TOXICITY AND EFFECTS OF AQUEOUS LEAF EXTRACT OF *ANNONA SENEGALENSIS* PERS ON SOME ORGANS OF ALBINO RATS

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Abstract:
Plants are some of the great gifts nature has given to mankind. These have been utilized in diverse ways among which are for food and medicinal purposes. This study investigated the toxicity effects of the aqueous leaf extract of *Annona senegalensis* following prolong administration in albino rats. The study was carried out in the Faculty of Veterinary Medicine of University of Maiduguri, Borno State of Nigeria, West Africa. The acute toxicity was carried out using standard method as described by Karbar and modified by Aliu and Nwude. In this method, histopathological investigation were carried out as described by Drury and Carlton. Serum enzymes measurements were carried out using the method of Schmidt and Schmidt. The acute toxicity (LD_{50}) level obtained was 2400 mg/kg which corresponds with Organization for Economic Cooperation and Development (OECD) classification of nontoxic level, but harmful according to EPA United States of America. The study showed significant increase in alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST). This suggests presence of toxic effects of the extract on the liver. In the organs investigated, there were significant histopathological changes induced by the extract as shown on the microscopic plates. It was concluded that the extract has a dose dependent toxicity on liver and other organs. The use of this plant for food and treatment of various ailments should therefore be done with caution.
Keywords: *Annona senegalensis*, acute toxicity, organs, lesions and extract

Introduction

Humans use plants in many ways which food, clothing, shelter, fuel and as medicinal source for management of various diseases/ailments. Plants have been useful companion to human beings and animals due largely to the diverse uses for the above purposes. There are many bioactive ingredients in plants that account for their therapeutic activities where pharmaceutical industries find them useful in drugs manufacturing and so do folkloric medical practictioners (Anon, 2014; Orwa *et al*., 2009).

Annona senegalensis belongs to the family Annonaceae, genus *Annona* and species *senegalensis.* It is a species of flowering plant. The specific epithet, *senegalensis*, translates to mean "of Senegal", the country where the type specimen was first collected (Orwa *et al*., 2009). The genus *Annona* is from the Latin word 'anon', meaning 'yearly produce' (annual harvest) referring to the production habits of fruits of the various species in this genus (Pinto, 2005; Orwa *et al*., 2009).

One of the primary uses of this plant is for food, although it has applications in numerous aspects of human endeavour. The flowers, leaves and fruit are edible and culinary, while fruit pulp has a mild, pineapple-like flavour. Wild Soursop fruits are sold in local markets in Africa. The fruit has a pineapple-like odour and sweet taste (FAO, 1983). It keeps for only a few days. It is used in sherbets, ice creams and for making drinks (FAO, 1988). The flower is added to spice or garnish meals; leaves are eaten by humans as vegetables, or browsed by livestock (Anon, 2014). The leaves are also used to enhance general health tonic in the treatment of pneumonia, diseases of the eye, stomach and intestines (Cordeiro *et al*., 2005). The leaves have essential oils with parasiticidal, antidiarrhoeica, rheumatological and antineuralgic properties (Cordeiro *et al*., 2005). Boiled water infusions of the leaves have anti-spasmodic, astringent and gastric properties (Calzavara and Miiller, 1987; Khan *et al*., 1997), and help in treatment diabetes and gastric upsets (Calzavara and Miiller, 1987) and also are used in kidney ailments (Cordeiro *et al*., 2005). The cooked flowers and petals are used for treating eye inflammations; the treatment requires 2-3 washes a day (Calzavara and Miiller, 1987).

The plant has many properties as reported by some researchers which include antidiarrhoeal, antimicrobial, antioxidant, cytotoxic, antiparasitic, Anti-inflammatory, anticonvulsant andantimalarial effects.*In vivo t*rypanocidal, anti-snake venom, anti-nociceptive and anthelmintic properties have also been reported(Sahpaz *et al*., 1994; Johnson and Olatoye, 2002; Alawa *et al*., 2003;Adzu *et al*., 2003; 2005; Ajaiyeoba *et al*., 2006; Ogbadoyi *et al*., 2007; Suleiman *et al*., 2008; Ajboye *et al*.,2010; Yeo *et al*., 2011;Awa *et al*., 2012; Konate *et al*., 2012). The current study was carried out to investigate the acute toxicity and prolonged administration of the extract on some organs in albino rats.

Methodology

Study Area

The study area Maiduguri, is located in the semi-arid north-east region of Nigeria. It falls within latitudes 11º04'N and 11º44'N; and between longitudes 13º04'E and 13º44'E, covering an area of 543 km^2. The urban center is located in four Local Government Areas (LGA): Maiduguri Metropolitan Area, Jere LGA, Konduga LGA and to a smaller extent Mafa LGA. Maiduguri is within the vast open plain which is flat or gently undulating, developed on the young sedimentary rock of the Chad formation. This relatively flat terrain is sloping towards the Lake Chad and has an average relief ranging between 300 m and 600 m (Daura, 2001). Maiduguri climate is characterized by dry and wet seasons. Four distinct seasons are identified by natives of the city. These are: cool dry (harmattan season) from December to February; hot dry season from March to late May; raining season from June to September; and humid dry transitional period between September and November (Waziri, 2009). The timing is however not rigid owing to climatic fluctuations of recent times. The mean annual rainfall for the period 1960 – 2003 was 577 mm (Bukar, 2006). The rainfall is single peak type rising gradually from

late June to maximum in August and with 60% to 70% of the annual rainfall within two months of July and August (Daura, 2001).

Collection and Identification of Plant Sample

The leaves of *Annona senegalensis* were collected from Girei Local Government Area of Adamawa State. The leaves were identified and authenticated by a Taxonomist in the Department of Forestry and Wild Life Management of the Modibbo Adama University of Technology, Yola. Voucher specimen (PG/15/CHM/008) was deposited in the Departmental herbarium (Department of Chemistry). The leaves of the plant were dried under shade, ground into powder and kept until required.

Experimental Animals

Female and male albino rats were obtained from the Laboratory Animal House of the Biochemistry Department, University of Maiduguri and kept in the Postgraduate Veterinary Anatomy Research Laboratory, University of Maiduguri. The rats were given pelletized growers mash 120 g daily (Vital feeds Nigeria Ltd) and water *ad libitum*. A total of 100 rats were used for the research.

Plant Extraction

The *Annona senegalensis* aqueous leaf extract was obtained using soxhlet extractor and distilled water as the solvent. The extract was evaporated to near dryness on a water bath, weighed and kept at 4°C in refrigerator until required (Evans, 2009c; Zade and Dabhadkar, 2013).

Acute Toxicity Study

Aqueous extract of *A. senegalensis* leaf was used for this study. Thirty (30) rats consisting of males and females were randomly divided into 6 groups of five rats each in separate cages. The rats were marked and housed individually and kept for 7 days in the laboratory to allow them acclimatize. The rats were fed with standard feed (Vital feed, Nigeria) and water *ad libitum*. Group 1 rats served as the control and distilled water was given orally. Groups 2 - 6 rats were treated orally with varying doses of 100, 200, 400, 800, 1600 and 3200 mg/kg respectively of the aqueous extract once. The rats were observed within 24 hours for signs of toxicity and mortality. Dead rats were posted and histopathology was carried out to determine any pathological changes. The median lethal dose (LD_{50}) was calculated using the arithmetic method of Karbar (1931) as modified by Aliu and Nwude (1982).

LD_{50} = apparent least dose lethal to all in a group

$$= - \frac{\sum(a \times b)}{N}$$

Where, N = number of animals in each group

a = dose difference

b = mean mortality

Effects of Prolonged Administration of Aqueous Leaf Extract of A. senegalensis on Organs Weight of Albino Rats.

Determination of the Effects of the Extract on some Serum Enzymes

Estimation of Serum Alkaline Phosphatase (ALP)

Serum alkaline phosphatase was estimated by the method of phenolphthalein monophosphate (Babson, 1965; Babson *et al*., 1966; Klein *et al*., 1969).

Principle: Serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which, at alkaline pH values, turns into a pink colour that can be photometrically determined.

Procedure: One (1) ml each of water and 1 drop each of the substrate was measured into two different test tubes using pipette and labelled sample and standard respectively. The contents of each of the two test tubes were shaken and incubated at 37°C for 5 minutes. Thereafter, 0.1 ml of serum sample was added to sample test tube and 0.1ml of standard solution was added to standard test tube, mixed and incubated at 37°C for 20 minutes. The ALP level in the serum was calculated using the following formula:

$$\frac{SA\ \ O.D}{ST\ O.D} \times 30 = IU/Lof\ Alkaline\ Phosphatase$$

Estimation of Serum Alanine Aminotransferase (ALT)

The serum alanine aminotransferase was estimated colorimetrically using ALT reagent kit (Randox Laboratories Ltd., Antrim, UK) as described by Reitman and Frankel (1957); Schmidt and Schimdt (1963).

Principle: α-oxoglutarate + L-alanine ⟶ L-glutamate + pyruvate

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure: In determining the ALT, 0.1 ml of the serum was measured into a sample test tube using a pipette and 0.5 ml of buffer added into the same test tube and a second test tube (sample blank) respectively. The contents were mixed and incubated for 30 minutes at 37°C. Five (5) ml each of 2, 4-dinitrophenylhydrazine was measured with a pipette into the sample blank and sample test tubes. Each test tube was then shaken and allowed to stand for 20 minutes at 25°C. Thereafter, 5ml of sodium hydroxide was added to the content of each tube, mixed and the absorbance of the sample test against sample blank taken after 5 minutes using a spectrometer (Boeringer 4010, West Germany) at 546 nm. The activity of ALT in the serum was then read from the standard table in IU/L (Randox Lab Ltd. U.K.).

Estimation of Serum Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST) was determined by colorimetric method using commercial kit (Randox Lab Ltd Ardmore, U.K.) as described by Schmidt and Schmidt (1963).

Principle: α-oxoglutarate + L-aspartate⟶ L-glutamate + oxaloacetate

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine and the absorbance read at 546nm.

Procedure: One micro-liter (0.1µl) of the serum was measured into a sample test tube using a pipette and 0.5 ml of buffer was added to the same test tube and a second test tube (sample blank) respectively. The contents were shaken and incubated at 37°C for 30 minutes. Using a pipette, 0.5 ml of 2, 4-dinitrophenylhydrazine was measured into the sample blank and sample test tubes and the content of each tube was shaken and allowed to stand for 20 minutes at 25°C. Thereafter, 5 ml of sodium hydroxide was added to the contents of each tube, shaken and the absorbance of the sample test against sample blank read after 5 minutes using a spectrometer (Boeringer 4010, West Germany) at 546 nm. The activity of AST in the serum was then read from a standard table.

Gross and Microscopic Study

Gross Pathology: Necropsy of all the rats were carried out every week. The brain, heart, kidneys, liver, lungs, spleen and stomach were dissected out and weighed (g) immediately.

Histopathology: The small intestine, spleen, liver, stomach, kidneys and heart were collected every week and fixed for histology. The samples were taken from both the control and treated groups for comparison. The organs were prepared for histological investigations and standard methods were used (Drury *et al*., 1967). The tissues were dehydrated in ethanol

and were cleared in xylene. The tissues were pre-impregnated in paraffin and xylene kept in an oven at 63ºC before embedding in pure molten paraffin which was allowed to solidify and then sectioned at 4-5µm thickness using a microtome blade. This was floated on warm water at 45ºC and was then mounted on egg albumin smeared glass slides which were allowed to dry in an oven. The slides were subjected to staining with Haematoxylin and Eosin (H and E) for histological observation through the aid of a light microscope (Drury *et al*., 1967; Carlton, 1980).The lesions observed under the microscope were photographed using canon digital camera power shot (A470).

Results

Acute Toxicity Study (LD_{50})

Mortality was not observed in any of the doses except at 3200 mg/kg which produced 100 % death of the animals. There was slight sedation, dyspnea, reduced appetite, coma and eventually death in group six (3200 mg/kg). The calculated LD_{50} is 2400 mg/kg.

Effects of Aqueous Leaf Extract of Annona senegalensis on serum enzymes in albino rats.

The effects of aqueous leaf extract of *Annona senegalensis* on some serum enzymes in albino rats is presented in Table 1. There was a dose dependent decrease ($P<0.05$) in the mean values of ALP in week 1 at the doses of 300 and 400 mg/kg (429.00±3.39 and 432.40±9.84 IU/L) and week 2 in the doses of 200 and 400 mg/kg (401.80±34.83 and 361.60±9.74 IU/L). However, it significantly ($P<0.05$) increased in the doses of 100, 200, 300 and 400 mg/kg (402.80±19.37, 474.80±12.17, 537.20±52.63 and 534.40±6.77 IU/L) at week 3. There was no significant change in ALP value in week 4 compared to the control.

Table 1: Effects of aqueous leaf extract of *Annona senegalensis* on some serum enzymes in albino rats.

Parameters/ Weeks of admi nistration		Extract Dose (mg/kg) Control(0) (Distil water)	100 (IU/L)	200	300	400
ALP	1	469.00±7.87^a	480.20±2.17	483.40±17.57	429.00±3.39^c	432.40±9.84^c
	2	533.40±21.78^a	549.80±9.86	401.80±34.83^c	559.00±56. 89	361.60±9.74^c
	3	345.00±59.43^a	402.80±19.37^b	474.80±12.17^b	537.20±52.63^b	534.40±6.77^b
	4	337.20±48.26	315.80±5.40	311.80±7.43	339.60±40.53	321.00±49.80
ALT	1	23.000±3.16	26.000±2.65	22.800±3.49	23.800±1.10	25.400±2.88
	2	26.600±2.62^a	31.400±2.30	38.600±5.13^b	32.400±2.07^b	30.400±2.41
	3	31.000±3.08	31.000±2.12	30.200±3.27	35.600±5.46	34.400±4.51
	4	38.000±4.80^a	48.800±2.86^b	24.600±1.67^c	20.600±2.61^c	21.400±2.61^c
AST	1	61.000±3.16^a	65.000±3.67	64.200±7.01	65.000±5.48	74.800±4.92^b
	2	60.400±3.44^a	62.200±3.03	75.200±3.77^b	53.400±6.84^c	73.800±3.11^b
	3	50.400±5.73^a	63.400±4.10^b	92.400±5.18^b	60.600±4.34^b	55.000±2.74
	4	36.600±3.13^a	43.400±4.98^b	95.400±4.04^b	58.000±5.10^b	54.400±3.78^b

Different superscripts in the same row are significantly different at $P<0.05$.

Key: a = for control groups
b = significantly increased
c = significantly decreased

Effects of Prolonged Administration of Aqueous Leaf Extract of A. senegalensis on Organs Weight of Albino Rats.

The effects of prolonged administration of aqueous leaf extract of *A. senegalensis* on organs weight is presented in Table 2. There was significant ($P<0.05$) decrease in brain weight at 400 mg/kg (1.380±0.08 g) in week 1, but there was no significant difference at the doses of 100, 200 and 300 mg/kg when compared to the control. Whereas in weeks 2, 3 and 4 it did not produce any statistically significant change in all the treatment groups when compared to the control (Table 2). There were no significant differences in the mean weights of the heart and kidney in all the treatment groups as compared to the control throughout the treatment periods (Table 2).

The mean weights of the liver in week 1 was significantly decreased at the doses of 200 and 400 mg/kg (3.560±0.13 and 4.680±0.16 g respectively) but produced no significant change at 100 and 300 mg/kg as compared to the control. In week 2, there were significant decreases at 200, 300 and 400 mg/kg (6.540±0.23, 5.840±0.20 and 4.780±0.19 g respectively) but no significant difference at 100 mg/kg as

compared to the control. There were significant decreases in the mean weights of liver at 400 mg/kg (4.780±0.19 g) and significant increase at 200 mg/kg (6.480±0.13 g) as compared to the control in week 3. The mean weights of the liver in week 4 was significantly increased at 200 and 300 mg/kg (5.980±1.05 and 5.980±0.39 g) and was not significant at 100 and 400 mg/kg when compared to the control (Table 2).The mean weights of the lungs in week 1 significantly decreased at 100 and 400 mg/kg (1.160±0.11 and 1.320±0.08 g respectively), significantly increased at 200 mg/kg (3.100±0.24 g) and produced no significant difference at 300 mg/kg as compared to the control. In week 2, the mean weights of the lungs significantly decreased at 100, 200 and 300 mg/kg (1.500±0.12, 1.180±0.08 and 1.500±0.12 g respectively) and not significant at 400 mg/kg as compared to the control. There was significant increase at the dose level of 100 mg/kg (1.840±0.11 g) and no significant difference at doses 200, 300 and 400 mg/kg when compared to the control of the mean weights of the lungs at week 3. The mean weights of the lungs in the 4th week of the treatment significantly increased at dose400 mg/kg (1.640±0.18 g) and did not produce any significant change at 100, 200 and 300 mg/kg doses when compared to the control. The mean weights of the spleen did not produce any significant change throughout the treatment periods in all the treatment groups except in week 1 where there was a significant increase at the dose of 200 mg/kg (1.120±0.08 g) as compared to the control.

The mean weights of the stomach significantly decreased in week 1 at the doses of 100, 200, 300 and 400 mg/kg (1.360±0.11, 1.100±0.10, 2.140±0.11 and 1.720±0.15 g respectively). The stomach weight significantly increased in week 2 at the dose of 400 mg/kg (2.500±0.20 g)when compared to the control. There were no significant changes in weeks 3 and 4 in all the treatment groups as compared to the control (Table 2).

Table 2: Effects of prolonged administration of aqueous leaf extract of *Annona senegalensis* on organs weight in albino rats (g)

Parameters	Dose (mg/kg)	Period of Administration (weeks) 1	2	3	4
		organ weights (g)			
Brain	control(0)	1.700±0.16[a]	1.340±0.44	1.580±0.08	1.100±0.07
	100	1.520±0.11	1.540±0.21	1.580±0.08	1.240±0.09
	200	1.440±0.13	1.580±0.08	1.600±0.07	1.560±0.09
	300	1.520±0.13	1.340±0.11	1.400±0.14	1.240±0.15
	400	1.380±0.08[c]	1.300±0.12	1.420±0.16	1.400±0.12
Heart	control(0)	0.5000±0.16	0.6400±0.11	0.6600±0.06	0.5400±0.06
	100	0.5400±0.06	0.5800±0.08	0.7200±0.08	0.5800±0.11
	200	0.5600±0.09	0.7000±0.12	0.7000±0.07	0.6800±0.05
	300	0.5600±0.06	0.6400±0.09	0.7400±0.11	0.5600±0.06
	400	0.6800±0.15	0.5800±0.11	0.6000±0.07	0.4200±0.13
Kidneys	control(0)	1.100±0.12	1.340±0.18	1.220±0.08	1.140±0.11
	100	1.120±0.05	1.380±0.22	1.220±0.05	1.140±0.11
	200	0.900±0.14	1.300±0.16	1.260±0.11	1.120±0.11
	300	1.260±0.11	1.140±0.11	1.240±0.09	1.200±0.07
	400	1.000±0.07	1.040±0.13	1.280±0.16	1.100±0.07
Liver	control(0)	6.320±0.35[a]	7.180±0.31[a]	5.980±0.19[a]	5.020±0.94[a]
	100	5.980±0.23	7.100±0.24	6.120±0.15	5.640±1.06
	200	3.560±0.13[c]	6.540±0.23[c]	6.480±0.13[b]	5.980±1.05[b]
	300	6.480±0.11	5.840±0.20[c]	5.680±0.29	5.980±0.39[b]
	400	4.680±0.16[c]	4.780±0.19[c]	4.780±0.19[c]	5.060±0.26
Lungs	control(0)	1.660±0.11[a]	1.840±0.11[a]	1.280±0.13[a]	1.240±0.09[a]
	100	1.160±0.11[c]	1.500±0.12[c]	1.840±0.11[b]	1.160±0.11
	200	3.100±0.24[b]	1.180±0.08[c]	1.220±0.08	1.440±0.09
	300	1.620±0.08	1.500±0.12[c]	1.480±0.11	1.200±0.07
	400	1.320±0.08[c]	1.980±0.19	1.160±0.13	1.640±0.18[b]
Spleen	control(0)	0.5000±0.07[a]	0.4800±0.08	0.6200±0.08	0.5400±0.06
	100	0.7000±0.07	0.7000±0.12	0.8000±0.14	0.4800±0.08
	200	1.120±0.08[b]	0.6600±0.11	0.6000±0.07	0.5600±0.06
	300	0.4200±0.08	0.7000±0.12	0.6800±0.15	0.4600±0.11
	400	0.5600±0.09	0.6600±0.11	0.5400±0.06	0.4600±0.15
Stomach	control(0)	2.420±0.08[a]	1.940±0.15[a]	2.120±0.18	1.600±0.14
	100	1.360±0.11[c]	1.540±0.21	2.360±0.11	1.640±0.17
	200	1.100±0.10[c]	2.120±0.18	2.120±0.13	2.420±0.11
	300	2.140±0.11[c]	2.220±0.19	2.320±0.11	2.120±0.18
	400	1.720±0.15[c]	2.500±0.20[b]	1.800±0.20	2.200±0.14

Different superscripts in the same column are significantly different at $P<0.05$.

Key: a = for control groups
b = significantly increased
c = significantly decreased

Histopathological Effects of Prolonged Administration of Aqueous Leaf Extract of A. senegalensis on some Tissues of Albino Rats.

The histopathological effects of prolonged administration of *Annona senegalensis* aqueous leaf extract on tissues in albino rats are presented in plates 1-7.

The aqueous leaf extract of *Annona senegalensis* administered at the 300 mg/kg resulted in necrosis of the small intestine characterized by sloughing of the surface epithelium as indicated on plate 1 (arrows).

The aqueous leaf extract of this plant at the dose of 400 mg/kg produced a focal area of moderate lymphocyte depletion from the white pulp in the spleen (plate 2).

The same dose of the extract (400 mg/kg) on the liver produced areas of non suppurative inflammation, multifocal hepatocellular degeneration, necrosis and lymphocytic perivascular cuffing (plate 3).

The aqueous leaf extract of *Annona senegalensis* (400 mg/kg) on the stomach produced necrosis characterized by sloughing of the surface epithelium (plate 4).

The aqueous leaf extract of *Annona senegalensis* administration at the dose 400 mg/kg was observed to produce kidney lesions including multifocal intratubular deposition of proteinaceous materials and tubular necrosis (plate 5).

The effects of the aqueous leaf extract of *Annona senegalensis* administration at the dose of 400 mg/kg on the heart include multifocal areas locally extensive lymphocytic infiltration (plates 6 and 7).

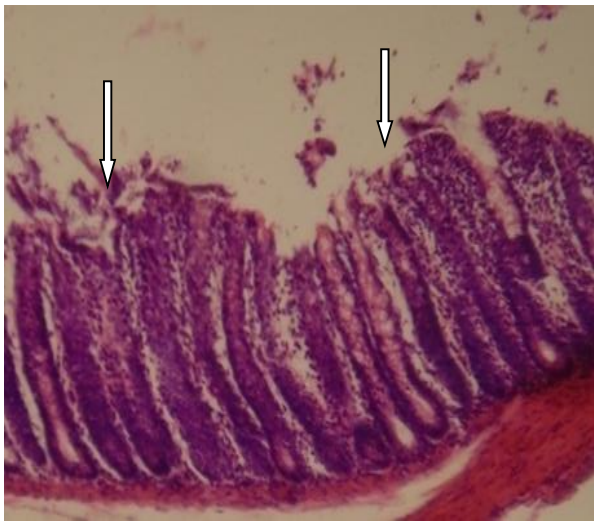

Plate 1. Photomicrograph of small intestine of an albino rat showing necrosis chracterised by sloughing of the surface epithelium (arrows) following treatment with 300 mg/kg aqueous leaf extract of *A. senegalensis*, H&E, X100.

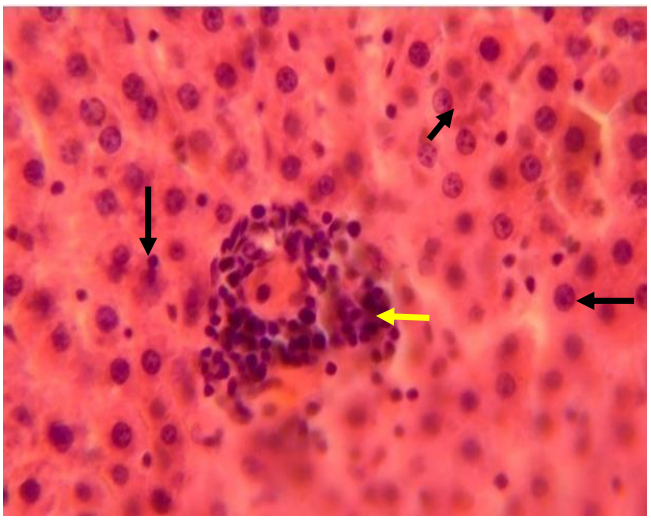

Plate 3 Photomicrograph of a liver of an albino rat showing areas of multifocal hepatocellular degeneration (white arrow) and necrosis (black arrows) and a lymphocytic perivascular cuffing (yellow arrow) following treatment with 400mg/kg of aqueous leaf extract of *A. senegalensis*, H&E X400.

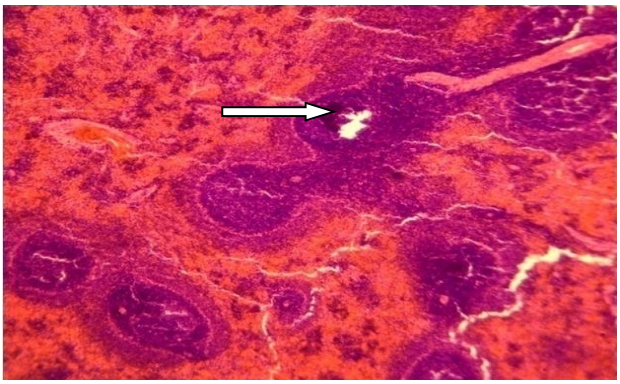

Plate 2. Photomicrograph of spleen of a rat showing focal area of moderate lymphocyte depletion from the white pulp (arrow) following treatment with 400mg/kg aqueous leaf extract of *A. senegalensis*, H&E, X100.

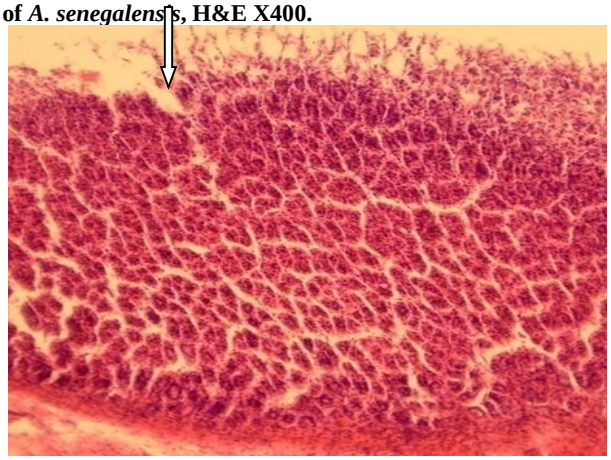

Plate 4 Photomicrograph of stomach of albino rat showing necrosis characterized by sloughing of the surface epithelium (arrow) following treatment with 400mg/kg aqueous leaf extract of *A.* senegalensis, H&E, X100.

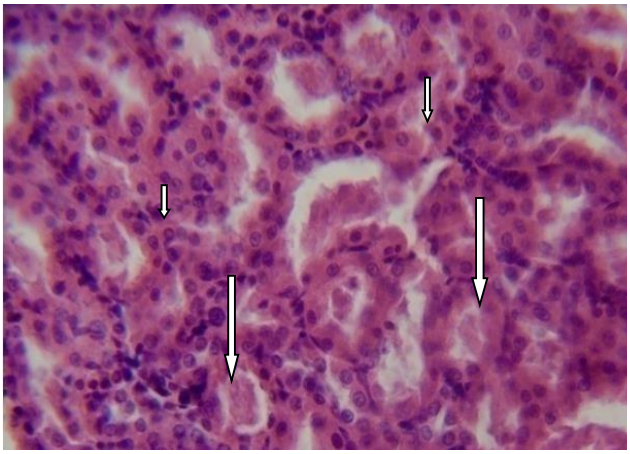

Plate 5 Photomicrograph of kidney of a rat showing multifocal intratubular deposition of proteinaceous materials (long arrows) and tubular necrosis (short arrows) following treatment with 400 mg/kg aqueous leaf extract of *A. senegalensis*, H&E, X400

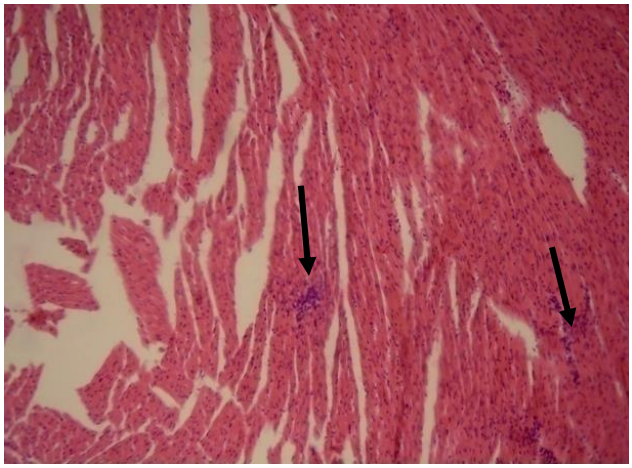

Plate 6 Photomicrograph of heart of an albino rat showing multifocal areas locally extensive lymphocytic infiltration (arrows) following treatment with 400 mg/kg aqueous leaf extract of *A. senegalensis*, H&E, X100.

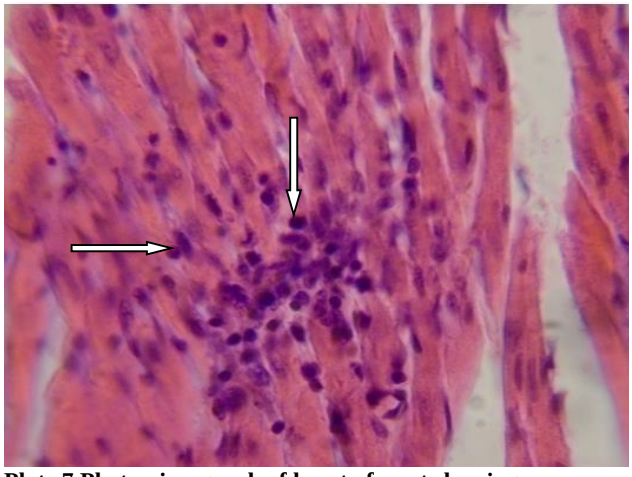

Plate 7 Photomicrograph of heart of a rat showing locally extensive lymphocyte infiltration (arrows) following treatment with 400 mg/kg aqueous leaf extract of A. senegalensis, H&E, X400.

Discussion

In this study, 3200 mg/kg was the dose that produced100 % mortality. The calculated LD_{50} of the aqueous leaf extract of *A. senegalensis* administered orally was 2400 mg/kg (Aliu and Nwude, 1982).The rats treated with 100, 200, 400, 800 and 1600 mg/kg doses produced no mortality, suggesting that the plant could be safe at those levels. It may therefore be considered nontoxic; although this does not predict the lethal dose in humans or other animals, it however provides a guide for choosing the dose for use in sub-chronic studies. Generally, the smaller the LD_{50} value, the more toxic the substance is and vice versa. These doses 100, 200, 400, 800 and 1600 mg/kg considered to be non harmful because the higher the LD_{50} the less toxic and the smaller the LD_{50} the more toxic the substance. This agrees with the report of Organization for Economic Cooperation and Development (OECD, 1998) which classifies: very toxic as<5 mg/kg, toxic as>5<50 mg/kg, harmful as>50 < 500 mg/kg and no label as>500 < 2000 mg/kg. On the other hand, the report of the Environmental Protection Agency (EPA) [OECD, 1998], United States classifies toxicity as follows: very toxic as ≤ 50 mg/kg, toxic as>50 ≤500 mg/kg, harmful as> 500 - 5000 mg/kg and no label as> 5000 mg/kg. From the above, every substance has to be handled with care. This extract is nontoxic based on OECD but for EPA (USA) it is harmful (OECD, 1998).

The result of this study further revealed significant ($P< 0.05$) increase in the activity of serum alkaline phosphatase (ALP) which is often employed to assess the integrity of the plasma membrane of the liver (Akanji, 1993). The increase in the serum ALP following administration of the plant extracts may be due to liver cell damage and or disruption of the liver plasma membrane as suggested by Nduka (1999). The result of this study demonstrates that *A. senegalensis*is is hepatotoxic at the experimental doses used and should be used with caution when administered over a long period of time.

Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) are important liver enzymes which play significant roles in the diagnosis of liver cytolysis (Shahjahan *et al*., 2004). The estimation of the levels of protein is used to examine the synthetic and excretory function of the liver. Measurement of the activities of enzymes in tissues and body fluids plays a significant and well known role in disease investigation and diagnosis (Malomo, 2000). Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked up by conventional histological techniques. Such measurement can also give an insight to the site of cellular tissue damage as a result of assault by plant extract (Adebayo *et al*., 2003).

In this study, the resultant increase in the activities of serum ALT and AST may indicate liver cytolysis, through which they are released into circulation. ALT is located in the cytosol of hepatocytes and the enzyme is considered a more sensitive marker of hepatocellular damage than AST. AST is found in the cytoplasm and mitochondria in different tissues such as the heart, liver, kidney, skeletal muscle, pancreas and erythrocyte (Hassan *et al*., 2008). The amino transferases are significant in amino acid metabolism as they help in retaining amino groups during the degradation of amino acids which are further used for the synthesis of new amino acids. Hence, they are involved in the biochemical regulation of the intracellular amino acid pool. When these enzymes are mobilized from the liver into free circulation, glutamate concentration may be affected with a resultant decrease in glutathione, one-third of which is formed from glutamate (Stryer, 1995). Detoxification is the essential role

of glutathione, and cells are exposed to attack by the active metabolite when it is depleted (Stryer, 1995). The liver being an organ of detoxification is the first organ that encounters all absorbed materials from the gastrointestinal tract. It has been shown to respond to toxicological insults in a number of ways including cellular degeneration and necrosis and bile duct hyperplasia and fibrosis (Mutalik *et al*., 2005; Garba *et al*., 2006; Saidu *et al*., 2007).

Organ weight is normally investigated as a sensitive indicator of chemically induced changes to the body. Biometric investigation has been shown to be a sensitive indicator of chemically induced toxicity (Peters and Boyd, 1966).The result of this study shows that prolonged administration of *A. senegalensis* does not affect the anatomical proportion of small intestine, spleen, kidney, liver and heart when compared to the control in the 100 and 200 mg/kg groups; it may be that the defensive mechanism of the animal has not been overcome and/or that the dose has not accumulated sufficiently to manifest any significant change.

The small intestine showed necrosis characterized by sloughing of the epithelium due to the activity of the extract, suggesting toxicity in the 300 mg/kg group. This was also observed in the stomach in the 400 mg/kg group due to the activity of the extract.

The significant increase of the liver weight (hepatomegaly) following the administration of the extract may be attributed to tissue degeneration, perivascular cuffing and necrosis; which may be due to the role of the liver in detoxification and excretion as was observed in the 400 mg/kg group.

The spleen showed lymphocytic depletion in the 400 mg/kg due to the activities of the extract. This could result from either hepatic or portal vein obstruction. The possibility of renal insufficiency or dysfunction occurring as a result of the toxic effect of the extract may be due to cumulative effect. The histopathological plate of the kidney showed intratubular deposition of proteinaceous materials and tubular necrosis in the 400 mg/kg group, suggesting renal dysfunction and or toxicity.

The heart showed lymphocytic infiltration in the 400 mg/kg group suggesting that the heart was reacting due to the toxic effect of the extract.

From the effect of the extract during prolonged administration it could be concluded that, in the 100 and 200 mg/kg groups there was no organ changes but in the 300 mg/kg group as showed in the small intestine and in the 400 mg/kg group there were organ changes due to toxicity. The histopathological lesions present in various organs such as small intestine, spleen, liver, stomach, kidney and heart showed degeneration and necrosis at different doses which may suggest that the extract has a dose dependent toxicity on these organs. The use of this plant for food and treatment of various ailments should therefore be done with caution as OECD label suggests that the plant is not harmful but with EPA's label it is as harmful (OECD, 1998).

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