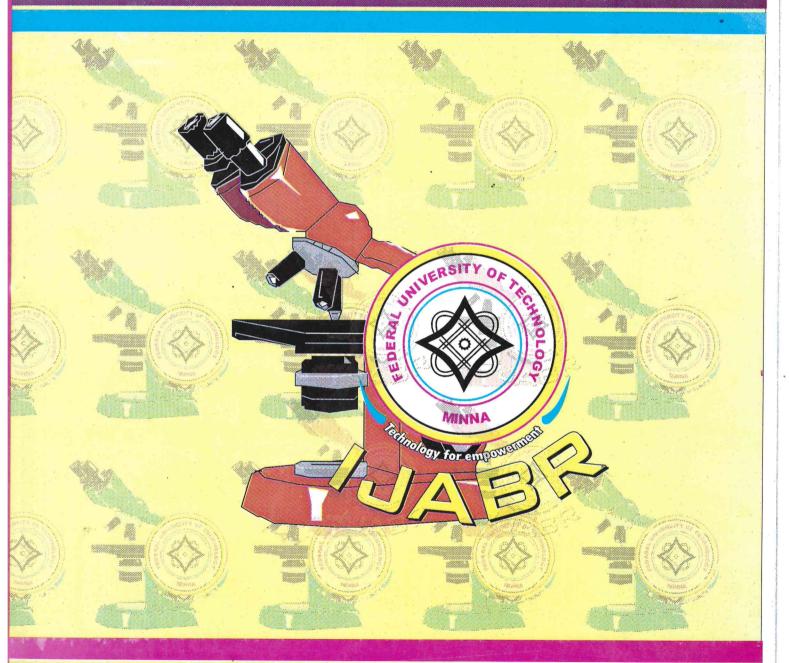
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Short Communication

MINERAL ELEMENTS AND HEAVY METALS IN SELECTED FOOD SEASONINGS CONSUMED IN MINNA METROPOLIS

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ABSTRACT

Eight samples of food seasonings: Ajinomoto, Maggi, Royco, Vedan, Knorr, Dinor, Onga stew, and Onga classic were obtained from Minna Central Market and were digested using standard laboratory techniques. Investigations on the concentrations of some mineral elements, Sodium, Potassium and Calcium and two heavy metals, Cadmium and Lead, were carried out using atomic absorption spectrophotometer. Royco had the highest calcium concentration $(0.022+0.001\mu g/g)$, with Onga classic having lowest $(0.002+0.00~\mu g/g)$. Potassium is highest $(0.020+0.016~\mu g/g)$ in Vedan, and lowest $(0.003+0.001~\mu g/g)$ in Ajinomoto. Sodium is highest $(0.021+0.011~\mu g/g)$ in Dinor, and lowest $(0.003+0.002~\mu g/g)$ in Knorr. Cadmium and Lead were both present in high concentrations in all the samples analysed. The presence of Cadmium and Lead in the food seasonings even at low concentrations could prove fatal through bioaccumulation.

Keywords: bioaccumulation, heavy metals, mineral elements, food seasoning, toxification

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INTRODUCTION

Food seasoning is a substance that adds flavour to food, for example salt, peppers, and other spices. Spices are vegetable substances of indigenous or exotic origin which are aromatic and have hot piquant tastes, used to enhance the flavour of foods or to add to them the stimulant ingredient contained in them (Cobley, 2002). Seasonings can also be used to replace common salt in a great variety of other industrially prepared food items as well as in the preparation of foods both in restaurants, catering, home kitchen etc.

Such seasonings are particularly suitable for soups, beefs, and other foods in which salty, and/or spiced seasonings are used. The ingredient mixture and seasonings when added to various food items change the food composition (Susan and Anne, 1998). The unique seasoning being vegetable product contains not only most of the substances in vegetable but also contains fractions which are not present in some vegetable products. It is this fraction that gives it the characteristic as a spice (Borget, 1993). Some mineral

elements such as magnesium, calcium and potassium are necessary for enzyme activities and are included in food seasonings. Ingestion of foods with these combinations leads to a significant decrease in both cholesterol level and blood pressure. Mineral elements are inorganic elements that serve a variety of functions such as co-factors in enzyme catalysed reactions, in the regulation of acid-base balance, in nerve conduction and muscle irritability and as structural elements in the body e.g calcium (Merril and Morton, 2001). Accumulation of these mineral elements by excessive consumption can lead to long-term health risk. The heavy metals on the other hand have different effects on organisms, depending on the stage of development of the organism. Some of these effects of metals are: carcinogenicity, neurotoxicity and reproductive failure. Metal such as cadmium derives its toxicological properties from its chemical similarity to Zinc (an essential micronutrient for plants, animals and man). Cadmium therefore replaces Zn in many of the reactions where Zn plays a role as a co-factor, thereby disrupting cellular activities. Lead on the other hand, manifests its toxicity in neurological, haematological, renal, endocrine and reproductive systems. Lead inhibits several of the enzymes involved in haem biosynthesis especially the synthesis of δ aminolevulinic acid by aminolevulinic acid synthase to porphobilinogen (Vallee and Ulmer, 2006). Spices as seasonings constitute a huge component of transboundary trade in areas such as India, China, Indonesia, East and West Africa. In all parts of Nigeria, seasoning is used to prepare foods and some of these seasonings are believed to aid uterus contraction in pregnant (Achinewy et al., 1995). Although the risk

of the food seasonings can only be identified after a long period, there is need for periodic assessment of their concentrations. The world Health Organisation (WHO, 1983) has therefore set an acceptable limit of 0.0 μ g/g for cadmium and lead in food seasonings and 0.4 μ g/g for calcium, potassium, and sodium. The National Agency for Food and Drug Administration Control (NAFDAC), has adopted these limits.

MATERIALS AND METHODS

Sample Collection

Eight samples of food seasonings were obtained from Minna Central Market. They include samples coded as: Ajinomoto, Royco, Maggi, Knorr, Dinor, Vedan, Onga stew and Onga classic.

Sample Digestion

2 g of food seasoning sample was weighed and transferred to the digestion tube. 10 ml of H_2O_2 , 5 ml of H_2SO_4 and 5 ml of HCl were added to the sample. The digestion bottle was placed on the block digestor while the temperature rose to $160\,^{\circ}C$. The sample was boiled for two hours. The digestate was allowed to cool, and filtered through Whattman No. 1 filter paper. The filtrate was transferred to the volumetric flask and made up to $100\,^{\circ}$ ml using distilled deionised water (Smith and Schenk, 1998). The samples were kept in plastic bottles till ready for analyses.

Sample Analyses

Atomic absorption spectrophotometer (Schimadzu AA650) was used for the estimation of cadmium, lead, sodium, potassium and calcium as described in the Pye Unican Atomic Absorption data book (Whiteside, 1984), and in the introduction to atomic absorption

spectrophotometer scientific equipment book.

Principle

Atomic absorption is a physical process involving the absorption by free atoms of an element of light, at a wavelength specific to that element. The sample to be analysed is digested and dissolved in an aqueous medium. The solution is placed in the instrument where it is heated to vaporize and atomize the elements.

A beam of radiation is passed through the atomized sample, and the absorption of radiation is measured at specific wavelengths corresponding to the element of interest. Information about the type and the concentration of element is obtained by measuring the location of the peaks in the absorption spectra (Peter, 1979).

RESULTS

Ajinomoto had a concentration $0.04 \mu g/g$ of Calcium and Sodium, 0.03µg/g of Potassium and Lead and 0.02μg/g of Cadmium respectively. Royco had 0.03µg/g of Lead and Cadmium, 0.02μg/g of Calcium and 0.01μg/g of Potassium and Sodium respectively. Maggi had 0.02µg/g of Cadmium and Lead and 0.01µg/g of Calcium, Potassium Sodium. Knorr, Dinor and Ongar Stew had 0.03µg/g of Lead, 0.02µg/g of Cadmium and 0.01µg/g of Potassium and Sodium. Vedan had 0.02μg/g of Potassium, Sodium, Cadmium and Lead and 0.01µg/g of Calcium. While classic had Onga the highest concentration of Lead 0.05µg/g, Calcium and Cadmium 0.02µg/g, and Potassium and Sodium 0.01 µg/g respectively (Table

Table 1: Concentration of Mineral Elements and Heavy Metals ($\mu g/g$) in Food Seasonings in Minna Central Market.

Food Seasoning	Calcium	Potassium	Sodium	Cadmium	Lead
Ajinomoto	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Royco	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
Maggi	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 + 0.01
Knorr	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.00
Dinor	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.01
Vedan	0.01 ± 0.00	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.00
Onga stew	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.02
Onga classic	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.05 ± 0.00

DISCUSSION

All the food seasonings sampled and analysed for heavy metals had values above the limit of $0.0 \mu g/g$ by NAFDAC. Bioaccumulation of this little but very significant concentration of lead in tissues of man can affect the activities of some enzymes like δ-aminolerulinic acid (involved in haem biosynthesis). superoxide dismutase (SOD), catalyse, glutathione S-transferase (GST) and glutathione. This accumulation may lead to reactions which generate reactive oxygen species (ROS), thereby leading to oxidative stress. Lead toxicity is known to inhibit the action of these enzymes because they have free sulphydyl groups. This is particularly noted with the precursors of haem, and leads to a decrease in haem synthesis. and hence to anaemia. Irrespective of the way a lead compound enters the body, it first penetrates the initial cellular barrier before reaching the intracellular fluid. The compound then penetrates the capillary blood vessels and thus enters the circulatory system which distributes it throughout the body (Petersdorf et al., 1999). Majority of the lead compounds do not cause damage at the site where they enter the body. The absorption process is the beginning of the path consisting of distribution, biotransformation, accumulation, and elimination of the lead compounds. In to provoke symptoms poisoning, lead and its metabolite must first penetrate a target organ which is susceptible to its action, and at the same time the concentration of the toxin must be sufficiently high and appears at the site at a definite time. The target organ is the point of anatomical preference for the appearance of the symptoms of poisoning by lead or its compounds (De Silva, 2007). The health implication of cadmium in man is that it shares the same oxidation state and structural

similarity with Zn (which is a beneficial heavy metal). And because of this, Cd readily replaces Zn in many reactions where Zn acts as a cofactor, thereby disrupting the cellular and enzyme activities. Following oral exposure of cadmium, the metal is transported in the blood by the erythrocytes or bound to low molecular weight proteins (e.g. metallothionein). Cadmium is taken up by liver cells, and is slowly released back into the plasma. Because of the small of size cadmium-metallothionein complex, it passes freely through the glomerulus, and into the renal tubule (Nordberg et al., 2001). Cadmium bound to metallothionein is efficiently taken up in the tubule by the pioncytosis. Within the renal tubular cells, the pioncytosis vacuoles fuse with lysosomes which degrade the metallothionein, thereby freeing the cadmium. The cadmium then combines with the newly synthesized metallothionein produced by the tubular cells, and accumulates in the kidney for a long time. Metallothionein is inducible in the liver and kidney by the cadmium, and other metals (Yousuf and El-Shahawi, 2000). Cadmium is stored in the kidney, and liver and very little is eliminated from the body until renal toxicity occurs. Thereupon the renal excretion increases. and levels of cadmium diminish in the particularly in the kidney (El-Hraiki et al., 1992). Calcium, potassium, and sodium in food seasonings were below the NAFDAC limit of 0.4 µg/g and can be supplemented from other food sources especially fruits.

CONCLUSION

The presence of Pb and Cd in the sampled food seasonings even at the lower concentrations may lead to bioaccumulation in tissues with time, thereby altering various biochemical

parameters in the liver and kidneys. Since the food seasonings are of vegetable origin, it is most likely that Pb and Cd were taken up by plants from the soil.

REFERENCES

Achinewy, J. and Denny, R. C. and Mendham, I. (1995). *Vogel's Quantitative Inorganic Analysis: Including Elementary Instrumentation Analysis.* 4th Edition. pp. 123-134.

Borget, K. R. (1993). Dietary and Environmental Lead: Human Health Effect. Elsevier Science Publishers. Amsterdam.

Cobley, L. (2002). Stimulants in food seasoning. *Encyclopaedia Americana*. 17: 15-30. Grolier Inc: USA.

De Silv, P. E. (2007). Determination of lead in plasma and studies on its relationship to erythrocytes. *British Journal of Industrial Medicine*. 38: 309-17.

El-Hraiki, M., Kessabi, Y. S. and Bernard, P. (1992). Contamination of Sea Food Products by Cadmium, Chromium, Mercury, and Lead in morocco. *Review medical vetinary*, 143:49-52.

Merril, J. C. and Morton, J. P. (2001). *Methods of Toxicology.* 41st Edition. Taylor and Francis Philadelphia.

Nordberg, K. Peterson, B. and Owen, V. (2001). Fish Mercury Distribution in Massachusetts, USA Lakes. *Environmental Toxicology and Chemistry*, 18 (17):1370-1379.

Peter, J. W. (1979). *An Introduction to Atomic Absorption Spectrophotometer*. Published by Pye Unicam Ltd. 1st Edition. pp 20.

Petersdorf, R. G., Martin, J. B. and Fauci, A. S. (1999). *Harrison Princilpe of Internal Medicine*. 2: 12th ed. McGraw-Hill, New York pp 2182-7.

Smith, D. L. and Schenk, W. G. (1998). *Association of Analytical Chemists*. Pp 55-59.

Susan, M. and Anne, P. (1998). *Tropical and Sub-tropical Foods*. 2nd Ed. MacMillan Publications New York 154 170.

Vallee, B. L. and Ulmer, D. D. (2006). Biochemical Effects of Mercury, Cadmium and Lead. *Journal of Biochemical Medicine*, 42: 54-60.

WHO (World Health Organisation), (1983). Evaluation of certain food additives and contaminants (Forty-first Report of Committee on Food Additives). WHO Technical Report Series No. 837. Geneva.

Yousuf, M. H. A. and El-Shahawi, M. S. (2000). Trace Metals in Lithrinus Lengan Fish from Arabian Gulf: Metal Accumulation in Kidney and Heart Tissues. *Bulletin of Environmental Contamination and Toxicology*, 62: 293-300.

Original Article

and a

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 LD₅₀ 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. *Corresponding Author: Tel: +2348036136862, alijigam@yahoo.com

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 analgesic, as 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 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 \pm 1.23 – 30.00 ± 2.14 g 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 [LD₅₀]

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.

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Table 1: Phytochemical Constituents of A. nilotica root.

Active Principle	Inference	
Alkaloids	+	
Morphine alkaloids		
Glycosides		
Cardiac glycosides		
Terpenoids	+	
Tannins	+	
Flavonoids	+	
Saponins	'	
Caffeine	2-12-1-	
+ = Present	- = not detected	

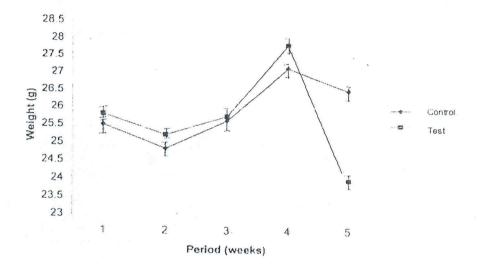


Figure 1: Weight variations in mice dosed with crude A. nilotica extracts over a 5 week period

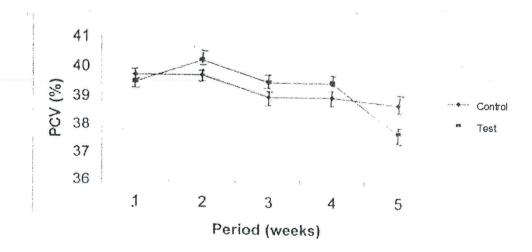


Figure 2: Variations in packed cell volume of mice dosed with crude *A. nilotica* extracts over a 5 week period

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

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

over a 5 week period				International Journal of Applied Biological Research 201						
Glutamate		WEEK	1	WEEK	2	WEEK	3	WEEK 4		WEEK 5
Oxaloacetate trans	aminase							W BBR 1		WEEK 2
(SGOT) (I.U)										
control:										
n = 2	0	37.25	H103	35.25	L1 2E	24751	1 25	20.55		
$(x \pm SEM)$ test:		07.20_	_ 1.05	33.23	1.23	34.75 <u>+</u>	1.25	33.75±	1.25	32.00 ± 1.47
n = 20		44.50	L007*	45.55			1900			
		44.50_	0.87	45.75 <u>+</u>	±0.85 [*]	51.75 <u>+</u>	1.93*	51.00 ± 1	1.87*	55.75±2.39*
-1-										
Glutamate										
Pyruvate										
Transaminase										
(SGPT) (I.U)										
	29.50±	0.65	30.00±	1.83	29.50 <u>+</u>	0.96	29.00 1	1.08	29.25 <u>+</u>	1 11
control:										. 1.11
n = 20	33.50±	1.04*	37.75±	0.85*	39.75±	-1 75*	42.50±	1 22*	4E 2E L	1.00*
$(x \pm SEM)$ test				23 535	07.701	_1.75	72.501	1.32	45.25 <u>+</u>	1.89
n = 20										

Alkaline Phosphatase
(ALP) (I.U) control

n = 20 51.50

$n = 20$ $(x \pm SEM)$ test $n = 20$	51.50±4.92	47.75 <u>+</u> 3.97	39.75 <u>+</u> 2.32	37.75±5.39	34.50 <u>±</u> 5.12
	50.75 <u>±</u> 1.25	48.75 <u>±</u> 1.11	40.75 <u>±</u> 1.11	38.00 <u>±</u> 0.823	41.00 <u>±</u> 1.29

^{* =} Significant (p<0.05)

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

Sodium (mmol/L) $\overline{(x} \pm SEM)$	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5
control: n = 20	124.50 <u>+</u> 4.03	126.50 <u>+</u> 4.65	127.25 <u>±</u> 3.60	130.25 <u>+</u> 5.72	134.00 <u>+</u> 2.58
test: n = 20	126.25±1.93	127.00 <u>+</u> 1.58	126.50±2.50	131.50 <u>+</u> 2.06	139.00 <u>±</u> 1.29
Potassium (mmol/L)					
control: n = 20	3.20 ± 0.32	3.40 ± 0.20	3.20 ± 0.32	3.55 <u>±</u> 0.30	3.35 <u>+</u> 0.28
$(\overline{x} \pm SEM)$ test $n = 20$	3.83 <u>±</u> 0.19	3.28 <u>+</u> 0.18	3.70 <u>±</u> 0.21	3.20 <u>+</u> 0.18	3.22 <u>±</u> 0.26
Chloride (mmol/L) Controls n = 20	101.25 <u>±</u> 1.80	103.50 <u>±</u> 3.30	105.25 <u>+</u> 3.40	105.00 <u>±</u> 2.48	101.50 <u>±</u> 3.01
$(x \pm SEM)$ test $n = 20$	102.50 <u>±</u> 4.87	103.75 <u>+</u> 1.75	105.50 <u>±</u> 2.63	109.00 <u>+</u> 3.42	110.50+090*
*Significant (n<0.05)					

^{*}Significant (p<0.05)



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

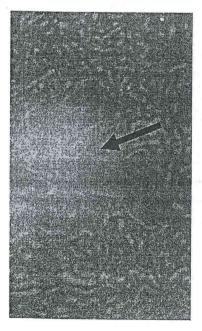


Plate IIa: Kidney tissue (Test) with ghost-like nephron

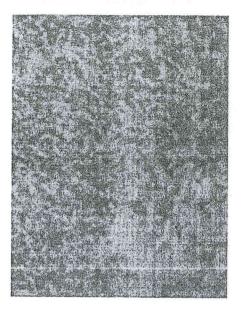


Plate Ib: Liver tissue (Test) with feathery disintegration of henatocytes.

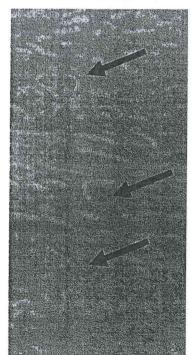


Plate IIb: Kidney tissue (Control) with intact nephrons

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 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). 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 cirrhosis, bone and intestinal diseases (Tietz, 1983). Such results should necessarily interpreted be 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 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*

1

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). Invivo 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.