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Acute and Sub-chronic Toxicity Studies of Aqueous, Methanol and Chloroform extracts of *Alstonia boonei* Stem Bark on albino mice

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Abstract: In a preliminary research, the authors reported that solvents extracts of *Alstonia boonei* (Egbu) possess strong antimalarial activity against NK-65 Chloroquine sensitive *Plasmodium berghei* infected mice with aqueous extract having the highest decrease in mean percentage parasitaemia. This research is therefore aimed at evaluating the acute and sub-chronic toxicity of solvents extracts of the plant on kidney. The ($LD_{50,}$ oral) was conducted in two phases. In the first phase, oral doses of 10, 100 and 1000 mg/kg body weight of the extracts were administered and mice observed for sign of toxicity and death. In the second phase, 1600, 2900 and 5000 mg/kg body weight of the extracts were administered and signs accompanying toxicity and possible death of animals were also monitored. Sub-chronic toxicity studies were carried out to assess the effect of the solvents extracts on kidney function indices after 28 days of oral administration of the extracts at 150, 250 and 500 mg/kg body weight. The (LD_{50}, oral) of all extracts was found to be greater than 5000mg/kg which is practically non- toxic according to standard scale of toxicity. The result of sub-chronic toxicity study showed a significant increase ($P<0.05$) in mean levels of creatinine, urea and serum electrolytes in all extracts administered groups in a dose dependent pattern compared to normal control. However, histopathological analysis of the kidney tissues shows no pathological changes between test groups and normal control. Thus, solvents extracts of *Alstonia boonei* modifies biochemical parameters (Crea, Urea and Electrolytes) but within the context of duration of this research, no significant pathology was observed in kidney tissues. *Alstonia boonei* stem bark should be used with caution.

Keywords: Acute toxicity, sub-chronic toxicity, kidney Function Indices and *Alstonia boonei*

INTRODUCTION

Traditional medicine (indigenous medicine, complementary medicine or folk medicine) describes medical knowledge systems which developed over centuries with various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as the health practices, approaches, knowledge and belief incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being. Traditional medicines include practices such as herbal medicine which is an aspect of indigenous medicines, the use of gathered plant parts to make teas, poultices or powders that purportedly effect cures. Herb is a plant or plant part used for its scent, flavor or therapeutic properties [1]. According to World Health Organization (WHO) reports, these medicinal plants, which are often referred to as traditional medicines, need to be evaluated, given due recognition and developed so as to improve their efficacy, safety, availability and wider application at low cost.

Alstonia boonei is one of the species of Alstonia plant. It is a very large, deciduous, tropical-forest tree belonging to the dogbane family, Apocynaceae. It is native to tropical West Africa, with a range extending into Ethiopia and Tanzania. Its common name in the English timber trade is cheese wood, pattern wood or stool wood). In Nigeria, it is locally called "Egbu/Egbu Ora" in Igbo language and "Ahun" in Yoruba language. The tree also finds many uses in folk medicine. Various pharmacological studies

have been carried out on this plant, it has been reported to possess antipyretic, analgesic and anti-inflammatory properties [2], anti-malarial [3], Immuno-stimulant property [4], antipsychotic and anxiolytic effect [5]. The stem bark of *Alstonia boonei* is locally used in South Eastern Nigeria for the treatment of malaria in the region. This research was therefore carried out to evaluate the acute and sub-chronic toxicity of solvents extracts of the plant on kidney.

Lethal toxicity (acute toxicity) is the ability of chemical to cause ill effect "relatively soon" after one oral administration or a 4- hour exposure of chemical in air. "Relative soon" is usually defined as a period of minutes, hours (24), or days (up to about 2 weeks) but rarely longer [6]. "LD" stands for "Lethal Dose" LD_{50} is the amount of materials given all at once, which causes the death of 50% of a group of test animals. The LD_{50} is one way to measure the short- term poisoning potential (acute toxicity) [7]. Toxicologist can use many kinds of animals but most often testing is done with mice and mice. It is usually expressed as the amount of chemical administered (e.g milligrams) per 100grams (for smaller animals) or per kilogram (for bigger subjects) of the body weight of the LD_{50} can be found for any route of entry or administration, but dermal and oral administration methods are the most common. The LD_{50} value obtained at the end of the experiment is identified as LD_{50} (oral), LD_{50} (skin) e.t.c as appropriate. The results of oral studies are important for drugs, food and accidental domestic poisonings. In general, the smaller the LD_{50} value the more toxic the chemical. Also, the larger the LD_{50} value the lower the toxicity [7]. LD_{50} value can be compared to other values using a toxicity scale. Confusion sometimes occurs because several different toxicity scales are in use. The two most common scales used are the "Hodge and sterner scale" and the "Gosselin, smith and Hodge scale [8]."

MATERIALS AND METHODS

Preparation of Plant Extract

The stem bark of Egbu plant (*Alstonia boonei*) was collected from Okpuje community, Northwest of Nsukka LGA of Enugu State (co-ordinates $6^{0}30^{1}N7^{0}30^{1}E$). The plant was identified and authenticated at the Herbarium of Plant Biology Department; Bayero University, Kano and was given a voucher number of (BUK/HAN/0258). The stem bark was washed, shade dried and ground to powder. 200g of sample was measured and transferred into each of the flasks containing $1000cm^3$ (1 liter) of methanol, chloroform and distilled water. The contents of the flasks were shaken and top covered with aluminum foil and kept for 72 hours (3 days). The herb-water mixtures were shaken daily to ensure proper extraction [9]. After 72 hours the extracts were filtered. The filtrates were concentrated under vacuum using a vacuum rotary evaporator, then measured and stored in screw capped vials under room temperature at the Postgraduate laboratory of Department of Biochemistry, Bayero University Kano. The volume of extracts to be administered was calculated according to dose and weight of the experimental animal using the relation below [10].

$$\text{Volume of extract (ml) to be administered} = \frac{\text{Weight of animal (kg) x dose (mg/kg)}}{\text{Concentration of extract (mg/ml)}}$$

Experimental animals

Healthy albino mice (weighing 16-20g) were purchased from the Animal house of the Department of Pharmaceutical Science, University of Jos and then kept under standard laboratory condition at the Department of Biological Science, Bayero University Kano for about two weeks for proper acclimatization. All authors hereby declare that Principle of laboratory animal care [11] and ethical guidelines for investigation of experimental pain in conscious animals [12] were observed during experimentation

Acute toxicity studies

LD_{50} of the aqueous extract was determined using the method of [13]. In the initial phase, nine mice were divided into three (3) groups of 3 mice each. The mice were administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg of aqueous extract of Khaya senegalensis orally. The mice were monitored for general behaviour and mortality for 24 hours.

In second phase, three mice were used and are grouped into three groups of one rat each, they were orally administered with 1600, 2900 and 5000 mg/kg body weight respectively. They were observed for the signs of toxicity which include: paw licking, salvation, rubbing of nose on floor, change in body weight and death within 24 hours.

Sub chronic toxicity

A total of 60 mice were randomly divided into 10 groups of 6 mice per cage. Group I serves as negative control, Groups II-IV animals were administered with aqueous extract at a dose of 150, 250 and 500mgkg-1 per day for four weeks, Groups V-VII animals were administered with methanol extract at a dose of 150, 250 and 500mgkg-1 per day for four weeks, while Groups VIII-X animals were administered with chloroform extract at a dose of 150, 250 and 500mgkg-1 per day for four weeks. On the 29th day, all the mice were euthanized and blood sample was collected in a dried centrifuge tube for biochemical analysis the carcasses were dissected and kidney tissues were collected for histological studies. The biopsies of kidney of the mice used in the research were fixed with 10% neutral suffer formalin, dehydrated with ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax. The microtome sections were stained with haematoxylin and eosin staining technique.

Histopathology examination was carried out by the method of Auwioro [14].

Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test after investigating the data for normality using Shapiro-Wilk test and for variances homogeneity to be sure that the data are normally distributed and variances would be homogenous using GraphPad Instat3 Software version 3.05 Differences of $P < 0.05$ were considered to be significant [15].

RESULTS AND DISCUSSIONS

Acute Toxicity Result

The LD_{50} result shown in Table 1 was determined orally in mice in two phases. In the first phase, no death was recorded which necessitated the second phase. Again there was neither mortality nor any sign of toxicity in the behavior of the animals in the second phase.

Table-1: Acute toxicity effect of aqueous, methanol and chloroform root extracts of *Curcuma longa L.* when administered orally to albino mice

Experiment	Dose(mg/kg)	Number of dead mice after 24 hours		
		Aqueous extract	Methanol extract	Chloroform extract
Phase 1	10	0/3	0/3	0/3
	100	0/3	0/3	0/3
	1000	0/3	0/3	0/3
Phase 2	1600	0/1	0/1	0/1
	2900	0/1	0/1	0/1
	5000	0/1	0/1	0/1

Sub-chronic Toxicity Result

The result of sub-chronic toxicity of the extracts on kidney function indices was shown in table 2. A significant increase ($p<0.05$) in serum creatinine and urea levels were observed in dose dependent pattern in all extracts administered groups compared to normal control. There was no significant difference ($p<0.05$) between values of electrolytes levels in extracts administered groups normal control group.

Table-2: kidney function indices of mice administered with solvents extracts *Alstonia boonei* after four weeks of extracts Administration

Groups	CREA(mg/dl)	UREA(mEq/l)	Na^+(mEq/L)	K^+(mEq/l)	Cl^-(mEq/L)	$HCO3^-$ (mmol/L)
Normal Control	0.008±0.001^{abcdefghi}	11.06±0.01^{abcdefg}	42.23±0.03	10.65±0.03	100.65±0.24	41.96±0.02
AQ 150mg/kg	0.038±0.01^{a}	10.36±0.01	46.65±0.03	8.24±0.03	99.73±0.03	40.20±0.01
AQ 250mg/kg	0.077±0.004^{b}	19.46±0.03^{a}	40.75±0.02	8.66±0.16	102.71±0.02	41.47±0.02
AQ 500mg/kg	0.033±0.001^{c}	21.40±0.01^{b}	47.18±0.01	9.37±0.02	100.50±0.04	38.15±0.04
MET 150mg/kg	0.024±0.005^{d}	12.87±0.08	44.11±0.13	9.50±0.01	101.05±0.12	39.23±0.04
MET 250mg/kg	0.033±0.001^{e}	21.06±0.01^{c}	43.55±0.03	9.95±0.03	106.42±0.02	43.15±0.01
MET 500mg/kg	0.052±0.001^{f}	24.16±0.02^{d}	47.91±0.02	8.32±0.04	100.45±0.04	40.41±0.01
CHL 150mg/kg	0.026±0.001^{g}	16.62±0.02^{e}	49.21±0.01	9.27±0.03	112.13±0.02	46.68±0.01
CHL 250mg/kg	0.055±0.002^{h}	20.91±0.01^{f}	44.15±0.01	10.37±0.01	106.92±0.04	39.12±0.01
CHL 500mg/kg	0.075±0.002^{i}	22.95±0.02^{g}	42.78±0.08	10.91±0.03	104.94±0.03	42.61±0.01

Values are presented as Mean ± standard deviation, (n = 6). Value with the same superscripts in a column are significantly different compared to each other ($P<0.05$). Key: CREA = Creatinine; Na^+= Sodium ion; K^+ = Potassium ion; Cl^- = Chloride ion; HCO_3^- = Bicarbonate ion. AQ = Aqueous extract; MET= Methanol extract; CHL= Chloroform extract.

Histopathological examination of kidney tissues

Plate 1 shows a photomicrograph of section of kidney of control mice showing no significant pathology. Plate 2, 3 and 4 shows photomicrograph of section of kidney administered with 150mg/kg, 250mg/kg and 500mg/kg of aqueous extract, Plate 5, 6 and 7 shows photomicrograph of section of kidney administered with 150mg/kg, 250mg/kg and 500mg/kg of methanol extract while Plate 8, 9 and 10 shows photomicrograph of section of kidney administered with 150mg/kg, 250mg/kg and 500mg/kg of chloroform extract.

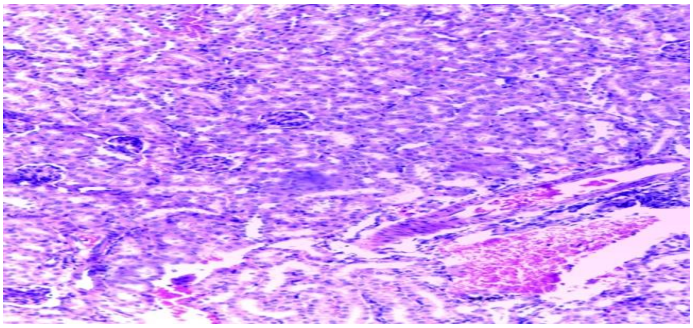

Plate 1: section of kidney of control mice showing no significant pathology with the cortex containing glomeruli and the medulla containing renal tubules (H and E, mag.×100).

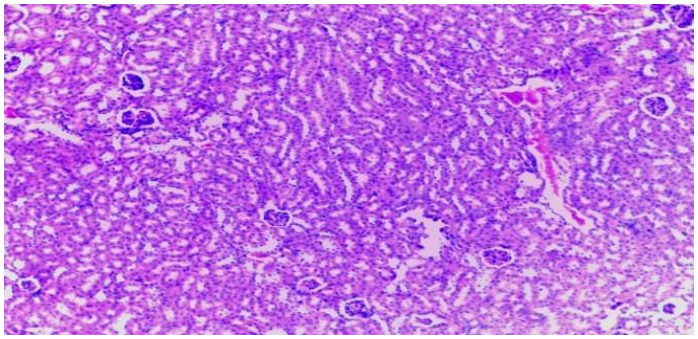

Plate 2: section of kidney of mice administered with 150mg/kg body weight of aqueous extract showing no significant pathology (H and E, mag.×100).

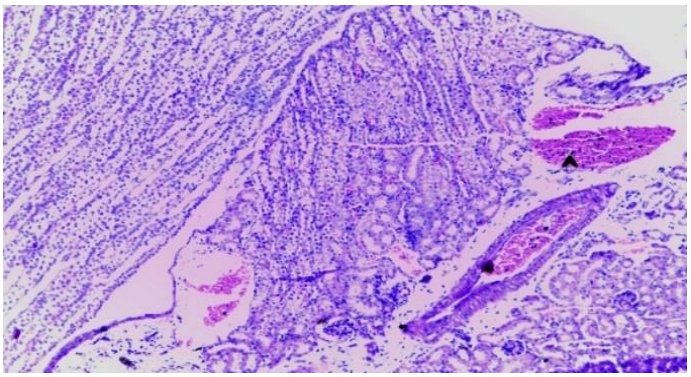

Plate 3: section of kidney of mice administered with 250mg/kg body weight of aqueous extract showing no significant pathology (H and E, mag.×100).

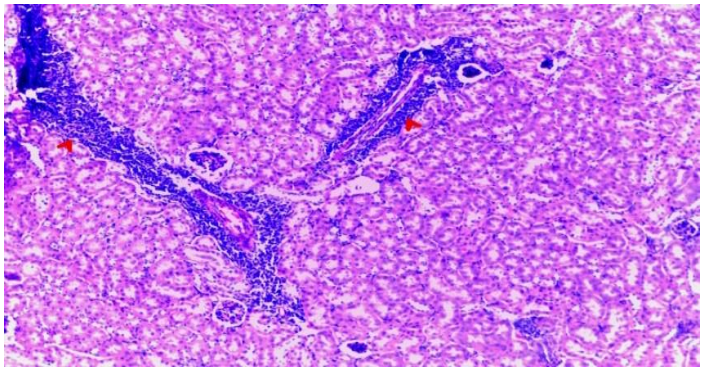

Plate 4: section of kidney of mice administered with 500mg/kg body weight of aqueous extract showing no significant pathology (H and E, mag.×100).

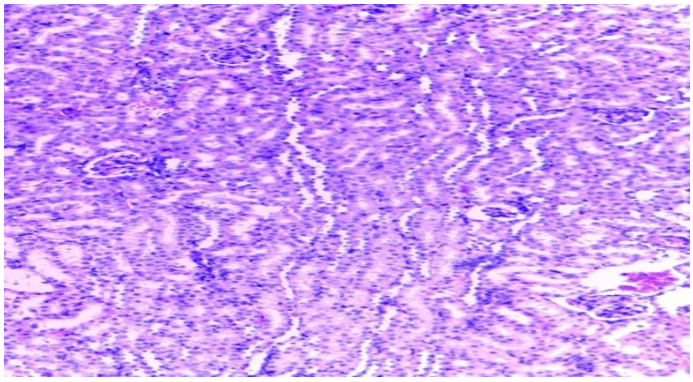

Plate 5: section of kidney of mice administered with 150mg/kg body weight of methanol extract showing no significant pathology (H and E, mag.×100).

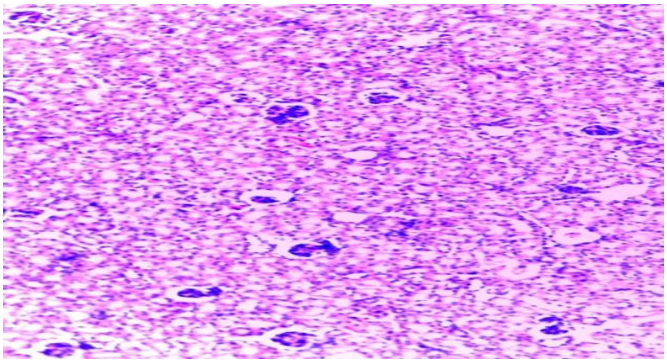

Plate 6: section of kidney of mice administered with 250mg/kg body weight of methanol extract showing no significant pathology (H and E, mag.×100).

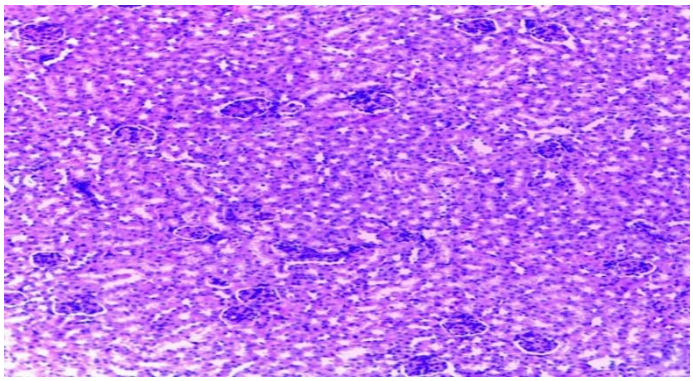

Plate 7: section of kidney of mice administered with 500mg/kg body weight of methanol extract showing no significant pathology (H and E, mag.×100).

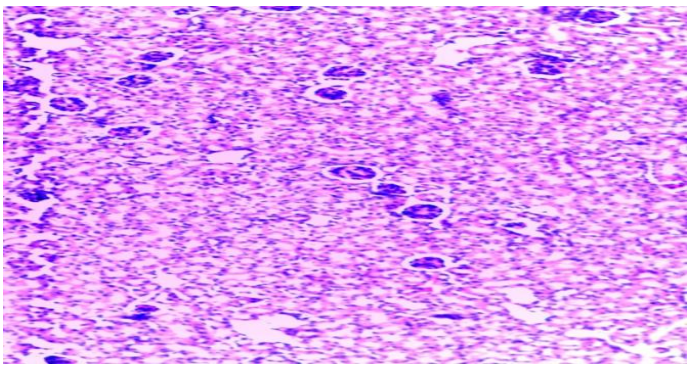

Plate 8: section of kidney of mice administered with 150mg/kg body weight of chloroform extract showing no significant pathology (H and E, mag.×100).

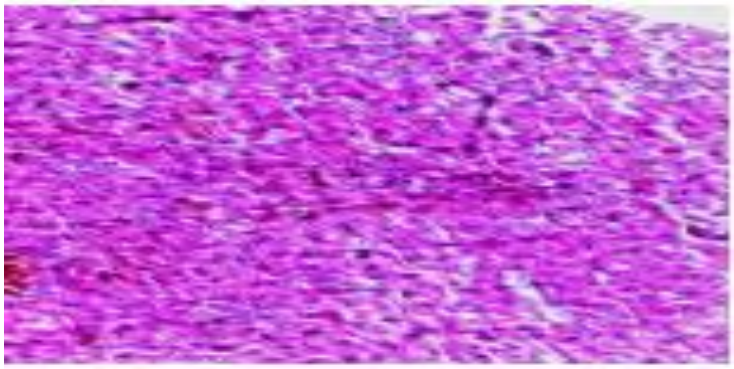

Plate 9: section of kidney of mice administered with 250mg/kg body weight of chloroform extract showing no significant pathology (H and E, mag.×100).

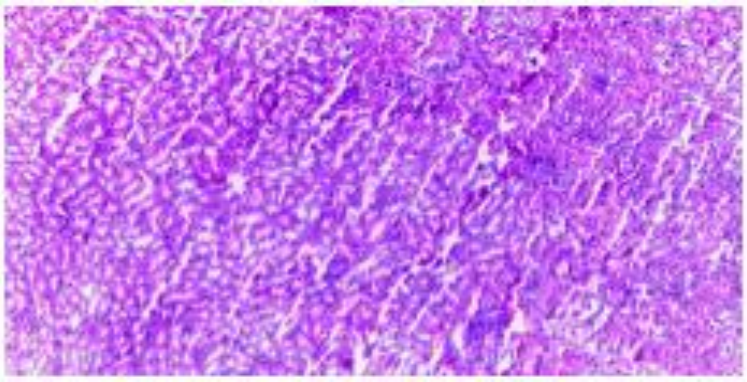

Plate 10: section of kidney of mice administered with 500mg/kg body weight of chloroform extract showing no significant pathology (H and E, mag.×100).

DISCUSSION

The study established the acute toxicity study (LD_{50}) of the aqueous, methanol and chloroform stem bark extracts to be greater than 5000mg/kg interpreted as practically non-toxic according to standard scale of classification [16]. These findings were supported by the absence of negative behavioral changes such as restlessness, excitement, respiratory distress, convulsion and/or coma. The absence of death in any phase suggests that extracts of *Alstonia boonei* are practically non- toxic.

Kidney plays important role in homeostasis i.e maintaining fairly constant internal environment through its osmoregulatory function such as regulation of electrolytes, maintenance of acid-base balance and regulation of blood pressure through maintenance of salt - water balance in blood [17]. It receives about 1200 ml of blood per minute [18] containing a lot of chemical compounds. Therefore, damage to the kidneys can be determined by measuring the level of urea, electrolyte and creatinine in blood as an indicator of kidney damage. Urea is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney. 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 [19], it is excreted exclusively through the kidney [20]. 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, also Electrolytes are substances that become ions in solution and acquire the capacity to conduct electricity. The balance of the electrolytes in human bodies is essential for normal function of cells and organs.

Elevated creatinine level may signify impaired kidney function or kidney disease. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidney. Elevated creatinine level could also be caused by dehydration or inadequate intake of water or intake of drug. Kidney is the main organ to filter the creatinine which is the metabolic waste of muscle. Blood urea nitrogen (BUN) is a normal waste product in the blood from body metabolism. It is normally removed from the blood by kidneys but when kidney function slows down, the BUN level rises. In this research, there was significant increase in a dose dependent pattern in creatinine and urea level in groups administered with aqueous, methanol and chloroform extracts compared with normal control group. On the other hand, no significant changes were observed in mean serum electrolytes levels between the extracts

administered groups and the normal control. The result therefore shows that the therapeutic use of extracts of *Alstonia boonei* for malaria treatment may possess detrimental effect to the kidney which is both dose and time dependent.

CONCLUSION

It may be concluded that solvents extracts of *Alstonia boonei* is practically non-toxic with oral LD_{50} greater than 5000mg/kg body weight. However, the extracts can be toxic to the kidney on long time exposure even at lower concentration, it should be used with great caution. Further studies are recommended for the toxic effect of the extracts on other organs.

REFERENCES

1. WHO (2014). Legal status of Traditional Medicines and Complementary/Alternative Medicine. A worldwide review. WHO publishing1: 23-26
2. Olajide, A. O., Awe, S. O., Makinde, J. M., Ekhelar, A. I., Olusola, A. Morebise, O. (2000). Studies on the Anti-inflammatory, Antipyretic and Analgesic Properties of *Alstonia boonei* Stem Bark. *Journal of Ethnopharmacology* 71: 179 – 186.
3. Ojewole, J. A. O. (1984). Studies on Pharmacology of Echitamine, An Alkaloid from the Stem Bark of *Alstonia boonei* L. (*Apocynaceae). International Journal of Crude Drug Research* 22: 121 - 143.
4. Taiwo, O. B., Kroes, B. H., Beukelman, C. J., Horsten, S. T. A. J., Makinde, J. M. (1998). Activity of Stem Bark of *Alstonia boonei* De wild on Human Complement and Polymorph Nuclear Leucocytes. *JE* 17: 13-15.
5. Elisabetsky, E., Costa-Campos, L. (2006). The Alkaloid Alstonine: A Review of its Pharmacological Properties. *Evidence-Based Complemetary and Alternative medicine* 3: 39-48.
6. Adumanya, O.C.U., Uwakwe, A.A. and Essien, E.B. (2014). Acute toxicity (LD_{50}) study of Methanol extract of *Salaciasene galensis* (dc) leaf on albino mice. *Int. J. Curr. Res. Chem. Pharma. Sci.* 1(4):20-23.
7. Gadanya, A.M., Sule, M.S. and Atiku, M.K. (2011). Acute toxicity study of "gadagi" tea on rats. *Bayero Journal of Pure and Applied Sciences.* 4(2):147-149.
8. Senin, R. (2006). Acute toxicity study; Available:http:/www.ccohs.ca/oshanswers/ chemicasl/Ld.html
9. Abosi, A.O. and Raseroke, B.H. (2003). *In vivo* antimalarial activity of *vernonia amygdalina. Journal of Biomedical. Science.* 60: 89-91.
10. Muhammad, I. U., Alhassan, A. J., Sule, M. S., Idi, A., Mohammed, A., El- ta'alu A. B., Dangambo M. A. and Abdulmumin, Y. (2016). Anti-Hyperglycemic Activity of Solvents Extract of *Khaya senegalensis* Stem Bark in Alloxan Induced Diabetic Rats. *Journal of Advances in Biology & Biotechnology*. 6(2): 1-8.
11. NIH. (1996) Guidelines for the care and use of laboratory animals. National Academic Press, NIH Publication. 85: 23.
12. Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 16(2), 109-110.
13. Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology.* 53: 275-287.
14. Auwioro, O.G. (2010). Histochemistry and Tissue Pathology: Principles and Techniques 2nd Edition. University Press Delta State University, Abraka Nigeria *ISBN, 978356279:* 561- 568.
15. GraphPad Instat3 Software (2000).Available: www.graphpad.com. Retrieve on 23rd February 2017.
16. Hodge, A. and Sterner, B. (2005). Toxicity Classes. In Canadian Centre for Occupational Health and Safety. http://.www.ccohs.ca/oshanswers/chemicals Id_{50}.htm 4-8.
17. Tortora, G.J. and Derrickson, B. (2006). *Principle of anatomy and physiology*. 11th ed. United States of America: John Wiley and sons. 918-921.
18. Moore, E.M., Bellomo, R. and Nichol, A.D. (2012).The meaning of acute kidney injury and its relevance to intensive care and anaesthesia. *Anaesthesia and intensive care* 40(6): 929–48
19. Liao, M.T., Sung, C.C., Hung, K.C., Wu, C.C., Lo, L. and Lu K.C. (2012). Insulin resistance in patients with chronic kidney disease. *Journal of Biomedicine and Biotechnology* 1–5.
20. Weber D.K., Danielson K., Wright S. and Foley J.E. (2002). Hematology and serum biochemistry values of dusky-footed wood rat (neotoma fuscipes) *J Wildlife Dis*. 38:576-582.