World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

ANIMAL MODEL ASSESSMENT OF THE NUTRACEUTICAL VALUE OF AQEUOUS EXTRACT OF *AZADIRACHTA INDICA* STEM BARK

Ihimire I. G.*, Alaiya H. T. and Onobun D. O.

Ambrose Alli University, Faculty of Life Sciences, Department of Biochemistry, P.M.B 14, Ekpoma, Edo State.

Corresponding Author: Ihimire I. G.
Ambrose Alli University, Faculty of Life Sciences, Department of Biochemistry, P.M.B 14, Ekpoma, Edo State.

Article Received on 23/11/2020 **Article Revised on 13/12/2020** **Article Accepted on 03/01/2021**

ABSTRACT

This study investigated the effect of administration of acute and sub-lethal dose of *Azadirachta indica* in animal model. Eighteen (18) mature Wistar albino rats were used to investigate lethal dose LD_{50} and establish safe dose (SD) in 24 hours. Another 18, divided into 6 Groups A – E were used to investigate the biochemical effect of administration of different doses of the extract. Group A served as control and received only water daily. Group B received 1 mL of extract 547.72 mg/kg body weight being the safe dose established. Group C – F respectively were given 492.95 mg, 602.49 mg, 657.26 mg or 711.89 mg/kg body weight in 1 mL for 14 days. Water and feed were provided ad libitum. On the 15th day, the animals were humanely sacrificed, blood collected by cardiac puncture and some organs/tissues were excised. Standard methods were used to assess in triplicate Some biochemical parameters. Mean values of these analytes were calculated and compared with Turkey-Kramer multiple comparison test. Results were presented as mean ± standard error of mean. Administration of the dose recorded comparable final weight as control, 184.87 ± 1.97 g and also liver, heart, spleen and kidney weight too. Group administered the safe dose i.e. Group B recorded evidence of liver protection effect with lower AST value compared to control. This study provided rational biochemical evidences for adequate usage of the extract.

KEYWORD: Safe dose, Acute, Sub-lethal, Rational and Biochemical.

INTRODUCTION

A nutraceutical or bioceutical is a pharmaceutical alternative.[1] In the United States, nutraceutical are largely unregulated as they exist in the same category as dietary supplements and food alternatives by FAD, under the authority of Federal Food, Drug and Cosmetics Act.[2] Depending on the jurisdiction, products may claim to prevent chronic diseases, improve health, delay the aging process, increase the expectancy or support the structure or function of the body.[3]

Many of these new products are being promoted to treat various diseases are mainly of plant origin.[4]

Azadiractha indica (Family Meliaceae) is popularly known as Neem (English) or Dongoyaro (Yoruba, Western Nigeria) in Nigeria.[5] The plant is perhaps one of the most studied and widely used medicinal plant of all ages. Biological and pharmacological activities are attributed to extracts and products like oil from the different parts of the plants and are as diverse as antiplasmodial, antitrypanosomal, antioxidant, anticancer, antibacterial, antiviral, lasvicidial, fungicidal, antiulcer, spermicidal, nematicidal, immunocontraceptive, insecticidal and antifeedant.[6] Specifically, extracts of the bark has been reported to elicit anti-inflammatory effect.[5]

In this study, considering the numerous benefits reported for the extracts, toxicity assessment, LD_{50} and safe dose assessment were investigated in animal model. Subsequently, biochemical effects of acute and sub-lethal doses of administered safe doses were studied. Mbaya and colleagues reported on the dose related clinical signs of toxicity – anorexia, dehydration, malaise, respiratory depression, coma and death and histopathological changes in the trachea, bronchi, bronchioles, lungs and kidney in adult albino rats intraperitoneally administered doses ranging from 100 – 3200 mg/kg body weight of crude ethanolic extract of *A. indica* stem bark.[7] The approach in this study simulated nutraceutical usage of herbal extract, it also initially established safe dose as required for drug usage and investigated some biochemical consequences of acute and sub-lethal dosage of the extract.

MATERIALS AND METHODS

Materials

Centrifuge (80 - 3 Lab Science, England), Spectrophotometer (721 visible spectrophotometer, PEC

medical, USA), Rotary evaporator (RES 2X Lab Science, England), Weighing balance (Shimadzu TX323L, England), Pickstone Oven (Thetford, England), Hammer mill (Gen Creston 14-5805, Germany), Freezer (Haier thermocool Chest Freezer, China). Other devices, instrument and chemicals were all of analytical laboratory grade.

Collection of *Azadiractha indica* Stem Bark

Trees in three different locations, Ewu, Esan West Local Government Area, Edo state Nigeria were authenticated by a taxonomist, Botany Department, Ambrose Alli University, Ekpoma, Nigeria fresh bark were harvested from them.

Extraction

Aqueous extracts of the *Azadiractha indica* stem bark was prepared according to the method described by Ashata *et al.* (2012) with slight modification.[8] Sample was dried at 40 ^{o}C in an air drought oven (Pickstone Oven) at 40 ^{o}C to a constant weight. Subsequently, 40 g was pulverized with a hammer mill to pass through sieve size of 1.0 mm diameter and soaked in 100 ml of distilled deionized water for 72 hours. It was thereafter filtered with Whatman filter paper No1 and concentrated at 45 ^{o}C to half of the filtered extract with a rotary evaporator (RESs 2X Lab Science, England). The concentrate was stored in a freezer and used for investigation either defrosting/cooling to room temperature.

Determination of acute toxicity

The modified Lorke's method was used.[9] It involved two phases. In the first phase nine (9) rats divided into three groups were respectively administered doses of test substance: 10, 100 or 1000 mg/kg body weight. Signs of toxicity or mortality was observed for 24 hours. The second phase involved also nine rats grouped into 3 based on body weight. These were respectively administered different doses of the test extract: 4000, 5000 or 6000 mg/kg body weight. They were observed for signs of toxicity or mortality and LD50 calculated from the formula

$$LD_{50} = \sqrt{D0 \times D100}$$

D_0 = dosage of group that elicited minimum evidence of toxicity

D_{100} = dosage of group that elicited more toxic effect than DO

Safe dose = safe dose was calculated as 1/10 of LD50

Biochemical acute and sub-lethal dose effect study

Eighteen (18) male rats of wistar strains were randomly grouped into six (A – F) of three animals each according to their body weight via

Group	Treatment	Extract concentration	Volume
A	Control	NIL	1 ml H_2O
B	Safe dose	547.72 mg	1 ml solution
C	10 % less than safe dose	492.95 mg	1 ml solution per kg body weight
D	10 % above safe dose	602.49 mg	1 ml solution per kg body weight
E	20 % above safe dose	657.26 mg	1 ml solution per kg body weight
F	30 % above safe dose	711.89 mg	1 ml solution per kg body weight

Animals were housed in animal house, Biochemistry Department Laboratory, Ambrose Alli University, Ekpoma in conditions of diurnal variation of daylight and darkness characteristics of tropical rainforest in the month of May. Water and food were supplied ad-libitum Conditions for management were as accepted for adequate animal care.[10] Administration of the extracts was for 14 days.

Treatment of Experimental Animals

On the 15th day, the animals were weighed, anaesthetized with chloroform and humanely sacrificed by dissection.[11] Blood was collected by cardiac puncture. Different organs collected were weighed and portions of certain parts/organs were excised and homogenized in 0.1M phosphate buffer pH 6.9 e.g muscle from right thigh, kidney, spleen, liver and bone. The respective homogenate were used for biochemical analysis.

Biochemical Analysis

Analytical grade laboratory weighing balance (Shimadzu TX323L, England) was used in weighing rats and excised different portions.

The aspartate aminotransferase (AST) activity in the plasma of liver homogenate of rats were determined by Reitman and Frankel method.[12]

The concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenlyhydrazine was used. The level of alkaline phosphate in tissue homogenate or liver muscle and bone were determined with manufacturer's guide.[13]

Alanine aminotransferase (ALT) activity in the plasma of the experimental rats were assessed following the principle described by Reitman and Frankel.[12] It was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine.

The concentration of Malondialdehyde (MDA), the end product of lipid peroxidation in the respective liver homogenates were examined using method of Varshney and Kale.[14] MDA formed/g protein was computed with a molar extinction coefficient of 0.1564 $M^{-1}cm^{-3}$.[15]

The level of calcium in tissue homogenate of muscle or bone respectively were assessed based on the reaction of calcium ion (Ca^{2+}) with O-cresolpthalein complex in alkaline solution that forms an intense violet coloured complex having maximum absorbance at 578 nm (Agappe Diagnostic, Switzerland).

The level of potassium in serum of respective experimental animals were determined employing the capacity of sodium tetraphenylboron in a specially prepared mixture to produce a colloidal suspension with turbidity proportional to serum potassium concentration in the range of 2 – 7 mEq/L at 500 nm.[16]

Serum/Plasma

Processing blood for sera/plasma procedure reported by Dacie and Lewis was used.[17] Whole blood obtained by cardiac puncture into sterile tubes was left for 2 hours then kept at 4 ^{o}C in refrigerator overnight for clot retraction and centrifuged (Baird and Tatlok, Mark IV England).

For the plasma, blood obtained by cardiac puncture in heparin syringe was centrifuged at 3000 rpm for 5 minutes to obtain clear slightly yellow supernatant fluid.

Statistical Analysis

Data obtained from the study were subjected to a one way analysis of variance (ANOVA) and analyzed with Tukey-Krammer multiple comparison post hoc test using GraphPad Prism Version 7.0 statistical software. Results were recorded as mean ± standard error of mean of triplicate determination.

RESULTS

The result of investigations into the impact of administration of different doses of the extract of *A. indica* in rats are presented in table I, II, III and IV.

The conditions observed with the rats in the acute toxicity study are reported in Table I. There was no observable effect on rats administered 10-1000 mg/kg body weight. Those administered 4000 mg/kg body weight recorded mild discomfort, rubbing of the mouth and nose but recovered after 3 hours. Evidence of toxicity reflected in discomfort irritability and erratic behavior was first noticed in rats administered 5000 mg/kg body weight. Erratic and irritable behavior became more severe on administration of 6000 mg/kg body weight. Their condition persisted longer than 24 hours.

In Table II, the effect of aqueous extracts of *A. indica* on organosomatic indices are represented. The final highest body weight of rats was observed in safe dose group. This value was not significantly ($P > 0.05$) different from those of rats in control group. Rats in group E and F recorded comparable values. The liver weight of safe dose group and those of control group at the end of the study were comparable. Other study groups recorded significantly ($P < 0.05$) lesser weight compared to these. The heart weight of all studied group were not significantly ($P > 0.05$) different. Only the weight of kidney of rats in control and group administered 20% recorded significant ($P < 0.05$) difference. The rats in group D recorded the highest lung weight. Lung weight of control group and those administered safe dose were comparable. Those observed in other studied groups were significantly ($P < 0.05$) less than observed in control or safe dose group.

The result of enzymatic activity in some tissues are presented in Table III. The highest plasma AST was observed in group D. Safe dose group recorded significantly ($P < 0.05$) less than observed in control group. Group E recorded higher values compared to control or safe dose group. AST observed in control group was comparable to values observed respectively in group D and F. The least hepatic AST was observed in control group. The highest plasma ALT was observed in safe dose group. The least was observed in control group. The values observed in control group was not significantly ($P > 0.05$) less than in group C. ALT observed in liver of safe dose group were higher than as observed in control that recorded comparable values as group C. The least ALT value in liver homogenate was observed in group D. The highest ALP in liver homogenate was observed in group D. The least was observed in control group with values as observed in safe dose group being significantly ($P < 0.05$) higher.

In Table IV, ion content in some tissues and enzyme activities are presented. As shown in the table, the highest ALP in bone tissue was observed in control group. Also it can be observed that administration of any dosage of the extract recorded significantly ($P < 0.05$) low values compared to as observed in control group. MDA activity observed in any of the group except those in group C and E recorded values less than observed in group administered safe dose. Group administered safe dose recorded values significantly ($P < 0.05$) less than observed in control. The level of Ca^{2+} observed in muscle tissue of control group compared with values observed in safe dose group. These were less than observed in respective group C. Very high values were observed in respective groups administered other different doses. Control subject group recorded the least Ca^{2+} in bone tissue. The highest Ca^{2+} was observed in bone tissue of group administered safe dose. Other study subjects recorded significantly ($P < 0.05$) lower values than observed in safe dose group which were also significantly ($P < 0.05$) greater than observed in control subjects. Comparable levels of serum K^{+} was observed in

control, group B and group E or group F. Others recorded significantly less values respectively.

Table I: Determination of acute toxicity.

Administered dose mg/kg body weight	Observation
10	No observable effect
100	No observable effect
1000	No observable effect
4000	Mild discomfort and rubbing of the mouth and nose, recovered after 3 hours
5000	Irritable, rubbing of mouth and nose, recovered after 7 hours
6000	Sluggish and erratic/irritable rubbing of mouth and nose. Did not recover in 24 hours.

Table II: Organosomatic indices.

Groups	Final Weight (g)	Liver (g)	Heart (g)	Spleen (g)	Kidney (g)	Gain %
A	184.87 ± 1.969^{a}	6.00 ± 0.28^{a}	0.71 ± 0.06^{a}	0.48 ± 0.03^{a}	0.63 ± 0.06^{a}	1.43 ± 0.05^{a}
B	193.50 ± 9.26^{a}	7.10 ± 0.07^{a}	0.64 ± 0.07^{a}	0.53 ± 0.10^{a}	0.66 ± 0.02^{ab}	1.33 ± 0.06^{a}
C	130.60 ± 0.45^{b}	4.33 ± 0.06^{bcf}	0.57 ± 0.04^{a}	0.59 ± 0.04^{a}	0.83 ± 0.02^{a}	2.23 ± 0.02^{a}
D	150.33 ± 8.42^{bc}	5.06 ± 0.31^{a}	0.54 ± 0.06^{a}	0.48 ± 0.01^{a}	0.76 ± 0.13^{a}	1.20 ± 0.15^{a}
E	179.87 ± 7.07^{a}	5.36 ± 0.41^{adf}	0.74 ± 0.14^{a}	0.53 ± 0.05^{a}	1.03 ± 0.05^{b}	1.20 ± 0.01^{a}
F	167.83 ± 3.46^{ac}	5.60 ± 0.20^{ac}	0.62 ± 0.09^{a}	0.51 ± 0.05^{a}	0.76 ± 0.13^{a}	1.00 ± 0.05^{a}

*Data is presented as Mean ± Standard Error of Mean (SEM). Values in the same columns with different alphabetic superscripts are considered statistically significantly different ($P < 0.05$). subjects in **Group A** served as the control group and was administered 1ml of distilled water for 28 days, **Group B** was administered 547.7mg/g body weight of AEAI, **Group C** was administered 492.95mg/g body weight in AEAI, **Group D** was administered 602.49mg/g body weight of AEAI, **Group E** was administered 657.26mg/g body weight of AEAI, **Group F** was administered 611.89mg/g body weight AEAI. **[AEAI = Aqueous Extracts of Azadirachta indica]**.*

Table III: Enzymatic activities in some tissues.

Groups	Plasma AST (U/L)	Hepatic AST (U/L)	Plasma ALT (U/L)	Hepatic Alt (U/L)	Hepatic ALP (U/L)	Muscle ALP (U/L)
A	72.5 ± 0.00^{a}	12.72 ± 0.77^{a}	6.0 ± 0.00^{a}	13.68 ± 0.83^{a}	130.64 ± 10.37^{c}	556.60 ± 18.67^{a}
B	42.50 ± 0.00^{b}	19.72 ± 0.31^{b}	15.70 ± 0.72^{b}	29.17 ± 1.30^{b}	1104.90 ± 33.78^{a}	529.00 ± 13.55^{a}
C	57.50 ± 4.33^{e}	40.96 ± 0.20^{c}	7.30 ± 1.3^{a}	14.50 ± 0.00^{a}	1322.00 ± 45.38^{b}	605.36 ± 75.93^{a}
D	128.33 ± 3.33^{c}	18.67 ± 0.42^{bd}	10.0 ± 0.00^{ac}	6.00 ± 0.00^{c}	1382.80 ± 24.53^{b}	222.64 ± 39.11^{bc}
E	90.00 ± 2.50^{d}	16.83 ± 0.23^{c}	10.0 ± 0.00^{ac}	14.50 ± 0.00^{a}	741.52 ± 29.57^{d}	129.72 ± 29.77^{c}
F	60.00 ± 2.50^{ae}	19.37 ± 0.31^{bf}	10.76 ± 0.67^{c}	14.50 ± 0.00^{a}	1127.00 ± 48.71^{a}	388.24 ± 15.31^{ab}

*Data is presented as Mean ± Standard Error of Mean (SEM). Values in the same columns with different alphabetic superscripts are considered statistically significantly different ($P < 0.05$). subjects in **Group A** served as the control group and was administered 1ml of distilled water for 28 days, **Group B** was administered 547.7mg/g body weight of AEAI, **Group C** was administered 492.95mg/g body weight in AEAI, **Group D** was administered 602.49mg/g body weight of AEAI, **Group E** was administered 657.26mg/g body weight of AEAI, **Group F** was administered 611.89mg/g body weight AEAI. **[AEAI = Aqueous Extracts of Azadirachta indica]**.*

Table IV: Ion contents and further enzyme activities in some tissues.

Groups	Bone Tissue ALP	Hepatic MDA x 1 mol	Ca^{2+} muscle (mg/dl)	Ca^{2+} Bone (mg/dl)	Serum K
A	2151.00 ± 30.95^{a}	64.00 ± 0.00^{b}	5.83 ± 0.08^{d}	1.55 ± 0.01^{f}	4.09 ± 0.03^{a}
B	1480.30 ± 33.48^{bd}	54.33 ± 1.45^{c}	5.99 ± 0.03^{d}	18.02 ± 0.01^{a}	4.22 ± 0.22^{a}
C	1812.40 ± 50.19^{abd}	75.67 ± 0.88^{a}	9.36 ± 0.02^{c}	3.30 ± 0.01^{c}	3.33 ± 0.07^{b}
D	786.60 ± 12.03^{c}	22.33 ± 1.20^{d}	21.89 ± 0.08^{b}	4.48 ± 0.01^{b}	2.59 ± 0.29^{c}
E	1592.50 ± 164.42^{d}	75.67 ± 0.87^{a}	22.46 ± 0.03^{a}	3.47 ± 0.01^{d}	3.81 ± 0.02^{a}
F	1671.60 ± 142.12^{d}	34.67 ± 0.67^{e}	22.38 ± 0.07^{a}	3.58 ± 0.01^{c}	4.23 ± 0.02^{a}

*Data is presented as Mean ± Standard Error of Mean (SEM). Values in the same columns with different alphabetic superscripts are considered statistically significantly different ($P < 0.05$). subjects in **Group A** served as the control group and was administered 1ml of distilled water for 28 days, **Group B** was administered 547.7mg/g body weight of AEAI, **Group C** was administered 492.95mg/g body weight in AEAI, **Group D** was administered 602.49mg/g body*

weight of AEAI, ***Group E*** *was administered 657.26mg/g body weight of AEAI,* ***Group F*** *was administered 611.89mg/g body weight AEAI.* ***[AEAI = Aqueous Extracts of Azadirachta indica]***

DISCUSSION

The administration of herbal preparations without any standard scientific studies on their safety profile has caused concerns on their toxicity. Acute toxicity test gives clues on the range of dose that could be toxic.[18] It could also be used to estimate the therapeutic index - safe dose of drugs or xenobiotics.[19] The acute toxicity value - LD50 for rats administered aqueous extracts of *Azadirchta indica* was found to be 5477.23 mg/kg body weight. Hence the safe dose for administration is 547.72 mg/kg body weight.

The LD_{50} determined in this study is greater than reported on toxic manifestation on intravenous administration of aqueous leaf extract.[20] Kango reported toxicity in mice at 2000 - 5000 mg/kg in mice administered extract of *Azadirchta indica* stem bark.[2] This agrees with this study as toxicity was initially evident on administration of 5000 mg/kg body. Schorderet reported that LD50 values greater than 5000 mg/kg body weight are classified as substances with low toxicity.[22] Lorke considered, 1 mg/kg body weight as highly toxic; 10 mg/kg body as moderately toxic and 1000 mg/kg body weight as slightly toxic.[23] Hence, the aqueous extract of *Azadirchta indica* can be considered as a substance with low toxicity. According to Corbett and colleagues, the neem stem bark extract is moderately toxic.[24]

As shown in organosomatic indices, administration of safe dose recorded higher weight comparable to control group. Whereas, administration of other doses respectively recorded significantly ($P < 0.05$) lower weight. This type of change has been implicated in the normal functioning of the body.[25,26]

Relative organ weight do serve as an indicator of physiological and pathological status in man and animals.[27] Toxic substances induces abnormal metabolic reactions that affect primary organs such as heart, liver, spleen and lungs and alteration in their weight are signs of impairment in their normal functioning.[28] As shown in the organosomatic indices table, atrophy of the liver and spleen occurred with administration of safe dose in test rats. This was also observed in kidney index. Lloyd reported atrophy in liver, adrenal gland and spleen weight in a similar study.[29] No significant reduction in heart weight was observed in most test groups in this study. Reduction condition has been reported in toxicity studies in which dogs and rats were treated with high doses of angiotensin converting enzyme (ACE) inhibitor.[26] The condition observed in this study did not implicate deleterious effect on heart with administration of the extract at safe dose.

In Table III, result of enzyme study is presented. AST in safe dose group was significantly lower than observed in control group. Reduction in activity of AST implicates inhibition or inactivation of the enzyme.[30] It is also associated with inhibition of the cofactor, vitamin B6 following loss of total protein resulting from gastrointestinal injury or reduction in hepatic synthesis when there is reduced nutrition.[31] Groups administered dosage above the safe dose recorded high AST activity. Elevation of AST in plasma is usually associated with myocardial infarction, acute liver cell damage, viral hepatitis, carbon tetrachloride poisoning and damages to muscle tissue.[32,33] A low concentration of *A. indica* extract has been reported to possess hepatoprotective ability as observed with administration of safe dose.[34,35] Generally, the level of AST in liver homogenate of rats administered the respective doses were higher than observed in control. This can be ascribed to increasing *de novo* synthesis of the enzyme molecule or adaptation by their liver to the assault from the plant extract that led to higher activity.[36] Similar observation has been reported with administration of extract of *Morinda lucida* stem bark.[37] AST as a biomarker of hepatic integrity is to a certain extent a measure of hepatocellular damage that could have occurred in the adaptation process.[38]

The ALT values observed in plasma or liver homogenate recorded similar pattern with safe dose group recording high values compared to the control group. Studies have shown that increase in serum ALT level could result in hepatotoxic problems as liver damage.[39] The implication is that plant extract could contain some cytotoxic phytochemicals that could have led to damage and leakage of ALT. This is not supported by the non-quantitative ALT values observed in rats administered different higher doses. It is most likely to be due to part of the adjustment induced by that dosage in the biochemical regulation of intracellular amino acid pool.[40] Higher values of ALT in liver homogenate or plasma was observed in groups E and F. This confirms those doses as respectively being toxic as an increase in enzyme activities results in hepatotoxic problem as liver damage.[39]

In Table IV, the further effect on other enzymes and ion content of some tissue are reported. As shown, administration of the respective doses of the extract recorded decrease in alkaline phosphatase activity in bone, this will have adverse effect on metabolic process involving synthesis of nuclear proteins, nucleic acids and phospholipids as well as cleavage of phosphate esters.[41] Decrease in ALP in bone has been associated with disruption in the ordered lipid bilayer.of membrane structure detectable with increase in quantity of ALP in the blood.[41] This effect is most severe in group D. As shown in the table administration of safe dosage recorded significantly ($P < 0.05$) low lipid peroxidation compared to control considering MDA values. The effect did not seem to be dose dependent. This decrease can

offer protection against radical and reduce lipid peroxidation.

The level of Ca^{2+} in muscle of control rats compared with those administered the other different doses. This trend has been reported in earlier study in serum of rats administered different doses of extract of *A. indica* stem bark in male wistar rats.[42] The level of Ca^{2+} shown in the table confirm this trend i.e the increase in tissue Ca^{2+} with administration of the extract even the safe dose that recorded the highest level.

Calcium is an important ion present in the body found mainly in the bones. At normal level, it helps in enzyme activation, muscle contraction, coagulation of blood, regulation of some hormonal secretion and cell membrane permeability.[43] Increased Ca^{2+} level as observed is associated with hyperthyrodism, malignant tumor, acute osteoporosis and adrenal insufficiency.[13] These adverse effects can manifest significant alterations in the levels of biomolecules such as enzymes, metabolic products, normal functioning and histomorphology of organs.[44] These need to be investigated to promote nutraceutical usage of product.

The result of the administration of the extract only recorded adverse effects on K^+ on administration of 10% below safe dose or 10% above safe dose. Comparable K^+ levels were observed on administration of other doses respectively. This did not agree with that reported by Ahmad and colleagues on administration of higher dosage of aqueous leave extract of *Vitex doniana*.[45] Clinically, electrolytes such as K^+ is among the parameters that are useful in determination of kidney function.[46] The elevation or depletion of the level of the ion may be an indicator for a kidney problem in response to which medical checkup for kidney function is required.[47] Administration of the safe dose did not implicate kidney dysfunction. Low value observed on administration of 10% below safe dose or 10% above safe dose did. The value observed in group C though low was comparable to those of group A or group B respectively.

CONCLUSION

This study presents evidence to support usage of *A. indica* stem bark extract at safe dose (SD) at sub-lethal or acutely toxic doses ALP index suggest that the extract could lead to disruption in the ordered lipid-bilayer of membrane, adversely affect nuclear protein synthesis and cleavage of phosphate esters. When adequately administered as in group C i.e the safe dose, kidney function is not compromised, body weight gain is comparable to control, lower lipid peroxidation is reflected in hepatic MDA index and liver protection effect is reflected in plasma AST value that is lower compared to as in control subjects.

REFERENCES

1. Sarris J, Murphy J, Mischoulou D, Papakostas GI, Fava M, Berk M, Ng CH. Adjunctive nutraceuticals for depression. A systematic review and meta-analysis. *American Journal of Psychiatry*, 2016; 173(6): 575-587.
2. Banach M, Patt AM, Giglio RV, Cicero AFG, Atanasou AG, Bajraktari G, Bruckert E, Descamps O, Djuric DM, Ezhou M, Fras Z, von Haehling S, Katsiki N, Langlois M, Latkovskis G, Mancini GBJ, Mikhailidis DP, Mitchenko O, Moriarty PM, Munter P, Nikolic D, Panagiotakos DB, Paragh G, Paulweber B, Pella D, Pitsavos C, Reiner Z, Rosamo GMC, Rosenson RS, Rysz J, Sahebkar A, Serban MC, Vinereanu D, Vrablik M, Watts GF, Wong ND, Rizzo M. The role of nutraceutical in stating intolerant patients. *Journal of American College of Cardiology*, 2018; 72(1): 96-118.
3. Hasler CM. Regulation of functional foods nutraceuticals: A global perspective. IFT Press and Blackwell Publishing, 2005; 55: 1177-1182.
4. Abayomi S, Eyitope O, Adedeji, O. The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *African Journal of Traditional Complementary and Alternative Medicine*, 2013; 10(5): 210–229.
5. Isaac AB, George IN, Oladimeji JA, James DH. A bioactive flavonoid from *Pavetta crassipes.* K. Schum. *Organic and Medicinal Chemistry Letters*, 2011; 1: 14.
6. Devmurari VP, Jivani NP. Hepatoprotective activity f methanolic and aqueous extracts of *Azadirachta indica* leaves. *International Journal of PharmTech Research*, 2010; 2: 1037-1040.
7. Mbaya AW, Ibrahim UI, Thankgod, O, Ladi, S. Toxicity and potential anti-trypanosomal activity of ethanolic extract of *Azadirachta indica* (Maliacae) stem bark: An in vivo and in vitro approach using *Trypanosoma brucei*. *Journal of Ethnophamarcology*, 2010; 128: 495–500.
8. Ashafa AOT, Orekoya LO and Yakubu MT. Toxicity profile of ethanolic extracts of *Azadirachta indica* stem bark in male wistar rats. *Asian Pacific Journal of Tropical Biomedicine*, 2012; 2: 811-817.
9. Chinedu E, Aromeh D, Ameh F.S. A new method for determining acute toxicity in animal models. *Toxicology International*, 2013; 20(3): 224-226.
10. National Research Council (NRC). Guide for the care and use of laboratory animals (8th Edn). The National Academic Press, Washington DC., 2011; 1-246.
11. Rowett HGQ. Dissection guide III. The rats with notes on the mouse. John Murray, London, 1977; 38-43.
12. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalate and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 1957; 28: 56-58.
13. Biggs HG, Moorehead WR. 2-Amino-2-methyl-propanol as the Alkalizing Agent in an Improved

Continuous-Flow Cresophtalein Complexone Procedure for Calcium in Serum *Clinical Chemistry*, 1974; 20: 1458-1460.
14. Varshney R, Kale RK. Effects of calmodulin antagonist on radiation-induced lipid peroxidation in microsomes. *International Journal of Radiation Biology*, 1990; 58(5): 753-743.
15. Adam-Vizi V, Seregi A. Receptor independent stimulatory effect of noradrenaline on Na,K-ATPase in rat brain homogenate. Role of lipid peroxidation. *Biochemical Pharmacology*, 1982; 31(13): 2231-2236.
16. Terri AE, Sesin PG. Determination of serum potassium by using sodium tetraphenylboro method. *American Journal of Clinical Pathology*, 1958; 29(1): 86─90.
17. Dacie J, Lewis SM. Practical haematology (6th Edn). Churchill, Livingstone, London, 1986.
18. Zanuncio JC, Serrao JE, Mourao AA. Toxic effects of the Neem (*Azadirachta indica*) formulation on the stink bug predators, *Podisus nigrispinus* (Heteroptera: pentatomidae). *Scientific Reports*, 2016; 6(6): 30261.
19. Rang HP, Dale M, Ritter J. Pharmacology (4th Edn). Churchill Livingstone, New York, 2011; 16.
20. Pennington TD, Styles BT. A generic monograph of the *Meliaceae blumea*, 2015; 22: 419-540.
21. Kango D. In Neem (2nd Edn). Randhawa and Parmar, B.S. (Eds), 2016; 77-110.
22. Schorderet M. Pharmacology of fundamental concepts of therapeutic applications, Paris Edition. Frison-Roche, 1992; 1-1010.
23. Lorke D. A new approach to practical acute toxicity testing. *Archives of Toxicology*, 1983; 54: 275-287.
24. Corbett JR, Wright K, Baillie AC. The biochemical mode of action of pesticides (2nd Edn). Academic Press, London, 2014.
25. Shemonty H, Sikder MD, Massnoon A, Mustari H, Tasniya Z. Toxicological Studies of the Ayurvedic Medicine "*Naradiya Laksmivilasa* Rasa" Used in Sinusitis. *Biology and Medicine*., 2016; 8: DOI: 10.4172/0974-8369.1000359.
26. Tasniya NZ, Mamun S, Tanmony S, Imtiuj HC. Organ-body weight ratio toxicity studies of an Ayurvedic medicine chitamanichaturmukh ras used in vertigo. *International Journal of Pharmacy*, 2017; 7(4): 35-40.
27. Amresh GR, Singh PN, Rao CV. Toxicological studies of an Ayurvedic medicine. *Journal of Ethnopharmacology*, 2008; 116: 454-460.
28. Dybing E, Doe J, Groten J, K;einer J, Brien J. Hazard characterization of chemicals in food and diet. *Food and Chemical Toxicology*, 2002; 42: 237-282.
29. Lloyd RV. Adrenal corticol tumors, pheochromocytomas and paragangliomas mode in pathology, 2011; 24: 858-865.
30. Akanji MA, Natiu MO, Yakubu MT. Enzyme activities and histopathology of selected tissues in rats treated with potassium bromate. *African Journal of Biomedical Research*, 2008; 11: 87-95.
31. York MJ. Clinical pathology. In: A comprehensive guide to toxicology in nonclinical drug development (2nd Edn). Elsevier, Philadelphia, 2017; 325-374.
32. McPhalen CA, Vincent MG, Picot D, Jansonius JN, Lesk AM, Chothia C. Domain closure in mitochondrial aspartate aminotransferase. *Journal of Molecular Biology*, 1992; 227(1): 197-213.
33. Aulback AD, Amuzie CJ. Biomarkers in nonclinical drug development. In: Comprehensive guide to toxicology in nonclinical drug development (2nd Edn). Elsevier, Philadelphia, 2017; 447-471.
34. Akah PA, Ottiah VN, Onuagu E. Hepatotoxic effect of *A. indica* leaf extracts in Rabbits protozoa, 1992; 63: 311-319.
35. Nwachukwu N, Iweala EJ. Influence of extraction methods on the hepatotoxicity of *Azadirachta indica* bark extract on albino rats. *Global Journal of Pure and Applied Science*, 2009; 15: 369-372.
36. Yakubu MT, Akanji MA, Salau IO. Protective effect of ascorbic acid on some selected tissues of Ranitidine treated rats. *Nigerian Journal of Biochemistry and Molecular Biology*, 2001; 16(2): 177-182.
37. Agbor GA, Tarkang PA, Fogha JVZ. Acute amd subacute toxicity studies of aqueous extracts of *Morinda lucida* stem barks. *Journal of Pharmacology and Toxicology*, 2012; 7: 158-165.
38. Kirsch JF, Eichele G, Ford G, Vincent MG, Jansonius JN, Gehring H. Mechanism of action of aspartate aminotransferase proposed on the basis of its spartial structure. *Journal of Molecular Biology*, 1994; 174(3): 497-525.
39. Adeshina G, Jibo S, Agu VE, Joseph EO. Antibacterial activity of fresh juices of *allium cepa* and *zingiber officinale* against multidrug resistant bacteria, 2011; 2(2): 289-295.
40. Clark S, Youngman LD, Palmer A, Parish S, Peto R, Collins R. Stability of plasma analytes after delayed separation of whole blood implications for epidemiological studies. *International Journal of Epidemiological*, 2003; 32: 125-130.
41. Olaoluwa TA, Odutola O, Olugbenga OA, Funmilayo DO, Sunday OO, Aolayan AJ. Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities in selected tissues of rats fed on processed Atlantic Horse Mackerel (*Trachurus trachurus*). *Advances in Bioscience and Biotechnology*, 2015; 6: 139-152.
42. Vasudevan DM, Sreekumari S, Kannan V. Textbook of Biochemistry for medical students (8th Edn). Jaypee, London, 2016; 492-496.
43. Yakubu MT, Bukoye BB, Oladiji AT, Akanji MA. Toxicological implications of aqueous extracts of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Human and Experimental Toxicology*, 2009; 28: 591-598.

44. Ahmad IM, Wudil AM, Yunusa I. Effect of oral administration of aqueous leaves extracts of *Vitex doniana* on serum electrolyte levels in rats. *Pakistan Journal of Biological Sciences*, 2013; 16: 1819-1822.
45. Burton DR. Chemical base and electrolyte disorders. International Students Edition, Kagakusila Ltd and McGraw-Hill Co, New York, 1997; 191.
46. Oduntola AA. Rapid interpretation of routine chemical laboratory test. Nameo Nigeria Ltd, Nigeria, 1992; 879.