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HEAVY METALS IN BLOOD AND HISTOPATHOLOGICAL ANALYSIS OFWISTAR RATS EXPOSED TO FIVE NATRON (*KANWA*) VARIETIES SOLD IN KURMI MARKET KANO, NORTHERN NIGERIA

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ABSTRACT

Natron has been used for thousands of years as a cleaning product for both the home and body. In the present study, the effects of five natron varieties *farar kanwa, jar kanwa*, *manda*, u*nguru* and *mangul* sampled from famous Kano, Kurmi market were determined. Cadmium and Lead concentrations in blood samples of the wistar rats were determined following standard protocols Sub-acute toxicity of five natron samples were evaluated on 11 groups of Wistar albino rats of 3 per group each which were administered natron at various concentrations of 150mg/kg& 300mg/kg in each variety with the exception of control group. Values of Cd in blood ranged from 0.03-0.09µg/dL and for Pb ranged from 0.02 to 0.07µg/dL, which were below average values of CDC which were 5.00µg/dL and 1.26µg/dL for Cd and Pb respectively. However, histopathological examination of wistar rat liver and kidney tissues revealed alteration in their morphological features, as vascular congestion and fibrosis were observed in most sections examined.

Keywords: Evaluation; Heavy metals; Histopathology; Natron; Wistar rats

INTRODUCTION

For medicinal purposes, natron is ground and mixed with water and applied on tooth for relief of tooth ache. It is used as an expectorant for cough treatment due to its ability in inducing secretion of respiratory mucosa. It is used as antacid, for the relief of constipation, stomach ache and preventing flatulence (Bankole *et al.*, 2015).

Peripartum cardiac failure (PPCF) been a disease is a dilated form of cardiomyopathy that causes deterioration in cardiac function (Muhammad *et al*., 2014). Higher consumption of potash injures testicles and thereby causes infertility in men. Its effect is by suppressing steroidogenesis - a process by which sperm is formed and alter testicular tissues. The fatty acid profile of testes, which is mostly unsaturated, is also altered by excessive intake of potash (Segun, 2016). Bankole *et al.* (2015) reported progressive tubular and vascular changes, cellular necrosis and glomerular degeneration in the kidney of albino rats fed with various concentrations of potash mixed with growers mash for period of three weeks. These become imperative to examine changes associated with administration of Natron so as to have a better understanding of the effects on blood metal levels. Therefore, the study determined the levels of cadmium and lead in the blood and assess histopathological effects (sub-acute toxicity) of natron on some vital organs (liver and kidney) of treated animals.

MATERIALS AND METHODS

Collection of Natron Samples

Samples of natron were purchased from different vendors in Kurmi market, which is a market situated in the heart of Kano (Kano Municipal Local Government Area) (Ujorha, 2003). One of the traditional and historical products sold in this market is natron (*kanwa*) of many varieties and from different locations.For this study, the five different varieties of natron (*kanwa*) were sampled from different vendors, the samples were *farar kanwa*, *jar kanwa*, *manda*, u*nguru* and *mangul* as they are called locally in Hausa language.

Handling of Experimental Animals

Albino Wistar rats were obtained from the Animal House of the Department of Biological Sciences, Bayero University Kano, Nigeria. They were housed under standard environmental condition and acclimated to their environment one week before the experiment begins. They were fed with pelleted poultry feed (growers marsh) and allowed free access to drinking water. The animals were weighed using digital weighing scale and were found to be 120g-135g average weight. The rats were handled and treated according to rules and regulations of animal's ethics as secured by the committee of research ethics, College of Health Sciences, Bayero University, Kano.

Preparations of Natron Samples for Administration

The five different natron (*kanwa*) varieties samples were carefully pulverised and then sieved with small size mesh. The samples were then weighed on digital weighing balance and stored. For each variety, 20gwere dissolved in 500ml distilled water and followed by oral administration of a 0.4ml and 0.8ml equivalent to 150 and 300mg/kg respectively based on OECD, (2011) guidelines.

Experimental Design

Experimental animals were placed into 11goups of 3 rats per group as follow:

Group 1a & 1b administered 150mg/kg& 300mg/kg respectively of *farar kanwa*, group 2a & 2b challenged with

150mg/kg & 300mg/kg respectively of *jar kanwa*. Group 3a & 3b challenged with 150mg/kg & 300mg/kg respectively of *mangul*. Group 4a & 4b challenged with 150mg/kg & 300mg/kg respectively of *ungurnu*. Group 5a & 5b challenged with 150mg/kg & 300mg/kg respectively of *manda*. While group 6 serves as control, administered with normal saline. The above dosages were administered orally using 2ml syringe from the stock solutions of *farar kanwa, jar kanwa, mangul, ungurnu* and *manda* preparedonce daily for period of 14 consecutive days.

Sub-acute Toxicity of Natron Samples on Experimental Animals

Sub-acute toxicity is the exposure to toxic substances for one month or less (Klaassen, 1995). The animals were allowed to acclimatize a week to the commencement of the practical. The animals were distributed into 11 triplicated groups as shown in the experimental design. The prepared natron doses were administered orally using syringe for 14 consecutive days. This preparation was performed according to the guidelines of Organization of Economic Co-operation and Development (OECD, 2011). The rats were observed for toxic symptoms such as weakness, loss of appetite, difficulty in movement, nose bleeding, discharge from eyes and ears, noisy breathing, aggressiveness and mortality for the first two and 24 hours which were used as indicators for acute toxicity effect (Carol, 1995).

Collection of Blood Sample

After 14 days sub-acute treatment, the animals were allowed to fast overnight before been sacrificed. They were anaesthetised with chloroform vapour and blood samples were collected in labelled tubes for heavy metals analysis (Ogunka *et al*., 2012).

Determination of Cadmium and Lead in Blood Samples

The blood samples collected were then taken for heavy metals (Cd and Pb) analysis. The samples were digested using perchloric acid and nitric acid. The resultant solution was aspirated into available AAS (Atomic Absorption Spectrophotometer) Buck Scientific (Model VGP 210) for the determination of heavy metals (Cd and Pb) (AOAC, 1999).

Examination of Liver and Kidney of Experimental Wistar Rats

The liver and kidney tissues of albino Wister rats were fixed with 10% formalin and were taken to Histopathology Department of Amino Kano Teaching Hospital for examination. The tissues were examined following the method of Anwioro (2010).

Statistical Analysis

The data were analyzed using the statistical package for social science (SPSS version 20). Data were expressed as the mean ± standard deviation (SD).

RESULTS

Mean Concentrations of Cadmium and Lead in the Blood of Treated Wistar Rats

Table 1: Mean Concentrations of Cadmium and Lead in Blood Samples of Wistar rat Challenged with 150 and 300mg/kg.

Sample	Cadmium (µg/dL)		Lead (µg/dL)	
	150 (mg/kg)	300(mg/kg)	150 (mg/kg)	300(mg/kg)
F/ *Kanwa*	0.030±0.00	0.050±0.00	0.028±0.002	0.046±0.00
J/ *Kanwa*	0.043±0.00	0.090±0.00	0.044±0.004	0.063±0.00
Mangul	0.036±0.00	0.090±0.00	0.037±0.006	0.070±0.01
Ungurnu	0.063±0.00	0.060±0.00	0.064±0.006	0.076±0.00
Manda	0.060±0.00	0.060±0.00	0.061±0.004	0.076±0.00
Control	0.001	0.001	0.001	0.001
CDC Average values	5.00	5.00	1.26	1.26

Histopathological Effects of Natron on Liver

Plate I indicated the normal histological features of liver section of albino rat used as control, the hepatocytes were arranged as radiating cards forming hexagonal units containing central venules. This could be as a result of the fact that experimental animals in this group were not administered with any natron as such no observable effects were visibly seen.

Plate I showed the section of liver of experimental animals treated with 150mg/kg *farar kanwa* marked no significant pathology while section of liver treated with 300mg/kg f*arar kanwa* (Plate II) showed vascular congestion and fibrosis indicating that *farar kanwa* effect on liver is dosage dependent as abnormalities were observed due dosage increment.

Plate III and IV showed that the experimental animals treated with both 150mg/kg and 300mg/kg *mangul* induced marked abnormalities in liver sections such as vascular congestion and fibrosis. The result indicated that its consumption caused considerable damage to liver hepatocytes.

Also, the liver sections in Plate V and VI, experimental animals which were challenged with 150mg/kg and 300mg/kg *ungurnu* revealed hepatoxicity effects such as liver damage, cytoplasmic vacuolation, vascular congestion, and fibrosis. Moreover, Plate VII and VIII showed the liver

section of groups treated with 150mg/kg and 300mg/kg *Jar kanwa* revealed areas of liver damage, fibrosis and vascular congestion.

Furthermore, Plate IX and X, photomicrograph sections of liver of the animals treated with 15mg/kg and 300mg/kg *manda* revealed vascular congestion, fibrosis and liver damage indicating that consumption of *manda* at these concentrations cause hepatoxicity.

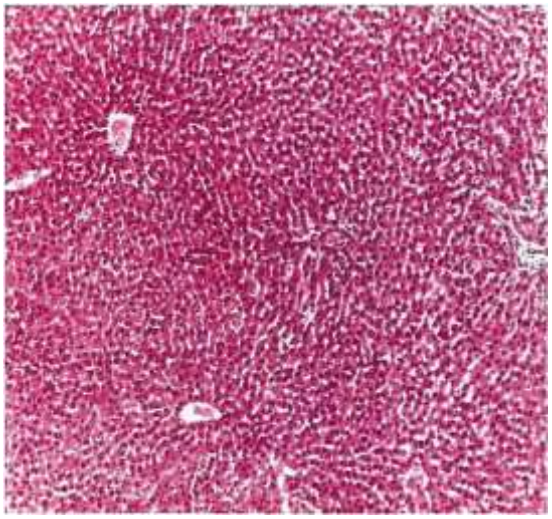


Plate I: Section Wistar rat liver challenged with 150mg/kg *farar kanwa*, showing no significant pathology (H & E mag x 100).

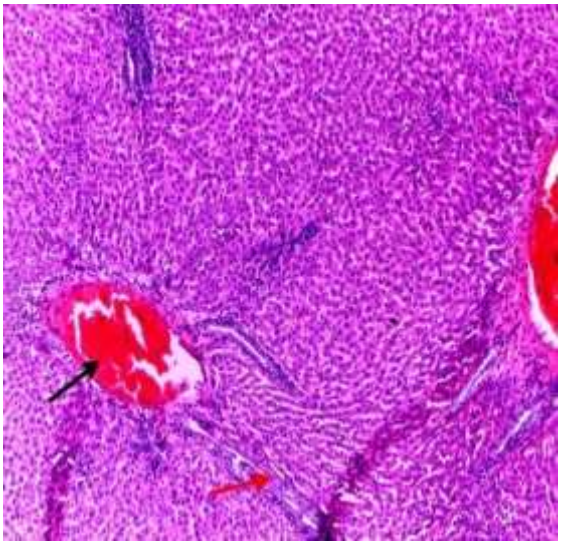


Plate II: Section of Wistar rat liver challenged with 300mg/kg*farar kanwa*, showing areas of vascular congestion and fibrosis (H & E mag x 100).

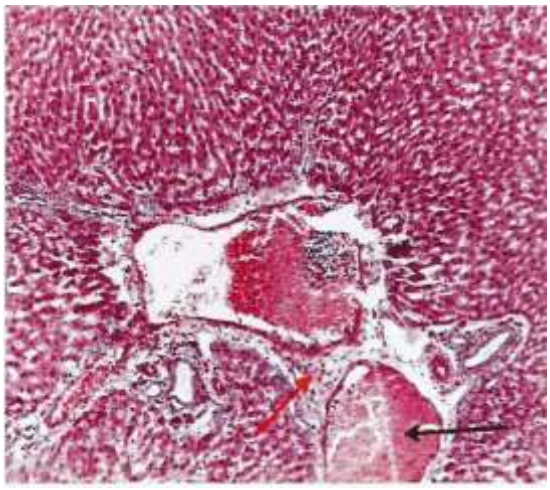
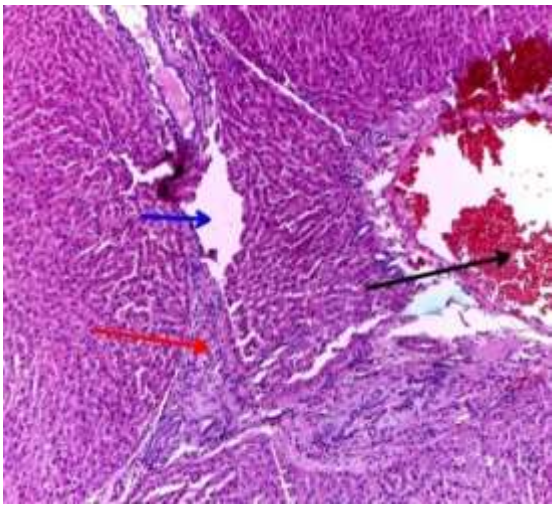


Plate III: Section of wistar rat liver, challenged with 150mg/kg of *mangul*, showing areas of vascular congestion and fibrosis (H & E mag x 100).



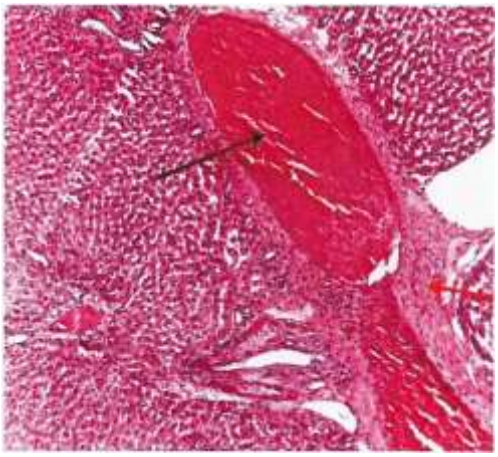


Plate V: Section of Wistar rat liver challenged with 150mg/kg *ungrunu*, showing vascular congestion and fibrosis (H & E mag x 100).

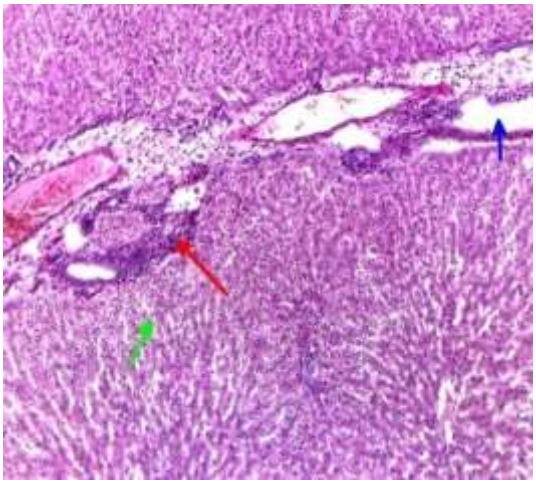


Plate VI: Section of Wistar rat liver challenged with 300mg/kg*ungurnu*, showing vascular congestion, fibrosis and liver damage (H & E, mag x100).

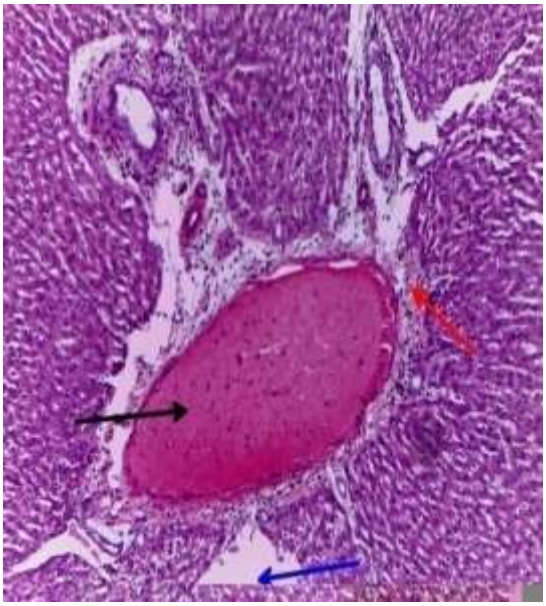


Plate VII Section of wistar rat liver challenged with 150mg/kg *jar kanwa* showing vascular congestion, fibrosis and liver damage (H & E, mag x 100).

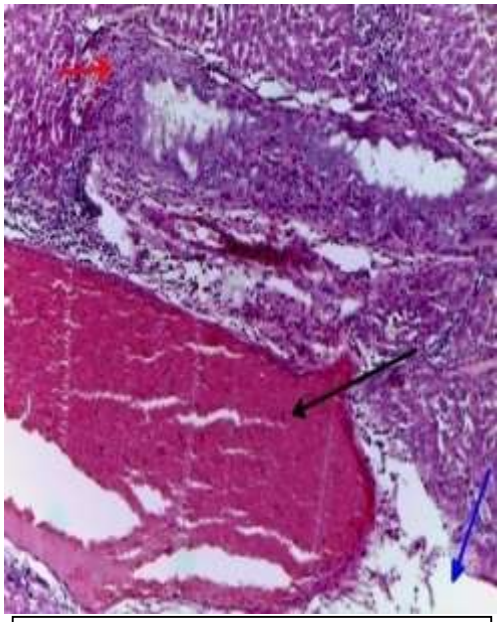


Plate VIII: Section of wistar rat liver, challenged with 300mg/kg *jarkanwa*, showing vascular congestion, fibrosis and liver damage (H & E, mag x 100).

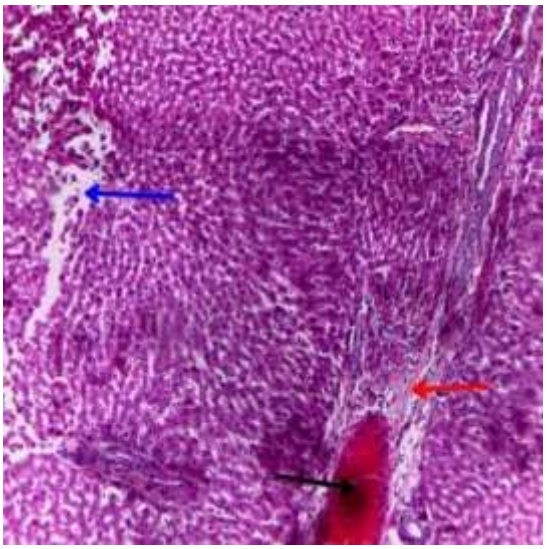


Plate IX: Section ofwistar rat liver challenged with 150mg/kg*manda*, showing vascular congestion, liver damage and fibrosis (H & E, mag x 100).

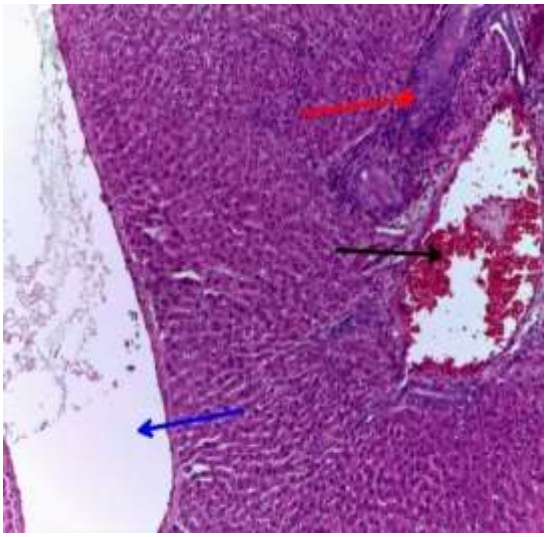
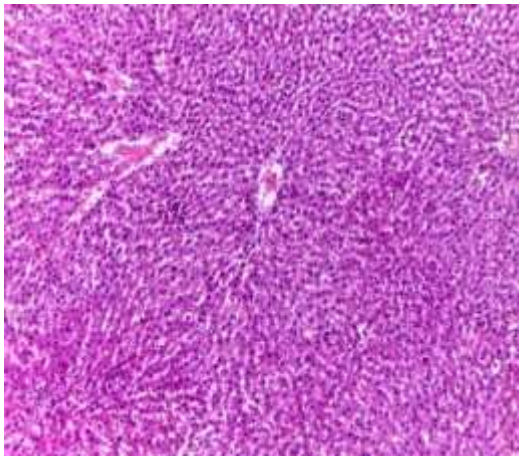


Plate X: Section of wistar ratliver challenged with 300mg/kg *manda*, showing liver damage, fibrosis and vascular congestion. (H & E, mag x 100)



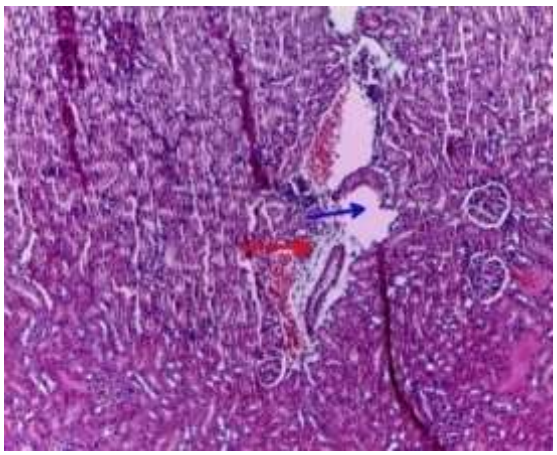


Plate XI: Section Of wistar rat liver, showing normal liver tissue with hepatocytes arranged as radiating card forming hexagonal units containing central venules
(H & E, Magx100)

Histopathological Effects of Natron on Kidney

Plate XII showed the results of histopathology of the section of *R. novergicus* kidney used as control showing hematoxylene and eosin (H & E) reactions on normal renal tissue with cortex containing glumeruli and the medulla containing renal tubules intact. Plate X III was section of rat kidney administered with 150mg/kg *farar kanwa* showed no significant pathology, while Plate XIV; 300mg/kg *farar kanwa* showed areas of renal damage and fibrosis indicating that effect is dose dependent.

Plate XIV and XVI showed sections of rat kidney treated with 150mg/kg and 300mg/kg *mangul* respectively, all showing areas of renal damages and fibrosis indicating that consumption of *mangul* cause considerable damage to renal tissue. So also plate XVII and XVIII are sections of kidneys of rat treated with 150mg/kg and 300mg/kg *ungurnu* revealing areas of renal damages and fibrosis after been challenged with different dosages of natron.

Furthermore, animals administered with 150mg/kg and 300mg/kg *jar kanwa* their kidney sections show abnormalities compared with control, as both concentrations administered caused fibrosis and vascular congestion as seen in Plate XIX and XX. While in plate XX1 and XX1I, no significant pathology was observed in the photomicrograph sections of kidney of the animals treated with both 150mg/kg and 300mg/kg *manda* indicating that this natron sample does not induce any nephrotoxicity to kidneys.

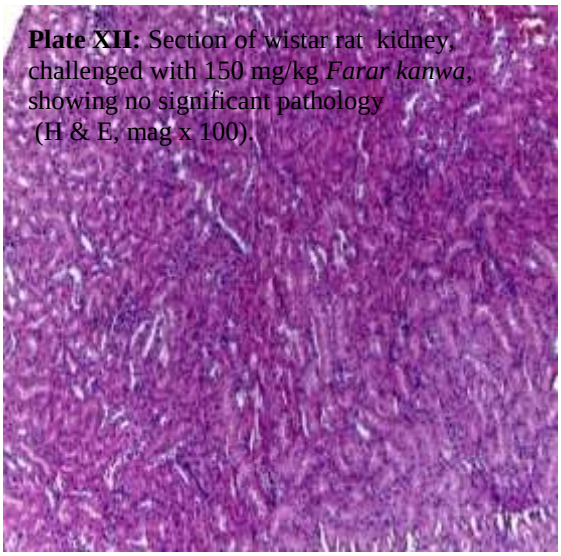


Plate XII: Section of wistar rat kidney, challenged with 150 mg/kg *Farar kanwa*, showing no significant pathology (H & E, mag x 100).

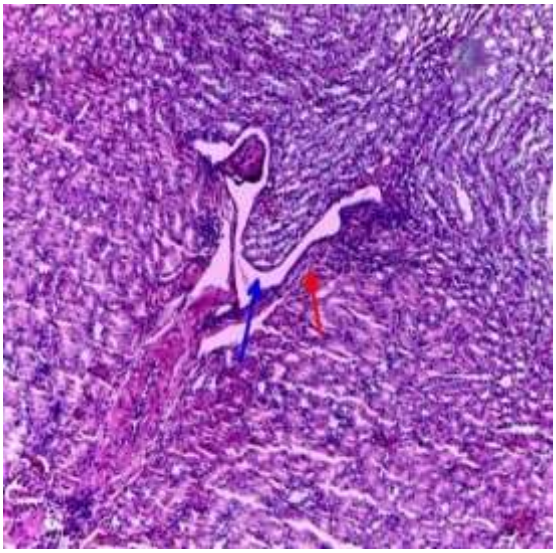


Plate XIII: Section of wistar rat kidney challenged with 300mg/kg *Farar kanwa*, showing areas of renal damage and fibrosis (H & E, mag x 100).

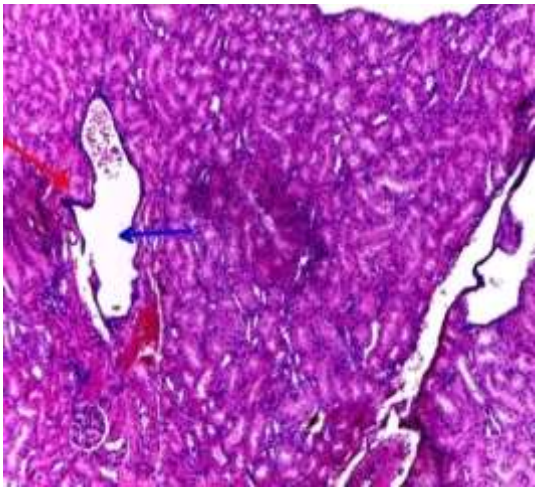


Plate XIX: Photomicrograph section of wistar rat kidney administered with 150mg/kg m*angul*, showing areas of renal damage and fibrosis (H & E mag x 100).

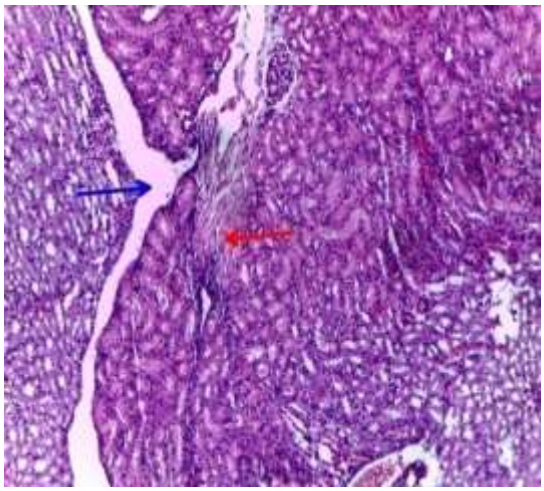


Plate XX: Section of Wistar rat kidney challenged with 300mg/kg m*angul*, showing areas of renal damage and fibrosis (H & E, magx100)

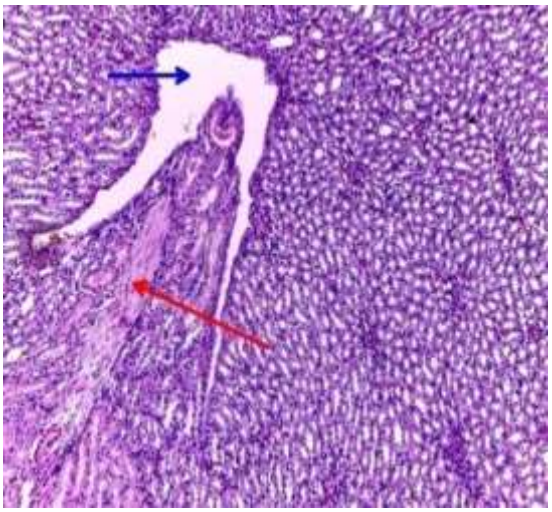


Plate XIV: Section of wistar rat kidney challenged with 150mg/kg *ungurnu* showing areas of renal damage and fibrosis (H & E mag x 100).

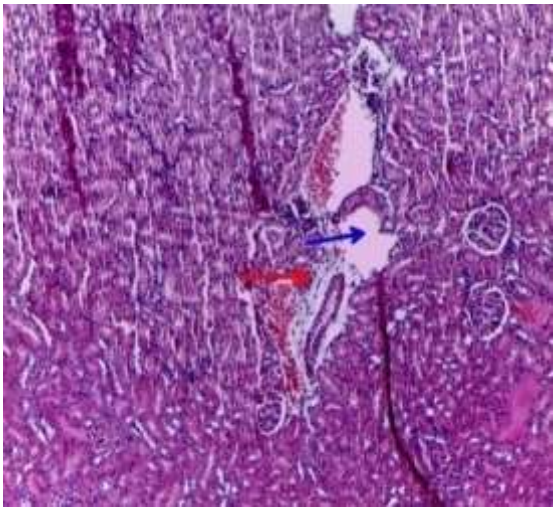


Plate XV: Section of wistar rat kidney challenged with 300mg/kg u*ngurnu*, showing areas of renal damage and fibrosis (H & E mag x 100).

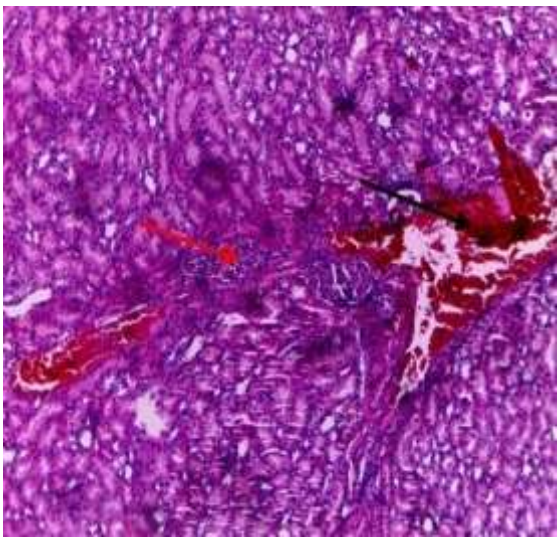


Plate XVI: Section of wistar rat kidney challenged with 150mg/kg *jar kanwa*, showing areas of fibrosis and vascular congestion (H & E, mag x 100).

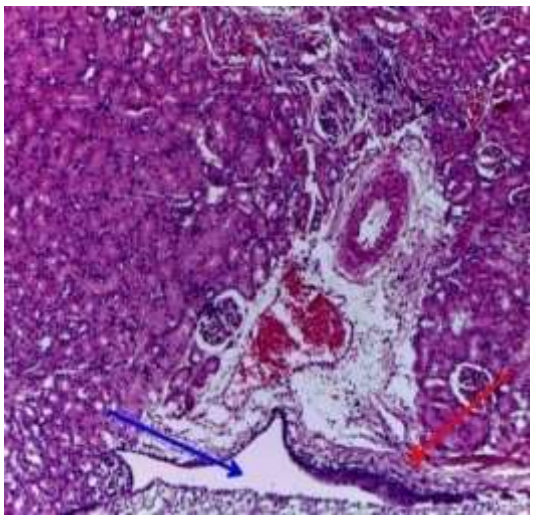


Plate XVII: Section of wistar rat kidney challenged with 300mg/kg *jar kanwa*, showing areas of renal damage and fibrosis (H & E, mag x 100).

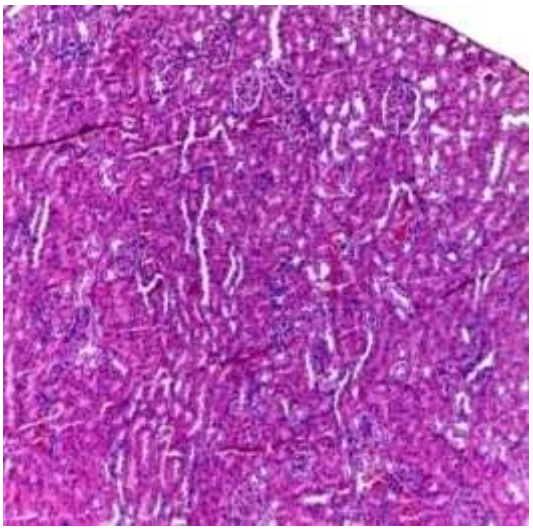


Plate XVIII: Section of wistar rat kidney challenged with 150mg/kg m*anda*, showing no significant pathology (H & E mag x 100)

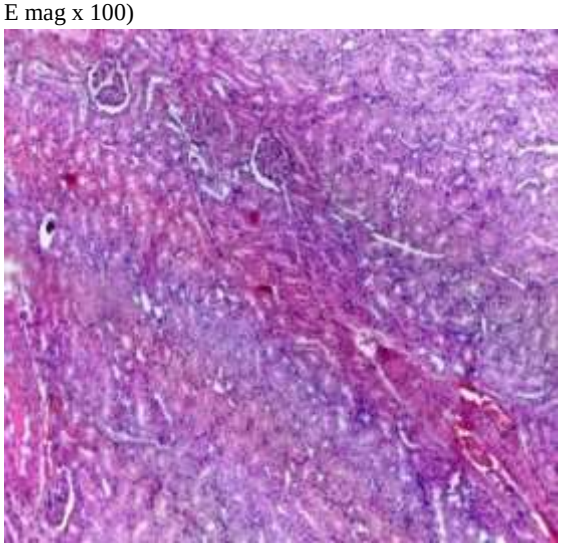


Plate XXVII: Section of wistar rat kidney served as control, showing normal renal tissue with cortex containing glomeruli and the medullar containing renal tubules intact (H & E, mag x 100).

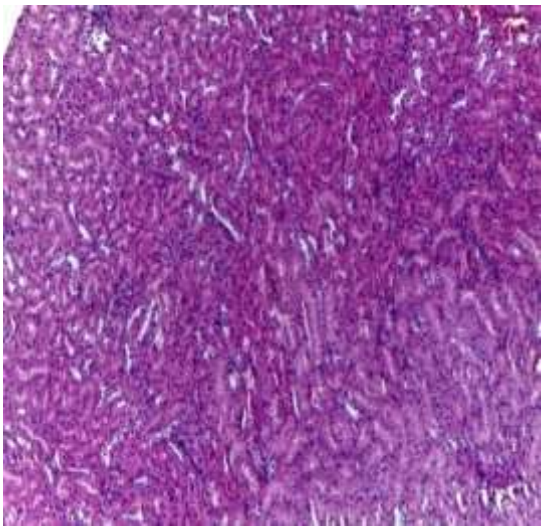


Plate XIX: Section of wistar rat kidney challenged with 300mg/kg m*anda* Showing no significant pathology (H & E, mag x 100).

DISCUSSION

The results obtained for blood cadmium and lead levels from animals challenged with 150 and 300mg/kg different natron varieties revealed insignificant values. These values were lower compared with CDC recommended average blood lead levels of 5.0µg/dL and average blood cadmium levels of 1.26µg/dL. Aderinola *et al.* (2009) obtained values of 0.55 and 0.67 in fish species of Lagos Lagoon. Therefore, very low levels of these heavy metals were detected, probably due to time of exposure is short, i.e. two weeks sub-acute test. In most of the literatures consulted the values are greater than 1µg/L to show the effect. Jialing (2014) indicated that the average lead level in children blood should be 60.29 µg/L and for cadmium it should be 1.2µg/L. The amount of blood toxic element can reflect the disease state of the person or the environment where that person resides or work (Farzin *et al.*, 2008).

Histopathological examination of treated albino rats kidneys and liver revealed remarkable changes to hepatocytes (liver cells) and renal tissues in most samples examined after 14 days sub-acute exposure with different natron, with exception of liver of rats challenged with*farar kanwa* which showed no significant pathology to liver tissues indicating that it is relatively safe. The findings showed that most natron consumed may have some deleterious effects on liver and by extension may affect the functions of liver,this is because cytoplasmic vacuolation, fibrosis and vascular congestion were observed in most sections. This agreed with findings of Muhammad *etal*. (2014) where albino rats administered with graded doses of *kanwa* revealed heart chambers dilation, hypertrophy and focal atrophy. Histopathological examination of kidneys revealed nephrotoxicity of most natron samples irrespective of their concentrations, as renal damages, fibrosis and vascular congestion were observed compared with control group, with the exception of groups of animals challenged with *farar kanwa* at 150mg/kg induced no significant pathology.*Fararkanwa* administration at 300mg/kg revealed kidneys damage indicating that its effect is dose-dependent. Likewise, rats challenged with *manda* revealed no significant pathology in kidneys sections, indicating that it is less toxic to renal tissues. Results obtained from this research supported the theory of target organs of Heywood (1981) that metabolisms of environmental chemicals occur mostly in the liver, while excretion occurs through the kidneys (Clark and Clark 1977; Parke, 1982).

CONCLUSION

The values of Cd and Pb in blood were below average values of CDC. However, histopathological examination of wistar rat liver and kidney tissues revealed alteration in their morphological features, such as vascular congestion and fibrosis. This shows that natron at higher doses can alter tissues morphology and induce hepatotoxicity and nephrotoxicity.

RECOMMENDATIONS

1. Similar studies involving other Natron types and varieties across the country should be conducted.
2. Other organs should equally be examined to grasp the complete histopathology effects from natron consumption.
3. In addition, natron should be consumed with caution as excessive use might prove to be detrimental.

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