aqueous extract of *Alstonia boonei* leaves on body weights, organ weights, and relative organ weightsof male and female Wistar Rats are presented in (Tables 4 and 5), respectively.

Table 4: Effects of aqueous extract of *Alstonia boonei* leaves on body weight, organ weight, and relative organ weight of male wistar rats

Parameters	Daily Dosage (mg/kg)			Control
	500	1000	2500	
Body weight (g)	178.33±33.83^{a}	235.00±8.66^{a}	187.67±18.76^{a}	210.00±22.55^{a}
Organ weight (g)	6.18±0.93^{a}	7.24±0.31^{a}	6.51±0.11^{a}	6.63±0.79^{a}
Relative organ weight (%)	3.86±1.05^{a}	3.08±0.02^{a}	3.53±0.30^{a}	3.16±0.14^{a}

Values are Means ± Standard Error of Mean (SEM) of the three Replicate determinations. Similar letters indicate means that are not significantly different from each other, but different letters are significantly different from each other.
Note: p< 0.05 – Significant; p> 0.05 - Not Significant; mg/kg - Milligram per kilogram

Table 5: Effects of aqueous extract of *Alstonia boonei* leaves on body weight, organ weight, and relative organ weight of female wistar rats

Parameters	Daily Dosage (mg/kg)			Control
	500	1000	2500	
Body weight (g)	191.67±6.67^{ab}	172.67±7.22^{a}	175.00±5.77^{a}	200.00±2.89^{b}
Organ weight (g)	6.51±0.36^{b}	5.07±0.24^{a}	5.76±0.10^{ab}	5.75±0.19^{ab}
Relative organ weight (%)	3.40±0.12^{b}	2.93±0.01^{a}	3.30±0.16^{b}	2.87±0.06^{a}

Values are Means ± Standard Error of Mean (SEM) of the three Replicate determinations. Similar letters indicate means that are not significantly different from each other, but different letters are significantly different from each other.
Note: p< 0.05 – Significant; p> 0.05 - Not Significant; mg/kg - Milligram per kilogram

Illustration Tables of the sub-acute toxicity effects of aqueous leaf extract of *Alstonia boonei* on liver enzymes, body weights, organ weights, and relative organ weights of male and female Wistar rats are presented in (Tables 2 – 5).

The results of histopathological studies of effects of aqueous extract of *Alstonia boonei* leaves on liver of male and female Wistar rats are presented in (Figs. 2 and 3, respectively).

In this study, the acute behavioural signs of toxicity observed in mice given 10 mg of aqueous extract of *A. boonei* kg^{-1} b.wt. and above were itching, shivering, resting, sleeping, pilo erection, increase heart rate, calmness, hyperactivity, grooming and wobbling. There was however no mortality at all the dose levels tested. In this study, the oral median lethal dose (LD_{50}) was estimated to be greater ($\geq$10000 $mgkg^{-1}$b.wt.) (Table 1). The reduced activity of the treated mice observed in this study showed that the extract possesses central depressant effect. The absence of death following oral administration of aqueous extract of *Alstonia boonei* leaves at 10000 mg kg^{-1} b.wt. observed in the mice suggests that the extract is practically non-toxic acutely. This is in agreement with the research work conducted by Barnabé *et al*. (2014); Iyiola*et al.* (2011).

However, in this study, the significant difference (p< 0.05) or increase in serum ALT levels observed in male Wistar rats at all the doses tested (500, 1000, and 2500 mg/kg) (46.33±1.33, 40.00±1.15, 44.00±2.31 µ/L) when compared with the control (30.67±2.40 µ/L) may indicate necrosis or membrane damage, since necrosis or membrane damage releases the enzymes ALT and AST into circulation therefore, they can be measured in the serum (Abdullah, 2011; Ramaiah, 2011). High levels of AST indicate liver damage (Bello *et al.,* 2016; Patrick-Iwuanyanwu *et al.,* 2012). ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Bello *et al.,* 2016; Hayfaa, 2016; Patrick-Iwuanyanwu *et al.,* 2012). This was confirmed by the results of histopathological studies in which male animals which received 1000 mg/kg showed liver with necrotic liver cells and 2500 mg/kg showed liver with extensive necrosis of liver cells (Fig. 2 C&D). No significant difference (p>0.05) was observed in serum level of AST in male and female Wistar rats at all doses tested when compared with their respective controls.

The insignificant (p> 0.05) increases in serum levels of AST observed in male Wistar rats at the doses of (500, and 2500 mg/kg) (101.00±9.07, 74.00±20.21 µ/L)when compared with their control (59.00±17.04 µ/L) and in female Wistar rats at all the doses tested (500, 1000, 2500 mg/kg) (108.00±0.00, 126.00±12.70, 110.50±4.33 µ/L) when compared with their respective control (104.00±2.31 µ/L)may indicate liver damage (Tables 3 & 4). This was confirmed by the histopathological studies for female Wistar rats at all the doses tested whereby female at 500 mg/kg showed mild necrosis of liver cells, 1000 mg/kg revealed female liver showing necrosis of liver cells, and also at 2500 mg/kg showed necrotic liver cells (Fig. 3).

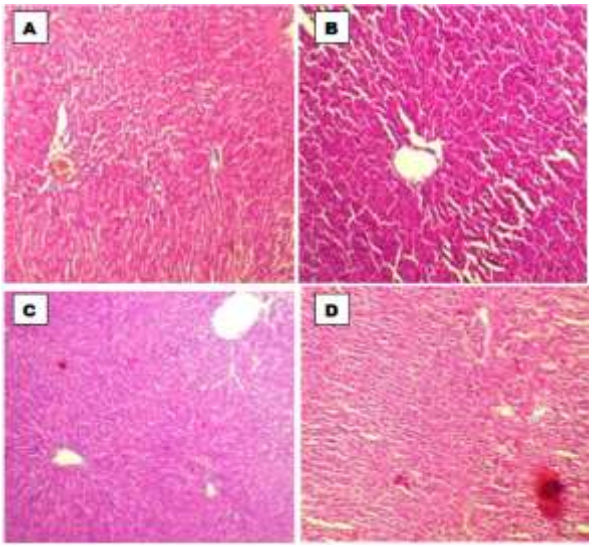
In this study, aqueous leaf extract of *A. boonei* caused significant increase (p< 0.05) in serum ALT in male Wistar rats at all the doses tested but causes an insignificant (p> 0.05) increase in serum AST levels in male and female Wistar rats except AST in male Wistar rats at the dose of 1000 mg/kg (39.00±1.73 µ/L) when compared with their respective control (Tables 3 & 4).

The ratio of AST/ALT may be employed in disease diagnosis, and when the ratio of AST and ALT is greater than one (AST**:**ALT > 1), it suggests myocardial infarction, while a ratio less than one (1) may be due to the release of ALT from the affected liver (Mugisha *et al.,* 2014). A mild or higher level of AST indicates liver injury or myocardial infarction (Mugisha *et al.,* 2014).

In this study, the ratio of (AST**:**ALT) was above 1 in all the treatment groups for male and female Wistar rats. Therefore, the mild or higher level of AST and ALT observed in male and female Wistar rats except AST in male Wistar rat at the dose of 1000 mg/kg (39.00±1.73 µ/L) when compared with control (59.00±17.04 µ/L) and ALT in female wistar rat at the dose of 500 mg/kg (43.33±6.06 µ/L) when compared with the

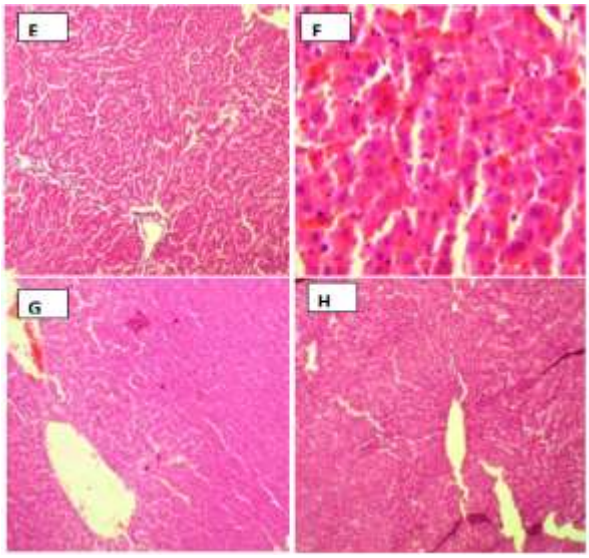
control (48.00±1.15 µ/L) may indicates liver injury or myocardial infarction, since a mild or higher level of AST indicates liver injury or myocardial infarction (Mugisha *et al.*, 2014).

This implies that the use of low and high doses of aqueous leaf extract of *A. boonei* for a longer period of time could cause significant damage to the liver as observed in this study. These results of serum chemistry were confirmed by the results of histopathological studies whereby necrosis of the liver cells was observed in all the female wistar rats at all the doses tested and in male wistar rats which received higher doses of (1000 mg/kg and 2500 mg/kg) showed liver with necrotic liver cells and extensive necrosis of liver cell, respectively (Figs. 2 and 3).



(**A**) Male liver control; (**B**) 500 mg/kg (male liver showing central vein with normal hepatocytes); (**C**) 1000 mg/kg (male liver with necrotic liver cells); and (**D**) 2500 mg/kg (Liver with extensive necrosis of liver cells)

Fig. 2: Diagrams showing the effects of *Alstonia boonei* aqueous leaf extract in a 28 days sub-acute toxicity study on male livers of albino wistar rats (H & E x 100)



(**E**) Female liver control (showing portal triad with normal hepatocytes); (**F**) 500 mg/kg (female liver with mild necrosis of liver cells); (**G**) 1000 mg/kg (female liver showing necrosis of liver cells); and (**H**) 2500 mg/kg (Liver showing necrotic liver cells)

Fig. 3: Diagrams showing the effects of *Alstonia boonei* aqueous leaf extract in a 28 days sub-acute toxicity study on female livers of albino wistar rats (H & E x 100)

However, in this study, AST enzymes showed significant increase ($p < 0.05$) in the treated animals especially in males at the doses of 500, 2500 mg/kg (101.00±9.07 µ/L and 74.00±20.21 µ/L respectively) when compared with control (59.00±17.04 µ/L) and in females at the dose of 500, 1000, 2500 mg/kg (108.00±0.00 µ/L, 126.00±12.70 µ/L, 110.50±4.33 µ/L) respectively as compared with the control (104.00±2.31 µ/L). This implies that prolonged administration of aqueous leaf extract of *A. boonei* at these doses may lead to hepatotoxic effect in both male and female Wistar rats as observed in this study(Tables 3 and 4), since the levels of AST and ALT enzymes are known to significantly increase in a toxic environment (Patrick-Iwuanyanwu *et al.*, 2012). This was confirmed by the results of histopathological studies as necrotic liver cells were observed (Fig. 2 C & D; Fig. 3 F, G & H).

In this study, the increase or elevation in the serum level of ALT and AST in the entire treatment groups except for male AST level at the dose of 1000 mg/kg (39.00±1.73 µ/L) and females ALT level at the dose of 500 mg/kg (43.33±6.06µ/L) is suggestive that this extract may possess hepatotoxic effect since ALT and AST are largely used in the assessment of liver damage by drugs or any other hepatotoxin (Ramaiah, 2011; Patrick-Iwuanyanwu *et al.*, 2012).

The decrease in level of AST at the dose of 1000 mg/kg (39.00±1.73µ/L) for males and decrease in ALT serum level at the dose of 500 mg/kg (43.33±6.06µ/L) for females when compared with theirrespective controls suggest that the plant extract is hepatoprotective at these doses in those particular animals. This is in agreement with the research work conducted by Ngueguim *et al.*, 2017; Barnabé *et al*., 2014, that aqueous extract of *A. boonei* caused an insignificant ($p > 0.05$) decrease in serum ALT and AST. The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage in those animals.

In this study, the insignificant ($p > 0.05$) increase in the serum level of alkaline phosphatase (ALP) in the male group administered the dose of 2500 mg/kg (77.35±0.00 µ/L) when compared with the control (70.72±6.02 µ/L) may be as a result of congestion or obstruction of biliary tract, which may occur within the liver, since an elevated serum ALP level is often associated with various disorders such as extra-hepatic bile obstruction, intra-hepatic cholestasis, infiltrative liver disease, hepatitis, and bone diseases (Adinortey *et al.*, 2012; Hayfaa, 2016; Thapa and Walia, 2007).

However, in this work, the insignificant ($p > 0.05$) decrease in serum ALP levels in treatment groups for all females and male 500 mg/kg, except male 1000 mg/kg (54.42±5.27 µ/L)when compared with the control groups(70.72±6.02 µ/L) rule out the occurrence of hepatobiliary diseases such as extra-hepatic bile obstruction, intra-hepatic cholestasis, infiltrative liver disease, hepatitis, and bone diseases, since ALP activity is related to the functioning of hepatocytes and an increase in its activity may be due to its increased synthesis in the presence of increased pressure (Patrick-Iwuanyanwu *et al.*, 2012).

In this study, there was no significant difference ($p > 0.05$) in serum level of total protein (TP) between the male and female Wistar rats at all doses tested when compared with their respective controls, since Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney and liver diseases and many other conditions (Thierry *et al*., 2011; Patrick-Iwuanyanwu *et al.*, 2012). This means that the extract at all the doses used in this study has no effect on serum level of total protein which is necessary for the repair of worn out body tissues.

In this study, the insignificant (p> 0.05) decrease in serum total bilirubin levels observed in male Wistar rats at all the doses tested and normal level of serum total bilirubin levels observed in female Wistar rat at the lowest dose of 500 mg/kg (0.62±0.04 mg/dL)when compared with their respective control groups rule out the occurrence of a disease condition of jaundice (Tables 3 & 4), since Bilirubin is a major breakdown product of haemoglobin in the liver, spleen and bone marrow, and an increase in total bilirubin in the blood results in a condition called jaundice (Adinortey *et al.*, 2012; Abdullah, 2011) and differential diagnosis of the types of jaundice is done by measuring the direct (conjugated) and indirect (unconjugated) bilirubin levels which determine whether the jaundice is pre-hepatic (haemolytic), hepatic or post-hepatic (obstructive) (Adinortey *et al.*, 2012; Thapa and Walia, 2007).

In this study, the normal levels and insignificant (p> 0.05) increase in serum total and direct bilirubin concentrations at all doses of the extract used in male and female Wistar rats except TB in female Wistar rats at the dose of 1000, 2500 mg/kg (1.03±0.03, 0.86±0.03 mg/dL) when compared with their control (0.62±0.02 mg/dL) and significant decrease (p< 0.05) in DB of females at the lowest dose of 500 mg/kg (0.13±0.04 mg/dL) when compared with their control (0.25±0.03 mg/dL) are indicative of non-adverse effects of the extract on haemoglobin metabolism pathways (Tables 3 and 4), since an increase in tissue or serum bilirubin concentrations occurs as a result of increased breakdown of RBC (haemolysis) or liver damage for example hepatitis or bile duct obstruction (Abdullah, 2011; Hayfaa, 2016).

In this study, there was no significant difference (p> 0.05) between body weights, organ weights, and relative organ weights of male and female Wistar rats at all the doses tested when compared with their respective controls except body weights in female Wistar rats at the highest doses of 1000, 2500 mg/kg and relative organ weights in female Wistar rats at the lowest dose of 500 mg/kg and highest dose of 2500 mg/kg. Therefore, aqueous leaf extract of *Alstonia boonei* at most of the doses tested in this study has no effects on body weights, organ weights, and relative organ weights of male and female Wistar rats.

In this study, aqueous leaf extract of *A. boonei* caused significant and insignificant increase and decrease in ALT and AST of male and female Wistar rats. There were also gross histopathological changes.microscopically; there was mild and extensive necrosis of liver cells. This is in agreement with research work done by (Silva *et al*., 2011; Barnabé *et al*., 2014) that *A. boonei* extract caused mild haptic liver injuries.

Alstonia boonei aqueous leaf extract in 28 days sub-acute toxicity study in male albino Wistar rats revealed that there was no gross histopathological changes in male liver cells at the dose of (500 mg/kg) which showed central vein with normal hepatocytes. The presence of necrotic liver cells was observed in male at a dose of (1000 mg/kg). While extensive necrosis of liver cells was observed in male Wistar rat which received extract dose of (2500 mg/kg) when compared with control (Fig. 2). This means that the extract at the concentrations of (1000 and 2500 mg/kg) has toxic effects on these groups of male and female animals.

Alstonia boonei aqueous leaf extract in a 28 days sub-acute toxicity study in female albino Wistar rats revealed that female liver controlshowed portal triad with normal hepatocytes. Mild necrosis of liver cells was observed in female which received the extract at a dose of (500 mg/kg). Necrosis of liver cells was observed in female animals which received a dose of (1000 mg/kg) while 2500 mg/kg showed the presence of liver with necrotic liver cellswhen compared with the control (Fig. 3). This means that the extract at all the concentrations has toxic effects on these groups of male and female animals.

The LD_{50} values of aqueous extract of *A. boonei* leaves was above 10000 mg/kg suggesting that the plant extract is experimentally safe, thus justifying its use in traditional medicine. Although the lowest and highest doses of the extract caused significant perturbations in males ALT at all the doses tested and females TB at the highest doses of 1000 mg/kg and 2500 mg/kg as observed in few of the serum assayed.

Conclusion

While a single dose and oral intake of *Alstonia boonei* aqueous extract caused no toxicity up to a dose of 10000 mg/kg b. wt. in acute toxicity test, toxic effects manifested in the long term treatments at all the doses in male and female animals at the highest doses of (1000 and 2500 mg/kg). Therefore, great caution should be exercised while utilizing *Alstonia boonei* leaves for its numerous health benefits as prolonged exposure to lower and higher doses may cause observable alterations in histopathological and biochemical parameters. Thus, prolonged use of low and higher doses of *A. boonei* aqueous leaves extract orally should be discouraged.

Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

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