Original Research Article

Hepatotoxicity of Ethanol Extract of *Adenium obesum* Stem Bark in Wistar rats

ABSTRACT

Aims: *Adenium obesum* is a known medicinal plant as well as a potent arrow poison. Therefore, the study aimed to evaluate the toxicity and histopathological effects of the oral administration of ethanol extract of *Adenium obesum* stem bark in the liver of exposed Wistar rats.

Place and Duration of study: Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria, between January 2011 and January 2012.

Methodology: Three female rats per group were orally administered single dose of 300mgkg^{-1}, 2000mgkg^{-1} and 5000mgkg^{-1} of the extract with distilled water placebo for the control and observed for signs of toxicity over a 14-day period. Progression from one extract dose to another was based upon the presence or absence of mortality, including the number of the mortality.

Results: Exposed rats did not show signs of toxicity and neither was there any mortality. The extract caused increased alanine aminotransferase activity but decreased aspartate aminotransferase and alkaline phosphotase activities, which were all non-significant ($p>0.05$). Congestion and fatty degenerative changes were seen in the liver of the exposed rats.

Conclusion: *Adenium obesum* did not cause major hepatic damage and therefore, it is a safe oral medicinal plant within the extract dose and exposure period used in the study in spite of the fact that the plant is a known potent arrow poison.

Keywords: Adeniun obesum, Wistar rats, toxicity, biochemical parameters, histopathology

1. INTRODUCTION

Medicinal plants have been used to treat variety of ailments worldwide [1, 2]. This is because medicinal herbs are integral parts of alternative therapy [3]. The World Health Organization (WHO) estimated that about 80 % of the world population presently uses herbal medicine for some aspects of their primary health care needs while plant products also play important roles in the health care system of the remaining 20 %, who mainly reside in developed countries [4]. No wonder herbal medicine has attracted public attention over the past 20 years especially as this type of medicine is easily accessible in some regions [5]. However, prolonged use of these plants is associated with toxic effects [6, 7] especially as most are used indiscriminately without adequate information on their safety or toxicity risk [8]. This calls for the continuous evaluation of their toxicity in attempts to elucidate on possible risks associated with the practice.

Adenium obesum is a deciduous pachycaul shrub with half buried and distinctly swollen base along with twisted branches that bears sparse leaves, which are shed prior to the appearance of its characteristic pink “showy” flowers [9, 10]. Although the plant grows mostly within the Sahel to Sudanese savannahs and also in Arabia [11, 12], it is also found worldwide where it’s cultivated for ornamental purposes [13]. The phytochemical screening of the stem bark of the plant revealed the presence of some alkaloids, flavonoids, saponins, tannins, glycosides, anthraquinones and steroids [14].

Adenium obesum is a medicinal plant, which is used to treat venereal diseases as well as skin diseases in the Sahel region [13]. This in addition to the bark being chewed as arbortificient [15, 12] even as a decoction of the plant’s root is specifically used as nose drops for rhinitis in Somalia [13]. The latex of *A. obesum* is used to treat decaying teeth, boils and septic wounds [13, 16]. Similarly, the latex and bark of the plant is used to treat bone dislocation, rheumatism, sprains, paralysis, swellings and wounds [17]. However, *A. obesum* is a known potent arrow poison [18, 13] as administered parenterally. There is therefore, a need to investigate the toxicity of this medicinal plant in order to optimize its

dosage, especially as pharmacology is simply toxicology at a lower dose [19] and vice versa. The fact that herbal toxicity represents a serious human health threat further makes the study very imperative [20]. Therefore, the study aimed to evaluate the toxicity and histopathological implications of the oral administration of ethanol extract of *A. obesum* stem bark in the liver of exposed Wistar rats as animal models for predicting possible effects in humans.

2. MATERIAL AND METHODS

2.1 Plant Extraction

Adenium obesum were gathered from the open fields of Rurum town, Rano Local Government Area, Kano State, Nigeria between the months of January – April, 2011. These were authentication with Voucher No. 1386 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (A. B. U.), Zaria, Nigeria. The barks were removed from the stems, sun-dried and pounded into powder for use.

The ethanol extraction was by the use of 21 L of ethanol (96.0 % vol. Sigma-Aldrich® Inc., St. Louis, MO 63178, USA) to soak 3.95 kg of the powdered stem bark over a 72-h period using the maceration method of Bently [21] and Ghani [22]. The method of Abu-Dahab and Afifi [23] was used to concentrate the filtrate to dryness in an evaporation dish at room temperature until constant weights were obtained.

2.2 Wistar Rat Toxicity Bioassay

A total of 12 female rats of 180.80 ± 4.55 g mean weight were obtained from the Animal Unit of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The rats were acclimatized for seven days in a well ventilated room under natural photo-period (12/12-h) while being housed in clean metal cages. Fresh drinking water was provided *ad libitum* along with NVRI pelletized rat feed (crude protein - 19.97 %, crude fibre - 6.89 %, Ash - 7.60 %, nitrogen free extract - 59.21 % and moisture - 12.98 %) during the period.

The toxicity bioassay was performed as described in the OECD guideline No. 423 [24] using single fixed doses separately in a stepwise procedure with the use of three female rats per step depending upon the presence or the absence of mortality and the number of the observed mortality over the 14-day period. The unexposed control rats were given 2 mL distilled water placebo. Experimental rats were observed for signs of toxicity during the first 30 minutes and daily thereafter throughout the 14-day observational period. The LD_{50} of the extract was established based on the OECD guideline No. 423 [24]. Similarly, changes in their body weights were used as a measure of toxicity [25].

2.3 Biochemical Analyses

Two millilitres of blood were collected from the exposed rats via vene-section under light chloroform anaesthesia at the end of the 14-day post oral dosing with the extract. These were dispensed into sample bottles not containing EDTA anticoagulant and centrifuged at 1,006 *g* for 10 minutes to obtain the serum after allowing them to clot. The Reference method by International Federation of Clinical Chemistry [26] was used to determine the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities using an autoanalyzer (Bayer Express Plus, Model 15950, Germany.). Enzymatic hydrolysis method as described by King and Armstrong [27] was used to determine the alkaline phosphatase (ALP) activity.

2.4 Histopathological Analyses

The liver of experimental rats was harvested after sacrificing them under light chloroform anaesthesia at the end of the 14-day observational period. The harvested tissues were fixed in 10 % neutral buffered formalin prior to paraffin embedding, sectioning at 5 µm and staining with haematoxylin and eosin [31, 32]. These were examined under light microscopy for histopathological lesions.

The nature and severity of lesions in the liver of the exposed rats were noted and determined semi-quantitatively based on the adaptation of the degree of tissue changes

(DTC) method by Poleksic and Mitrovic-Tutundzic [33] and Simonato *et al*. [34]. This involved the progressive classification of liver alterations in stages of tissue damage where the sum of the number of lesion types within each of the three stages is multiplied by the stage coefficient to give the numerical values of the DTC using the formula: DTC = (1 x ∑ *I*) + (10 x ∑ *II*) + (100 x ∑ *III*). Liver alterations that did not alter the normal functioning of the tissue were tagged Stage *I* alterations. Similarly, alterations that were more severe and impaired the normal functioning of the liver were tagged stage *II* alterations while those that were very severe and induced irreparable liver damage were tagged stage *III* alterations, respectively. The grading and interpretations of the results were as follows: 0 – 10 (normal liver); 11 – 20 (slightly damaged liver); 21 – 50 (moderately damaged liver); 50 - 100 (severely damaged liver); >100 (irreversibly damaged liver).

2.5 Statistical Analyses

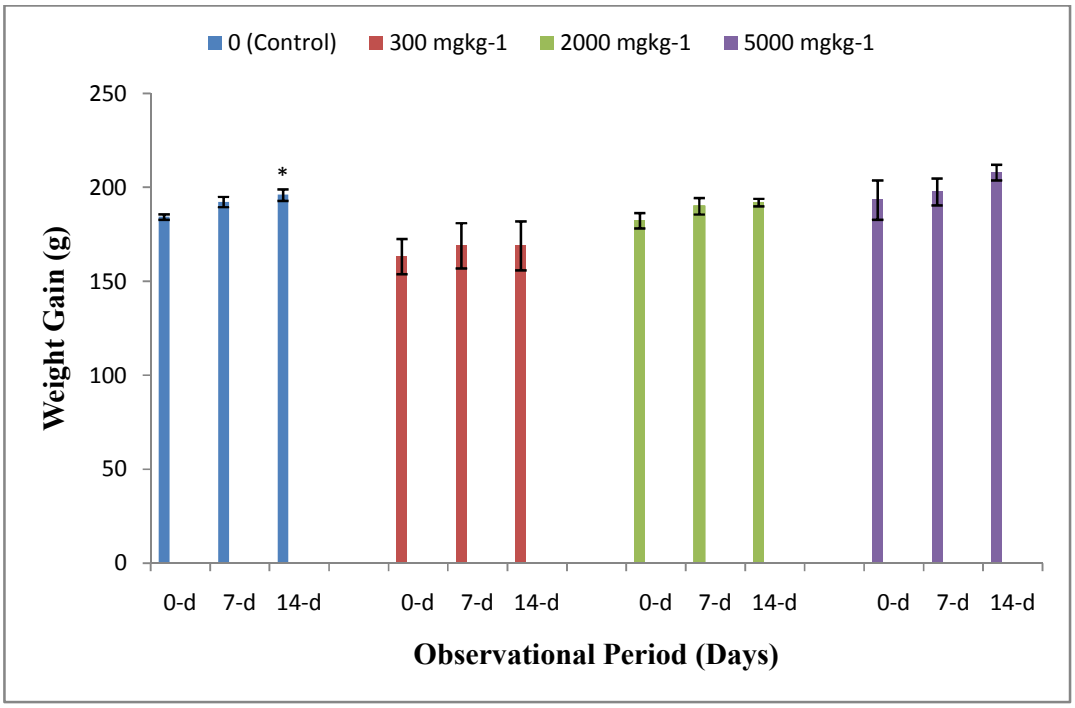
GraphPad Prism (GraphPad Prism, version 4.0, San Diego, California, USA.) was used to analyse the data (mean ± SEM) where a one-way analysis of variance (ANOVA) was performed for statistical significance at $p<0.05$, including Tukey's multiple comparison test to compare the differences between the various means. Differences between the DTC in the liver of the exposed groups and the control group were compared for statistical significance ($p<0.05$).

3. RESULTS

3.1 Toxicity Bioassay

There were no obvious changes in the skin and fur, eyes and mucous membranes of the exposed rats and neither were there changes in their behavioural patterns. Similarly, no obvious signs of tremors, salivation, diarrhoea, sleep, convulsions and coma were seen in the exposed rats, including the absence of mortality. The LD_{50} of the extract was therefore, > 5000 $mgkg^{-1}$ or ∞ (unclassified) based on the fixed LD_{50} cut off values [23]. There were gains

in body weights of experimental rats but this was significant ($p<0.05$) only in the unexposed control rats as shown in Fig. 1.



* $p<0.05$

Fig. 1: The effects of oral administration of ethanol extract of *Adenium obesum* stem bark on body weights of the expose Wistar rats.

3.2 Biochemical Analyses

Changes in some biochemical parameters of the exposed rats are as shown in Figs. 2. There were non-significant ($p>0.05$) decrease in the AST and ALP activities with increased ALT activity in the exposed rats. However, these biochemical changes were not concentration-dependent.

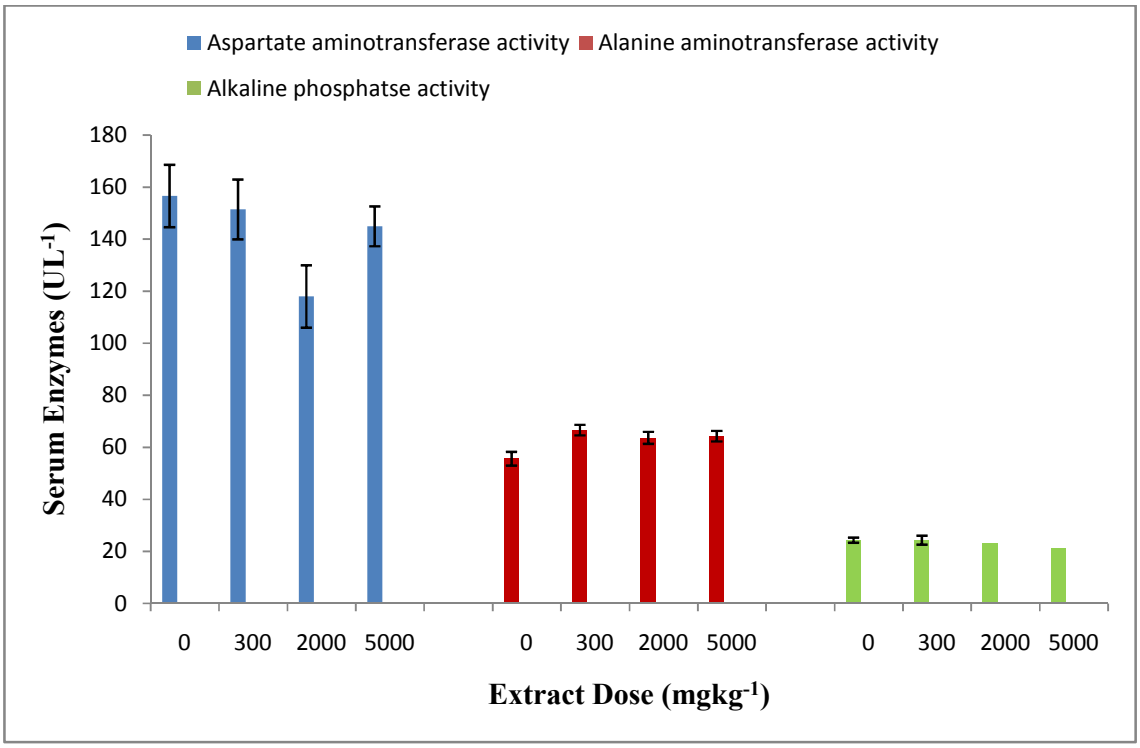


Fig. 2: The effects of oral administration of ethanol extract of *Adenium obesum* stem bark on aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities of the exposed Wistar rats.

3.3 Histopathological Analyses

Histopathological lesions were seen in the liver of the exposed and unexposed rats but these were comparatively to a lesser extent in the unexposed rats. The liver of the exposed rats showed congestions of the central vein along with hepatic fatty degenerations as shown in Figs. 3 and 4 with a cumulative DTC value of 3.33 ± 1.67, indicative of the normal functioning of the liver based on the DTC grading. The incidence of histopathological lesions in the liver of the exposed and unexposed rats is as shown in Table 1. However, there were no significant ($p>0.05$) differences between the DTC in the liver of the exposed groups and the unexposed control group.

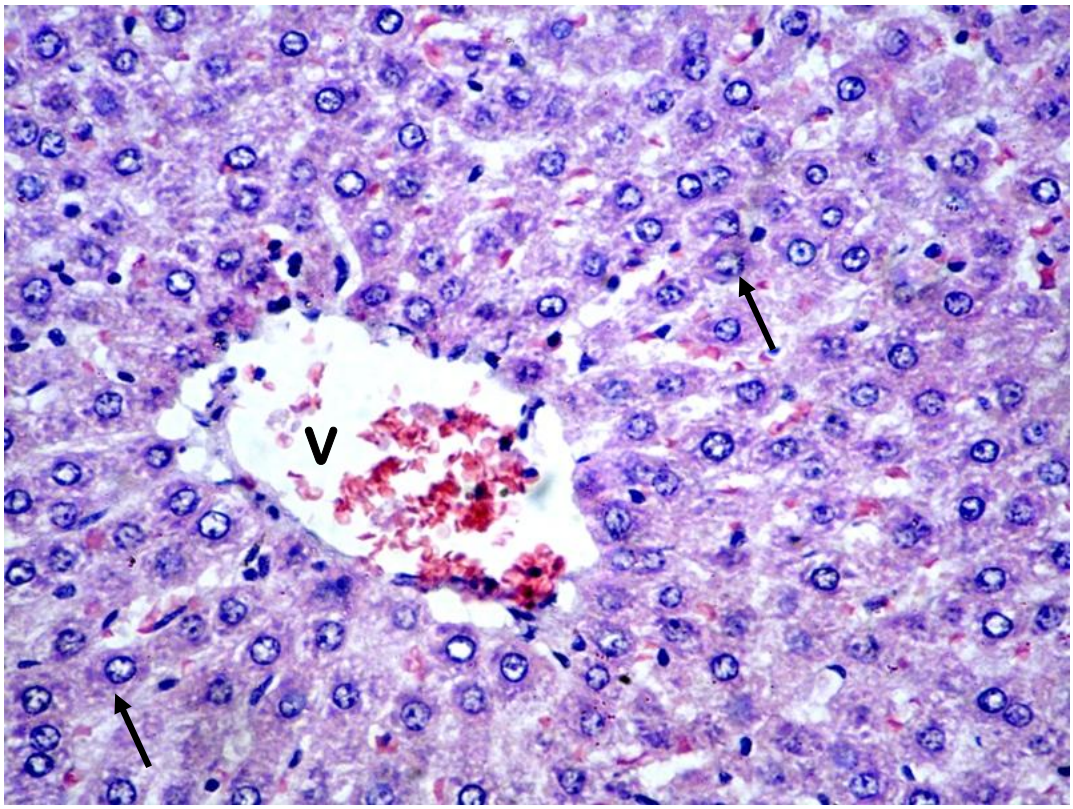


Fig. 3: Photomicrograph of the liver of the Wistar rats administered distilled water placebo (unexposed control). Note the central vein (V) and the hepatocytes (arrows). H & E x 397.

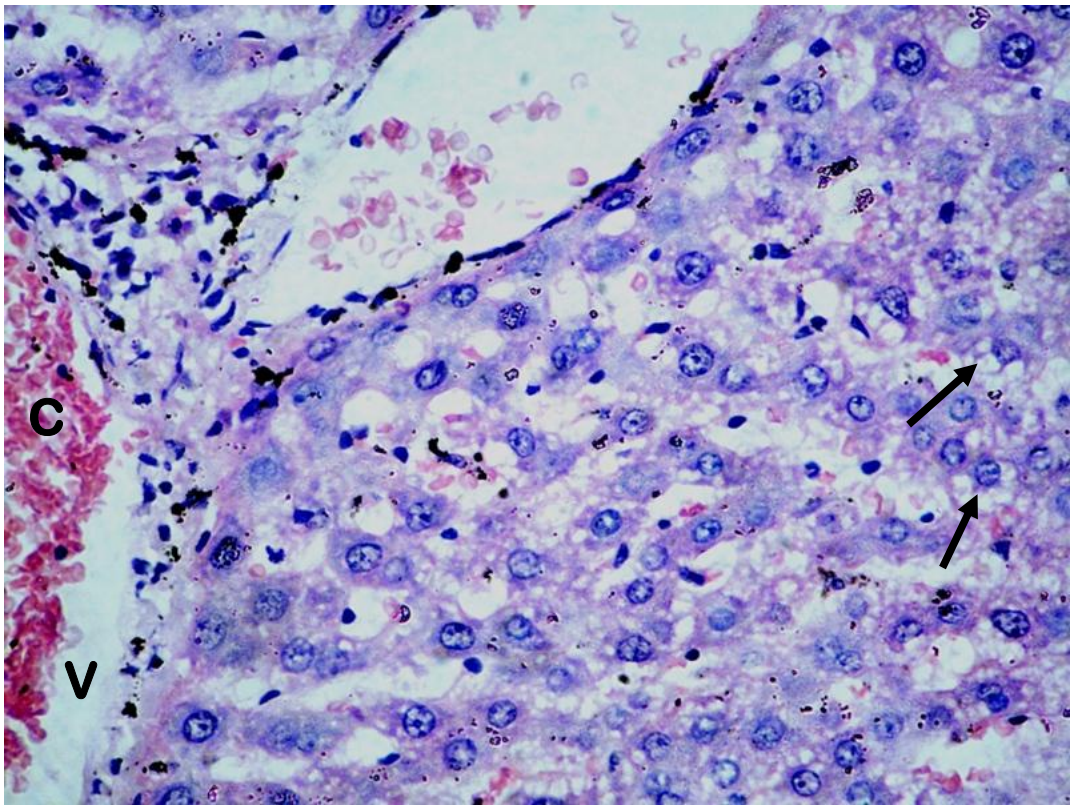


Fig. 4: Photomicrograph of the liver of Wistar rats dosed orally with 5000 mgkg^{-1} of the ethanol extract of *Adenium obesum* stem bark. Note the central vein (V), congestion of the central vein (C) and vacuolation of the hepatic cells (arrows). H & E x 397.

Table 1: The incidence of degree of tissue changes (DTC) in the liver of Wistar rats exposed to ethanol extract of *Adenium obesum* stem bark

Histopathological lesions	**DTC stage**	**Extract dose**			
		0 (Control)	**300 mgkg^{-1}**	**2000 mgkg^{-1}**	**5000 mgkg^{-1}**
Vacuolations	I	0	0	0	+
Congestion	II	0	+	+	+

(0) – absent; (+) – rare; (++) – low incidence; (+++) – high incidence

4. DISCUSSION

The absence of obvious signs of toxicity, including mortality was indicative of the very low toxicity of the extract in the exposed rats leading to very high LD50 value of > 5000 $mgkg^{-1}$ or ∞ (unclassified) based on the fixed LD_{50} cut off values [24] in spite of the fact that the plant is a potent arrow poison [18, 13]. The toxicity of the plant might be influenced by the route of administration as animals are normally exposed parenterally when the plant is used as arrow poison unlike the oral route of administration of the present study. This in addition to the fact that the toxicity of the plant is also depended upon the type and concentration of its phytochemical constituents, which are influenced by the age and parts of the plant used, genetic variation between species, climatic conditions and the soli profile of where the respective plants are found [35, 36]. The non-significant ($p>0.05$) weight gain in the exposed rats showed that the extract did not considerably affect their growth, indicative of the very low toxicity of the extract. This is because toxic chemicals or drugs adversely affect growth or weight gain in exposed animals [25].

The non-significant ($p>0.05$) increase in ALT activity showed that the extract did not cause considerable damage in the liver of the exposed rats. This is because serum enzymes are cytoplasmic and are only released into circulation in cellular damage [37] where ALT activity is more hepato-specific than AST activity [38]. Similarly, the non-significant ($p>0.05$) decrease in ALP activity showed that the extract did not cause hepatobiliary problems. The toxicological importance of the decreased AST activity is unknown [39] and well less understood compared to the significance of its increased activity [40]. However, Mgbojikwe [41] reported decreased ALT and AST activities but increased ALP activity in Wistar rats topically exposed to the aqueous extract of *A. obesum* stem bark.

The observed congestion and fatty degenerative changes might be due to the unique vascular, secretary, synthetic and metabolic features of the liver [42, 43]. This is because of its ability to degrade toxic compounds but can easily be overwhelmed by elevated

concentrations of these compounds resulting in its structural damage [44]. The hepatic fatty degeneration is indicative of metabolic disturbance, which is a normal feature of toxic exposures [45]. These changes are usually reversible except in some extreme cases where the functional efficiency of the affected liver might be affected [46. Similar congestion and fatty degenerative changes were reported in the liver of Wistar rats exposed to extracts of *Sorghum bicolor* leaf sheath [47]. The fact that the DTC in the liver of the exposed groups compared to the unexposed control group was non-significant ($p>0.05$) showed that the extract did not cause considerable liver damage or hepatotoxicity in the exposed rats.

5. Conclusion

In conclusion, ethanol extract of *A. obesum* stem bark did not cause major liver damage and therefore, is a safe oral medicinal plant within the limitations of the study's extract dose and exposure period in spite of the fact that the plant is a potent arrow poison. However, there is a need for further investigation over repeated and prolonged exposures.

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The work was examined and approved by the relevant ethical committee of the institution.

ACKNOWLEDGEMENT

The authors want to appreciate the efforts of Mr. Abdusalam Umar towards the work.

REFERENCES

1. Hostettmann K, Marston A, Ndjoko K, Wolfender J. The pontential of African plants as a source of drug. Curr Org Chem. 2000; 4: 973-1010.

2. Ahmad I, Agil F, Owais M. Modern phytomedicine: Turning medicinal plants into drugs. West-Sussex, England: John Wiley and Sons; 2006. Pp. 2-22.
3. Karim A, Nouman M, Munir S, Sattar S. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. Int J Pharmacol. 2011; 7: 419-439.
4. Hoareau L, Da silva EJ. (1999). Medicinal plants: A re-emerging health aid. Electronic J Biotechnol. 1999; 2(2): 56 – 70.
5. Humber, J.M. The role of complementary and alternative medicine: Accomodating pluralism. J Am Med Assoc. 2002; 288: 1655-1656.
6. Tédonga L, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Flejou JF, et al. Acute and subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Afr J Trad Compl Altern Med. 2008; 4(2): 140-147.
7. Ilodigwe EE, Akah PA, Nworu CS. Evaluation of the acute and subchronic toxicities of *Spathodea campanulata* P. Beauv. Int J Appl Res Nat Prod. 2010; 3(2):17-21.
8. Ouédraogo M, Zerbo P, Konaté K, Barro N, Sawadogo LL. Effect of long-term use of *Sida rhombifolia* L. extract on haemato-biochemical parameters of experimental animals. Br J Pharmacol Toxicol. 2013; 4(1): 18-24.
9. McLaughlin J, Garofalo J. *Desert Rose* (*Adenium obesum*). Miami-Dade County/University of Florida Cooperation Extension Services Fact-Sheet No. 17. 2002; Accessed 21 December 2010. Available: http://www.miami-dade.ifas.ufl.edu/pdfs/ornamental/ornamental_publications/desert-rose.PDF.
10. Zorloni A. Evaluation of plants used for the control of animal ectoparasitosis in southern Ethopia (Oromiya and Somali regions). Degree of Magister Scientiae Dissertation, South Africa: University of Pretoria; 2007.
11. Plaizier AC. A Revision of Adenium Roem and Schult. and of Diplorhynchus Welw. ex Fic. & Hiern (Apocynaceae). Wageningen, Netherlands: Mededelingen Landbouwhogeschool, Publication No. 80-12; 1980. Pp. 1-40.

12. Arbonnier M. Trees, Shrubs and Lianas of West African Dry Zones. GMBH, MNHN: CIRAD, MARGRAF Publishers; 2004. p. 161.
13. Oyen LPA. *Adenium obesum* (Forssk.) Roem. & Schult. In: Schmelzer GH, Gurib-Fakim A, editors. Plant Resources of Tropical Africa 11 (1): Medicinal Plant 1. Wageningen, Netherlands: PROTA Foundation/Backhuys Publishers/CTA; 2008. Pp. 46-49.
14. Tijjani A, Sallau MS, Sunusi I. Synergistic activity of methanol extract of *Adenium obesum* (Apocynaceae) stem-bark and oxytetracycline against some clinical bacterial isolates. Bayero J Pure Appl Sci. 2011; 4(1): 79-82.
15. Neuwinger HD. African Traditional Medicine: A Dictionary of Plant Use and Applications. Stuttgart, Germany: Medpharm Scientific; 2000. p. 589.
16. Bawden-Davies J. (2010). The Adenium Species. 2010; Accessed 21 December 2010. Available: http://www.ehow.com/about_6721472_adenium-species.html.
17. Anonymous. Plant Story: Desert Rose (*Adenium obesum*). 2011; Accessed 9 August 2011. Available: http://www.kew.org/science-conservation/save-seed-prospert/millenium-seed-bank/using-our-seeds/helping-communicate-worldwide/useful-plants-project/adenium-obesum/UPP-Adenium obesum.htm.
18. Jones DE. Poison arrows: North American Indian hunting and warfare. Austin: University of Texas Press; 2007. p. 27.
19. Sasidharan S, Darah I, Jain K. *In vivo* and *in vitro* toxicity study of *Gracilaria changii*. Pharm. Biol. 2008; 46: 413-417.
20. Chen XW, Serag ES, Sneed KB, Zhou SF. Herbal bioactivation, molecular targets and the toxicity relevance. Chem Biol Interact. 2011; 192 (3): 161–176.
21. Bentley AC. Textbook of Pharmaceutics. 8th ed. USA: Bailliére Tindall; 1977. Pp. 177-180.
22. Ghani A. Introduction to Pharmacognosy, 1st edn Zaria: Ahmadu Bello University Press Ltd.; 1990. p. 198.
23. Abu-Dahab R, Afifi F. Anti-proliferative activity of selected medical plants of Jordan against a breast adenocarcinoma cell line (MCF7). Sci Pharm. 2007; 75: 121-136.

24. Organisation for Economic Co-operation and Development (OECD). Guidelines for the Testing of Chemicals No. 423: Acute Oral Toxicity – Acute Toxic Class Method (adopted: 17th December, 2001). Paris: Organisation for Economic Co-operation and Development. 2001; 1-14.
25. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D, L-methylphenidate in Sprague-Dawley rats. Toxicol. 2002; 179: 183-196.
26. Schwartz MK, de Cediel N, Curnow DH, Fraser CG, Porter CJ, Worth HG, et al. International Federation of Clinical Chemistry, Education Committee and International Union of Pure and Applied Chemistry, Division of Clinical Chemistry: Definition of the terms certification, licensure and accreditation in clinical chemistry. J Clin Chem Clin Biochem. 1985; 23(12): 899-901.
27. King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activity. Can Med Assoc J. 1934; 31: 376-381.
28. Morgan JD, Iwana GK. (1997). Measurement of stressed states in the field. In: Iwana GK, Pickering AD, Sumpter JP, Schreck CB, editors. Fish Stress and Health in Aquaculture. Society of Exploratory Biology Seminar Series No. 62; 1997. Pp. 247-268.
29. Henry R, Canon DC, Winkelman JW. Clinical Chemistry: Principles and Techniques. Maryland, USA: Harper and Roe Publications; 1974. p. 543.
30. Tietz NW. Clinical Guide to Laboratory Tests. 2nd ed. Philadelphia, USA: W.B. Saunders Company; 1990. Pp. 554-556.
31. Roberts RJ. The patho-physiology and systemic pathology of teleost. In: Roberts RJ. editor. Fish Pathology. London: Bailliére Tindall; 1978. Pp. 55-91.
32. Bancroft JD, Cook HC. Manual of histological techniques and their diagnostic application. London: Churchill Livingstone; 1994. Pp. 289-305.

303 33. Poleksic V, Mitrovic-Tutundzic V. Fish gills as a monitor of sublethal and chronic
304 effects of pollution. In: Mulle R, Llyod R, editors. Sublethal and chronic effects of
305 pollutants on freshwater fish. Oxford: Fishing News Books; 1994. Pp. 339-352.
306 34. Simonato JD, Guedes CLB, Martinez CBR. Biochemical, physiological and
307 histopathological changes in the neotropical fish *Prochilodus lineatus* exposed to
308 diesel oil. Ecotoxicol Environ Saf. 2008; 69: 112-120.
309 *35.* Norton S. Toxic effects of plants. In: Klaassen CD, editor. Casarett and Doull's
310 toxicology: The basic science of poison. 5th ed. New York, USA: McGraw-Hill; 1975.
311 Pp. 841-853.
312 36. Sellappan S, Akoh CC, Krewer G. Phenolic compounds and antioxidant capacity of
313 Georgia-grown blueberries and blackberries. J Agric Food Chem. 2002; 50: 2432-
314 2438.
315 *37.* Sallie R, Tredger RS, Williams F. Drugs and the liver. Biopharma Drug Dispos, 1991;
316 12: 251-259.
317 38. Herfindal DR, Gourley ET. Textbook of therapeutic drug and disease management.
318 7th ed. Philadelphia, USA: Lippincott Williams and Wilkins; 2000. Pp. 83.
319 39. Ambali FS, Akanbi DO, Shittu M, Giwa A, Oladipo OO, Ayo JO. Chlorpyrifos-induced
320 clinical, haematological and biochemical changes in Swiss albino mice – Mitigating
321 effect by co-administration of vitamins C and E. Life Sci J. 2010; 7(3): 37-44.
322 40. Nanji AA. Decreased activity of commonly measured serum enzymes: Causes and
323 clinical significance. Am J Med Technol. 1983; 49(4): 241-245.
324 41. Mgbojikwe LO. Acaricidal properties of the aqueous stem bark extract of *Adenium*
325 *obesum*. PhD Dissertation. Jos: University of Jos; 2000.

326 42. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ.
327 Mechanisms of hepatotoxicity. Toxicol. Sci. 2002; 65 (2): 166-176.
328 43. Modi H, Patel V, Patel K. Hepatoprotective activity of *Aegle marrmelos* against
329 ethanol induced hepatotoxicity in rats. J Pharma Clin Res. 2012; 5 (4): 164-167.

44. Brusle J and Gonzalez G. The structure and function of fish liver. In: Munshi JSD, Dutta HM, editors. Fish morphology. India: Science Publishers Inc.; 1996.

45. Wolfe JC, Wolfe MJ. A brief overview of nonneoplastic hepatic toxicity in fish. Toxicol Pathol. 2005; 33 (1): 75-85.

46. Cotran RS, Kumar V, Collins T. Cellular pathology II: Adaptions, intracellular accumulations and cell aging. In: Cotran RS, Kumar V, Collins T, editors. Robbins's pathological basis of diseases. 6th ed. Philadelphia: W. B. Saunders Company; 1999. p. 39.

47. Ogunka-Nnoka CU, Uwakwe AA, Nwachoko NC. Serum enzyme and histological studies of albino rat treated with ethanol/potash extract of *Sorghum bicolor* leaf sheath. Indian J Drugs Dis. 2012; 1(3): 74-78.