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Original Article

EFFECTS OF CRUDE *Acacia nilotica* DEL. ROOT EXTRACTS IN MICE

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ABSTRACT

Acacia nilotica root extract is used for the management of malaria and other diseases in Northern Nigeria. An extract yield of 21.25% was obtained from dry *Acacia* roots on extraction with methanol. Alkaloids, glycosides, terpenoids, tannins and flavonoids were identified in the extracts. The safe dose of the crude extract was determined in mice to be 1000mg/kg with LD_{50} of 400mg/kg body weight. Forty mice were divided into two groups which served as test and control respectively and were assessed for the long term effects of crude acacia extracts over a five week period. The test groups were gavaged with 300mg/kg body weight of the crude extract daily, while the control groups were each given normal saline (20ml/kg b.w) over the study period. The results indicated significant ($P<0.05$) decreases in whole body weight and Packed Cell Volume (PCV). Serum Triglycerides, Glutamate Pyruvate Transaninase (SGPT), Glutamate Oxaloacetate Transaminase (SGOT) and Chloride were significantly ($P<0.05$) elevated. No significant ($P>0.05$) effects were obtained with Fasting Serum Glucose, Total Proteins, Alkaline Phosphatase (ALP), Sodium and Potasium ions. Histopathological examinations indicated no changes in cardiac, pancreatic, spleen and intestinal tissues. However, a feathery degeneration of hepatocytes and destruction of nephrons were observed.

Keywords – *transaminases, hepatocytes, Acacia nilotica, nephrons.*
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INTRODUCTION

Acacia nilotica Del. (Leguminoseae) is a spiny tree that averages 8 meters in height, reportedly native to Egypt but spread to the Arabian Penninsula, Indian subcontinent and most of Africa (Fagg, 1990).

It is referred to as "Gabaruwa" (Hausa) or "Bagaruwa" (Nupe) in the North of Nigeria where leaves, stem bark and root are useful as medicaments for different ailments (Tybrik, 1989). The pods are applied topically or inhaled as fumes when heated. Aqueous extracts are taken orally as treatment for diarrheoa, cough, fever, common cold and influenza (Al – Ghazali *et al.,* 1987). Roots are reported as useful in leprosy and as aphrodisiac (Sofowora, 1982). The stem bark is applied against ulcers, cancers, tumors, haemorrhoids, small pox, gall bladder disease, indurations of the liver and spleen (Zaki and Abdullah, 2000). Crude extracts are used in the treatment of malaria fever (Abdullahi *et. al.,* 2003, Jigam *et al.,* 2009). It is also reportedly useful as analgesic, antipyretic and anti – inflammatory agent. *Acacia root extract* was reported to be effective against *Plasmodium berghei* in mice (Jigam *et al.,* 2010).

A common problem with the use of crude plant extracts in ethnomedicine is the lack of thorough evaluation of the toxicity profiles of such species (Gamaniel, 2000). A blanket assumption should therefore not be made about their safety. It is also therefore necessary to ensure a thorough and detailed pharmacological and toxicological assessment of these plants. The present study was performed to investigate the potential toxicity of long term consumption of *A. nilotica* extracts in mice. This will help in advising individuals that use *Acacia* extracts in the treatment of different ailments.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used were of analytical grade and obtained from reputable Scientific and chemical companies. Dimethyl Sulphoxide (DMSO) was obtained from Sigma Chemicals St Louis, MO. USA; Suitable diagnostic Kits e.g. the Randox Glucose (Cat/Kat NR GL 2623), Randox Protein (TP245), Agappe Triglyceride kit (Cat 1121500 Kerala India), Dialab IVB Transaminase Kit and Alkaline phosphatase Kit (Dialab Cat. D95560) were used for the assay of serum biochemical parameters. Hemotoxylin – eosin (Sigma – Aldrin Europe) was employed in staining sectioned tissues.

Plant Materials

Fresh roots of *A. nilotica* were collected between June – September, 2009 from Tudun Fulani area of Minna, Northern Nigeria after identification by a staff at the Department of Biological Sciences, Federal University of Technology Minna.

Preparation of Extracts

40 g of air - dried roots were crushed into powdery form and extracted in the cold with 1.5 L methanol. The extract was filtered with Whattman 5 filter paper and solvents removed under reduced pressure in a rotary evaporator. Brown coloured pastes were obtained and weighed prior to further analysis.

Animals

Healthy male and female Swiss albino mice weighing between 20.00 ± 1.23 – 30.00 ± 2.14g were obtained from the National Institute of Pharmaceutical Research and Development (NIPRD) Abuja and used for the experiment. The rodents were housed in standard environmental conditions: temperature; 27±2°C; 70% relative humidity; free access to pellets and water and natural 12h day light/night cycles. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal care Guidelines and Protocol Review (CCAC, 1997).

Phytochemical Screening

Standard screening tests were used to detect the presence of alkaloids, flavonoids, tannins, saponins, glycosides, caffeine and terpenoids in the extract (Odebiyi and Sofowora 1978; Sofowora, 1982; Trease and Evans, 1989).

Safe dose and acute toxicity [LD_{50}]

Five groups of four mice each were used and the animals were gavaged extracts at doses of 200, 400, 600, 800, 1200mg/kg body weight (bw) respectively. The extracts were initially dissolved in small amounts of dimethylsulphoxide and then suspended in distilled water.

The control group was given normal saline (0.9% w/v NaCl) at 20 ml/kg body weight. The mice were observed over a 72 hour period for any adverse reactions and mortality was recorded.

LD_{50} was determined by the Lorke (1983) method.

Evaluation of the effects of long term dosage of Crude Extract in Mice

Forty mice were divided into two groups (A and B) of twenty each. Group A was used as test and gavaged with 300mg/kg bw extract daily while B was control and given 20ml/kg bw normal saline daily. All animals were monitored for different biochemical parameters over 40 days at weekly intervals.

Weights of mice were taken with Avery Balance (W and T) Avery Ltd, Birmingham, UK. Fresh organ (liver, kidneys, pancreas, spleen, intestine and heart.) weights were taken for each sacrificed mice using a metler PT1200 analytical balance. Packed cell volume (PCV) was determined using the micro haematocrit method (Green, 1976). Serum glucose was assayed based on the glucose oxidase reaction. Total proteins were evaluated based on the interaction of cupric ions in alkaline media with protein peptide bonds. Triglyceride assay was based on reactions by lipases, glycerol kinase, glycerol – phosphate oxidase and peroxidase which produced a red quinone dye read at 630nm (Annoni, *et al.*, 1982).

Transaminases (SGPT and SGOT) were analyzed based on the method of Wolf (1980). Alkaline phosphatase (ALP) was however determined on the basis of the conversion of P – nitrophenol to its intensely yellow coloured derivative, 4 – nitrophenoxide (Tietz, 1983).

Serum sodium and potassium were determined by flame photometry (Vogel, 1964). While chloride ions were by the Schales and Schales (1941) titrimetric method.

Histopathological evaluations were performed at the end of the 35 – day period of experiment. Lung, heart, liver, kidney, spleen, pancreatic and intestinal tissues were fixed in 10% buffered neutral formaldehyde for 48h, embedded in paraffin, and sectioned (6nm) with a microtome. Serial sections were stained with hematoxylin-eosin prior to microscopy.

Statistical Analysis

The biochemical data were subjected to some statistical analysis. Values were reported as Mean $\pm$ SEM while student's t-test was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 16.A value of $P<0.05$ was accepted as significant.

RESULTS

Results of the phytochemical screening of *A. nilotica* root extract are given in Table 1. Alkaloids, glycosides, terpenoids, tannins and flavonoids were detected, buts aponins and caffeine were not detected.

There was a decline in the total body weight of mice (Fig. 1). Packed Cell Volume (PCV) of the animals also dropped (Fig 2). Serum glucose and proteins in the test mice showed no significant difference ($P>0.05$) from the control but triglycerides were slightly elevated (Table 2).

Serum transaminases (SGOT and SGPT) were significantly ($P<0.05$) higher in test mice but Alkaline phosphate levels were normal (Table 3). Serum electrolyte values are given in table 4.

Sodium and potassium ions were not elevated but chloride levels were significantly ($P<0.05$) higher in the test mice.

Plates Ia and b and IIa & b represent liver and kidney tissues respectively showing pathologica alterations occurred in the two organs in test mice.

Table 1: Phytochemical Constituents of *A. nilotica* root.

Active Principle	Inference
Alkaloids	+
Morphine alkaloids	+
Glycosides	+
Cardiac glycosides	-
Terpenoids	+
Tannins	+
Flavonoids	+
Saponins	-
Caffeine	-

+ = Present

- = not detected

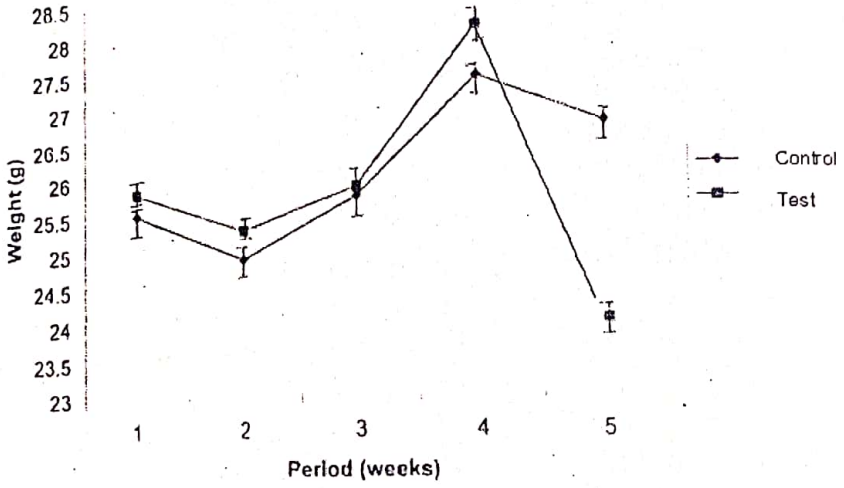

Figure 1: Weight variations in mice dosed with crude *A. nilotica* extracts over a 5 week period

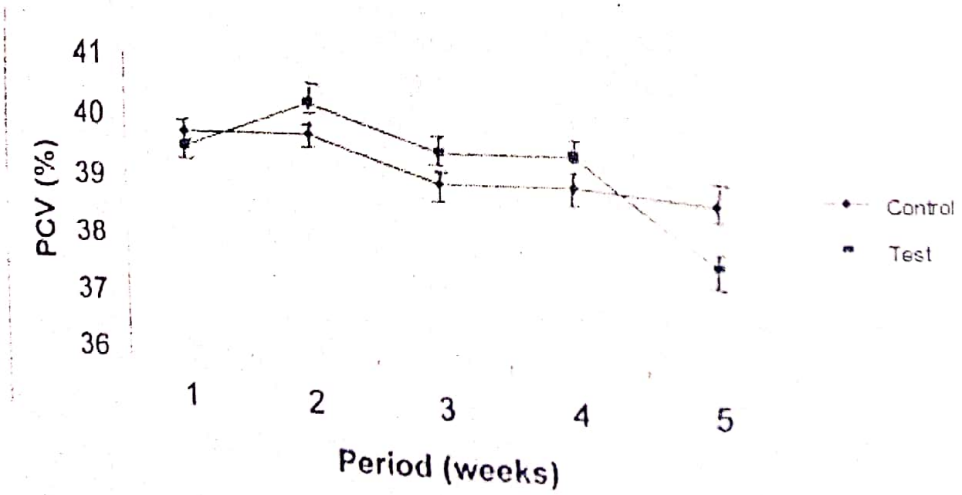

Figure 2: Variations in packed cell volume of mice dosed with crude *A. nilotica* extracts over a 5 week period

Table 2: Serum Glucose, Triglycerides and Total Protein levels in mice dosed with crude *A. nilotica* extract over a 5 week period

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
Glucose (mg/dL) control: $\bar{x} \pm SEM$ n = 20	93.58±5.55	99.29±10.39	94.08±15.51	94.99±9.85	107.64±2.08
test: n=20	92.57±6.28	95.00±15.62	109.87±10.10	108.47±5.32	109.03±2.63
Triglycerides (mg/dL) control: $\bar{x} \pm SEM$ n = 20	148.81±6.20	160.23±13.04	137.78±10.74*	142.39±18.82	150.97±14.45
test: n = 20	160±7.23	154.55±17.90	182.22±11.76	145.73±16.82	170.93±7.44
Total proteins (mg/dL) control: $\bar{x} \pm SEM$ n = 20	6.96±1.23	6.11±0.43	7.38±1.16	6.86±0.73	4.66±0.31
test n = 20	7.02±0.77	6.29±0.194	5.46±0.63	5.63±0.16	5.20±0.47

*Significant ($p<0.05$)

Table 3: Some serum enzyme levels in mice dosed with crude *A. nilotica* extract over a 5 week period

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
Glutamate Oxaloacetate transaminase (SGOT) (I.U)					
control: n = 20	37.25±1.03	35.25±1.25	34.75±1.25	33.75±1.25	32.00±1.47
($\bar{x}$ ± SEM) test: n = 20	44.50±0.87*	45.75±0.85*	51.75±1.93*	51.00±1.87*	55.75±2.36
Glutamate Pyruvate Transaminase (SGPT) (I.U)					
control: n = 20	29.50±0.65	30.00±1.83	29.50±0.96	29.00±1.08	29.25±1.11
($\bar{x}$ ± SEM) test n = 20	33.50±1.04*	37.75±0.85*	39.75±1.75*	42.50±1.32*	45.25±1.89*
Alkaline Phosphatase (ALP) (I.U) control n = 20	51.50±4.92	47.75±3.97	39.75±2.32	37.75±5.39	34.50±5.1
($\bar{x}$ ± SEM) test n = 20	50.75±1.25	48.75±1.11	40.75±1.11	38.00±0.823	41.00±1.2

* = Significant ($p<0.05$)

Table 4: Serum Electrolytes in mice dosed with crude *A. nilotica* extract over a 5 week period.

Sodium (mmol/L) ($\bar{x}$ ± SEM)	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
control: n = 20	124.50±4.03	126.50±4.65	127.25±3.60	130.25±5.72	134.00±2.58
test: n = 20	126.25±1.93	127.00±1.58	126.50±2.50	131.50±2.06	139.00±1.29
Potassium (mmol/L) control: n = 20	3.20±0.32	3.40±0.20	3.20±0.32	3.55±0.30	3.35±0.28
($\bar{x}$ ± SEM) test n = 20	3.83±0.19	3.28±0.18	3.70±0.21	3.20±0.18	3.22±0.26
Chloride (mmol/L) Controls n = 20	101.25±1.80	103.50±3.30	105.25±3.40	105.00±2.48	101.50±3.01
($\bar{x}$ ± SEM) test n = 20	102.50±4.87	103.75±1.75	105.50±2.63	109.00±3.42	110.50±0.90*

*Significant ($p<0.05$)

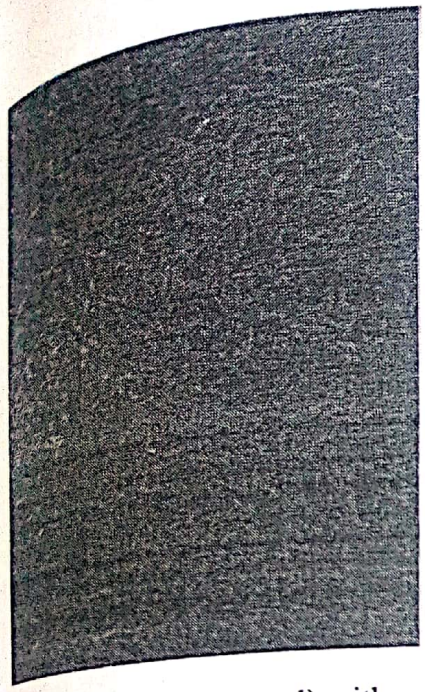

Plate Ia: Liver tissue (Control) with normal cellular hexagonal archs.

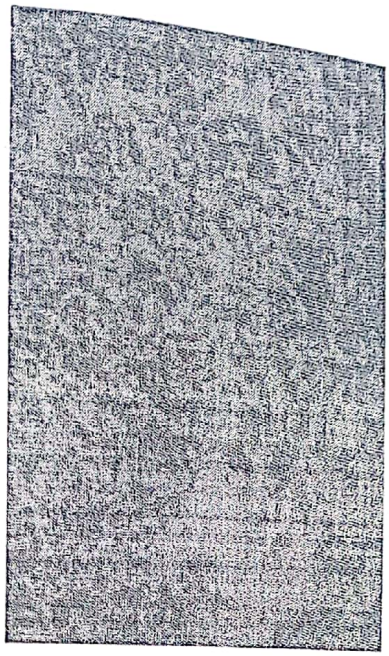

Plate Ib: Liver tissue (Test) with feathery disintegration of hepatocytes.

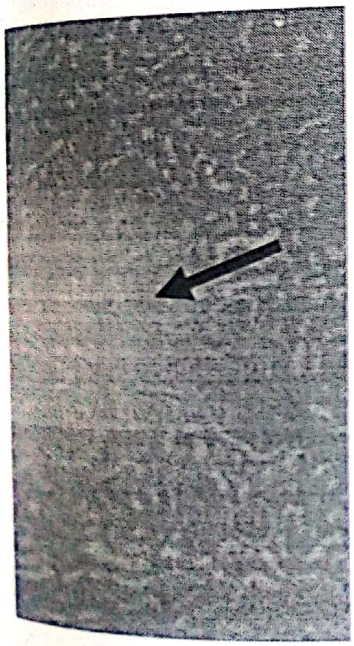

Plate IIa: Kidney tissue (Test) with ghost-like nephron

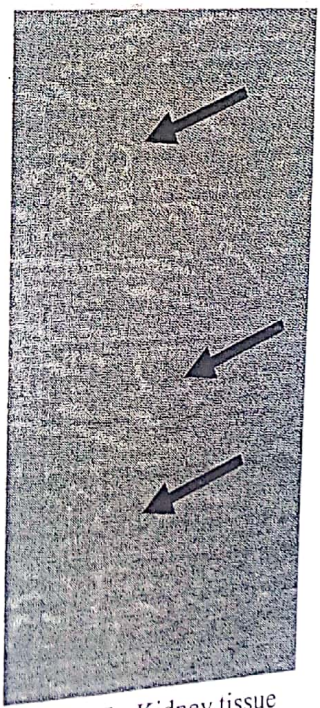

Plate IIb: Kidney tissue (Control) with intact nephrons

DISCUSSION

The detection of alkaloids, glycosides, terpenoids, tannins and flavonoids in crude extracts of the roots of *A. nilotica* is in conformity with earlier reports (El – Sayyad and Ross, 1983; Zaki and Abdullah, 2000).

The steady decline in whole body weights of mice in the present study is similar to earlier findings which attributed the effect to the presence of high tannin levels in Acacia. Tannins inhibit growth by decreasing the digestion coefficients of most nutrients and the coagulation of proteins (El-Sayyad and Ross, 1983; Sotohy *et al.*, 1997). This also further substantiated the significant decline in the packed cell volume of mice studied. Antinutritive factors chelate minerals e.g. iron and adversely affect the bioavailability of vitamins required for hemopoiesis (Shermer, 1967; Jigam *et al*; 2011).

Serum glucose levels of test mice were within normal values (Loeb and Quinby, 1989). No significant ($P>0.05$) difference existed between values for test and control mice which also corresponds with the findings of Zaki and Abdullah (2000). Alterations in serum glucose levels other than those associated with stress are uncommon and reflect an effect on the pancreatic islets of langerhans responsible for insulin production or anorexia (Gad, 2001). It can thus be concluded that crude *A. nilotica* extract is not toxic at least to the pancreas.

Triglycerides were significantly ($P<0.05$) elevated in the test animals over the control in the third week. The *Acacia* treatment had a hyperlipidemic effect in the course of the experiment. Other authors however reported no alterations in lipid levels when rodents were treated with *A. nilotica* (Zaki and Abdullah, 2000). Triglycerides are rich alternative sources of metabolic energy and are readily mobilized when the need arises (Martin *et al.*, 1975) as in the stressed state of experimentation to which these animals were subjected.

Total proteins generally declined in the test mice on *Acacia* treatment but controls were however within levels cited in the literature (Mitruka and Rawnsley, 1977). Zaki and Abdullah (2000) observed a significant ($P<0.05$) reduction in the levels of total proteins in rats fed 8% *Acacia* diets in four weeks, an effect that was however reversible upon termination of the treatment. Absolute alterations in total serum proteins are usually associated with decreased production by the liver or increased loss from the kidney (Gad, 2001). Thus long term intake of *Acacia* can predispose to hepatic and kidney disease

Serum Glutamate Oxaloacetate Transaminase (SGOT) levels in the test mice were consistently and significantly ($P<0.05$) elevated over the values obtained for the controls. Zaki and Abdullah (2000) reported fluctuations in the levels of SGOT in rats fed *Acacia* pods, the effect of which was reversible upon return to normal diets. Elevation in SGOT is usually associated with damages to skeletal and heart muscles. It is also high in kidney, liver and pancreatic disorders. The prognosis cannot be definitive unless other parameters and histopathological evidence are considered (Loeb and Quinby, 1989).

Serum Glutamate Pyruvate Transaminase (SGPT) was significantly ($P<0.05$) higher in test mice over the values for controls. Values for controls were within the normal range given by Okerman (1988). Elevations in SGPT are rarely observed except in parenchyma liver disease (Kachmar *et.al.*, 1973; Gad, 2001; Haschek and Rousseaux, 1991). The result obtained hence indicate that

SGOT and SGPT are sensitive to *acacia* intoxication.

Alkaline Phosphatase (ALP) values showed some fluctuations among the mice tested but there was no significant difference ($P>0.05$) with the controls. A general trend of decline was rather noted. Zaki and Abdullah (2000) earlier reported some slight fluctuations without much alteration in the levels of ALP in rats fed 2% and 8% *A. nilotica* respectively. Raised ALP levels are usually encountered in biliary disorders such as obstructive jaundice and cirrhosis, bone and intestinal diseases (Tietz, 1983). Such results should necessarily be interpreted in conjunction with other parameters e.g. bone alkaline phosphatase tends to be higher in young animals (Gad, 2001).

Mean serum sodium levels in the test mice showed some variations in relation to the controls. A general trend of increased concentration was noted in the test animals over the controls but was not significant ($P>0.05$).

In the case of potassium, a steady decline was noted among the test mice otherwise no significant difference ($P>0.05$) exists between the test and control groups. Chloride was generally elevated with a significant ($P<0.05$) difference between the tests and controls. Serum electrolytes interact with each other; a decrease in one is frequently tied for instance to an increase in one of the others (Gad, 2001). In the present study, the controls were within literature values (Mitruka and Rawnsley, 1977) but increased levels in the test animals is indicative of some respiratory or renal toxicity subject to histopathological and other test results (Boorman *et al.,* 1990). Biochemical parameters are rarely independent of each other rather, there are many of the parameters associated with toxic actions at particular target organs. For example, increases in ALP, SGOT and SGPT occurring together are strongly indicative of liver parenchyma cell damage and elevated electrolyte levels are associated with renal damage (Gad 2001).

The histopathological analyses of lungs, heart, liver, kidney, pancreas, spleen and intestinal tissues of mice treated with *A. nilotica* root extracts showed most to be devoid of any significant anatomical alterations or lesions except the liver and kidney. A feathery degeneration of hepatic tissue was noticeable and could be indicative of a compromise in liver functions (Boorman *et al.,* 1990). This finding combined with the earlier decrease noted in total proteins but elevation in SGOT and SGPT further indicates the hepatoxicity of *A. nilotica* if higher concentrations are consumed over a longer period (Haschek and Rouseaux, 1991). The destruction of some glomeruli noted with the kidney tissues and the elevation in serum sodium and chloride ions also points to the likelihood of the nephrotoxic effect of *A. nilotica* root extracts if the 300mg/kgbw dose regimen is consumed over a long period.

It can thus be concluded that long term consumption of *acacia* root in the treatment of diseases can predispose to organ damage.

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REFERENCES

Abdullahi M., Mohammed G. and Abdulkadir G. (2003). *Medicinal and Economic Plants of Nupe land*, Nigeria. Evans Books Nigeria. pp. 161.

Al – Ghazali, G. E., Bari, E. A., Bashir, A. K. and Salih, A. K. M. (1987). In: *Medicinal plants of the Sudan part II National*

Council for research, medicinal and aromatic plants research institute, Khartoum, Sudan. P. 14.

Annoni, G. G., Bottasso, R. M., Ciaci, D., Donato, M. F. and Tripoli, A. (1982). Clinical Investigation of Triglycerides. *J. Res. Lab. Med.* 9: 115.

Boorman, G. A., Eustis, S. L., Elwell, M. R. and Mackenzie, W. F. (1990). *Pathology of the Fischer Rat-Reference and Atlas.* Academic Press, San Diego. P 58 – 68.

Canadian Council on Animal Care CCAC. (1997) *Guidelines on: Animal use and Protocol Review.* P. 135.

Dafallah, A. A. and Al – Mustafa, Z. (1996). Investigation of the Anti – Inflammatory Activity of *Acacia nilotica* and *Hibiscus sabdariffa. AM. J. Chin. Med.*, 24 (3 – 4): 263 – 269.

El – Sayyad, S. M. and Ross, S. A. (1983). A Phytochemical Study of Some *Cassia* Species Cultivated in Egypt. *Nat. Prdts. Res.,* 46(3): 431 – 432.

EPA/OPPTS. (1988). A 90 – day Toxicity Tests in Rodents. OPPTS Test Guideline 870 – 3100. U.S. Government Printing Office, Washington, D.C. p. 38 – 47.

Fagg, C.W. (1990). Greaves *Acacia nilotica* Annotated Bibliography No. F42. Oxon UK: International. P. 5 – 10.

Gad, S. C. (2001). *Statistics for Toxicologist. Principles and Methods of Toxicology.* Taylor and Francis, Philadelphia. P. 351 – 353.

Gamaniel, K. S. (2000). Toxicity From Medicinal Plants and their Products. *Nig. J. Nat. Prdts. Med.*, 4: 4 – 8.

Green, J. H. (1976). *An Introduction to Human Physiology,* 4th Ed. Oxford Medical Publications London. Pp. 128 – 130.

Haschek, W. M. and Rousseaux, C. G. (1991). *Handbook of Toxicologic Pathology.* Academic Press, San Diego. P. 88 – 104.

Jigam, A. A., Akanya, H. O., Ogbadoyi, E. O., Dauda, B. and Evans, E. C. (2009). In-vivo Antiplasmodial, Analgesic and Anti – inflammatory Activities of the Leaf Extract of *Lippia multiflora mold. J. Med. Plts. Res.,* 3 (3): 148 – 154.

Jigam, A. A., Akanya, H. O., Ogbadoyi, E. O. and Dauda, B. (2010). Invivo Antiplasmodial, Analgesic and Anti – inflammatory Activities of the root extracts of *Acacia nilotica Del. Asian J. Exptl. Biol. Sci., 1(2):315-320*

Jigam, A. A., Dauda, B., Jimoh, T., Hauwa, N. Y. and Umar, Z. T. (2011). Determination of Copper, Zinc, Lead and some biochemical parameters in fresh cow milk from different locations in Niger State, Nigeria. *African Journal of Food Science,* 5 (3): 156 – 160.

Kachmar, B. C., Lewis, S. M. and Okerman, L. (1973). *Anatomy, Physiology and Biochemistry of Rabbits.* Academic Press New York. Pp 60 – 70.

Loeb, W. F. and Quinby, F. W. (1989). *The Clinical Chemistry of Laboratory Animals.* Pergamon Press, New York. P. 345.

Lorke, D. (1983). A new approach to practical acute toxicity. *Toxicology,* 54: 275 – 287.

Martin, H. F., Gudzinowicz, B. J. and Fanger, H. (1975). *Normal Values in Clinical Chemistry.* Marcel Dekker, New York. P. 50 – 73.

Mitruka, B. M. and Rawnsley, H. M. (1977). *Clinical, Biochemical and Haematological Reference values in Normal animals.* Mason, New York. P. 112 – 154.

Muthaura, C. N., Rukunga, G. M., Chhabra, S. C., Omar, S. A., Mwitari, P.G.,

Keter, L. K., Kirira, P. G., Kimani, C. N., Mungai, G. M. and Njagi, E. M. (2007). Antimalaria Activity of Some Plants Traditionally used in Treatment of Malaria in Kwale District of Kenya. *J. Ethno pharmacol.,* 2007; 112(3): 545 – 551.

Odebiyi, O. O. and Sofowora, E. A. (1978). *Phytochemical Screening of Nigerian Medicinal Plants II,* 41: 234 – 235.

Okerman, L. (1988). *Diseases of Domestic Rabbits.* Black Well Scientific Publication, Oxford, London. P. 13 – 15.

O'Neil, M. J., Bray, D. H., Boarman, P., Chan, K., Phillipson, J. D. and Warhurst, D. C. (1987). Activity of *Brucea javanica* fruits against *Plasmodium falciparum in vitro* and *P. berghei in vivo. J. Nat. Prdts.,* 50: 41 – 48.

Schales, O. and Schales, S. S. (1941). A Simple and Accurate Method for the Determination of Chloride in Biological Fluids. *J. Biol. Chem.*, 140: 879 –884.

Shermer, S. (1967). *The Blood Morphology of Laboratory Animals.* Academic Press, New York. P. 69.

Sofowora, A. (1982). *Medicinal Plants and Traditional Medicine in Africa.* John Wiley and Sons Ltd. USA. Pp. 142 – 145.

Sotohy, S. A., Sayed, A. N. and Ahmed, M. M. (1997). Effect of Tannin – Rich Plant (*Acacia nilotica)* on Some Nutritional and Bacteriological Parameters in Goats *German J. Sci.*, 104(10): 432 – 435.

Tietz, N. W. (1983). A Reference Method for the Measurement of ALP Activity in Human Serum. Study group on ALP. *J. Clin. Chem.,* 29: 751.

Trease, G. E. and Evans, W. C. (1989). Pharmacognosy. Eng. Lang. Bk. Soc. Bailliare Tindall Britain. Pp. 378 – 480.

Tybirk, K. (1989). Flowering, pollination and seed production of *Acacia nilotica. Nordic J. Bot.*, 9(4): 375 – 381.

Vogel, A. L. (1964). A textbook of quantitative inorganic analysis. Longmans and Co. London. P. 181.

Wolf, S. (1980). Assay of SPT. *Clin. Chem. Acta.*, 105: 147 – 172.

Zaki, H. A. and Abdullah, A. D. (2000). A study of the toxicology of *Acacia nilotica. AM. J. Chin. Med.,* 25(5): 280 – 282.