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TOXICOLOGICAL EFFECT OF SUDAN III AZO DYE IN PALM OIL ON KIDNEY PARAMETERS OF ALBINO RATS

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ABSTRACT

In recent years, there has been a rise in the utilisation of palm oil in Nigeria. This has resulted to increase demand and supply, with demand greater than supply. This gap between demand and supply has been accompanied with an increase in adulteration with Sudan III dye and another related compound. Thus this work was designed to investigate the toxicological effect of adulterated palm oil on the kidney of albino rats. Twenty rats divided into five different groups were used for the investigation. The palm oil was adulterated with Sudan III dye (0.1, 0.2 and 0.5 g/20ml) of palm oil. Group 1 serve as control, group 2 was administered with 1ml of unadulterated palm oil, while groups 3, 4, and 5 were administered with 1ml each of 0.1, 0.2 and 0.5 g of adulterated palm oil respectively for fourteen days, at the end of the experiment, the animals were sacrificed, blood and kidney samples were collected for biochemical and histological analysis. Creatinine, urea and electrolytes concentrations were determined. The result showed that Sudan III dye in palm oil could cause an increase in the parameters checked in a dose-dependent manner. The result of the histological examination revealed kidney debris (scattered remains) and casts (moulds) in some proximal tubules.

KEYWORDS: *Creatinine; urea; electrolytes; Sudan III.*

INTRODUCTION

Oil palm tree (*Elaeis guinensis*) is a tropical tree with fronds**:** a tree, bush or plant typically with a trunk without branches and a crown of pinnate or palmate leaves on top. It is a native to tropics, subtropics. For ages, its products have been useful to humanity. It is one of the most important oil-producing plant in West Africa (Otu, 2013). The fruit of oil palm tree produces two types of oil: red crude palm oil which is extracted from the monocarp and brownish crude oil from the seed (kernel). The red crude palm oil consists of mainly palmitic acid and oleic acid while the brownish crude oil consists mainly of Lauric acid. Palm oil is the richest natural source of carotenoids and tocotrienols, the carotenoids are responsible for the deep red colour (Gunstone, 2005; Gee, 2007; Otu,2013). Today, high demand for palm oil for both domestic and industrial utilisation has been accompanied with an increase in adulteration (Okogeri and Otika,2011; Otu, 2013). There is a widespread speculation in Nigeria that palm oil is being adulterated by producers and bulk buyers with the intentions of improving the quality of palm oil without considering possible effect on the quality of the palm oil and the health of the consumers. The adulterants repeatedly used are Azo-dyes (Imai, 1974; Axon *et al*.,2012; Otu, 2013).

Azo-dyes are organic compounds with the functional group R-N=N-R', the R and R' are usually aryl. They exist in the hydrazine form and are more likely to be broken down. They can be reduced by azo-reductase in intestinal bacteria cells and skin surface microflora. Azo-dyes are the major source of artificial colour in textile. These dyes are produced from the reaction of aryl diazonium salt ($Ar\text{-}N_2^+x^-$) with the secondary aromatic compound. Azo dyes give brighter colours than any other common dye; they are readily available and cheap. The simplicity of its reaction means the process can be scaled up and down very easily which is always a key factor in the cost of chemicals (Samar, 2013; Cristina, 2014). Azo dyes are used in industries (textiles, leather, cosmetics and plastics) for dyeing various materials (Song *et al*, 2004). Also, azo dyes are used in food industries as food addictive. They are classified as Sudan I, II, III and IV. These dyes have different colours and are used for different purposes. Sudan I is yellowish in colour, Sudan II has an orange colour while Sudan III and IV are red in colour. The most common Sudan Azo-dye used in

adulterating palm oil is the Sudan III dye, it is soluble in palm oil. The dye could also be used for colouring textiles, plastics wax, floor polish and as a biological stain for lipids, triglycerides and lipoproteins. (Alim *et al.*, 2016). It has been labelled carcinogenic by the International Agency for Research on Cancer (IARC) (IARC,1975; IARC 1978) and are not permitted to be used in food, but because this dye is bright in colour and low in cost it has been used intentionally to adulterate palm oil to enhance its colour (Susie *et al.*, 2016).

The kidneys are one of the more important tissues, because of its role in the filtration, metabolism, and excretion of compounds. It is a pair of bean-shaped organs on each side of the spine, below the ribs and behind the belly. Each kidney is about 4 or 5 inches long, roughly the size of a large fist.

MATERIALS AND METHODOLOGY

Sample: crude palm oil, Sudan III azo dye, albino rat
Reagents: petroleum ether, hydrochloric acid

Adulteration of Palm Oil

Twenty millilitres (20ml) each of the palm oil was measured and transferred into four (4) beakers of 75ml each and labelled sample A, B, C and D. Sample A was unadulterated palm oil, while samples B, C and D, were mixed with Sudan III azo-dye at different concentration (0.1, 0.2 and 0.5) g respectively.

Test for Adulteration of Palm Oil

The purchased palm oil was analysed in the laboratory to ensure the absence of adulterant. This analysis was done using petroleum spirit and concentrations of hydrochloric acid (4:1, 3:1, 2:1, and 1:1 hydrogen and water mixture). To 5ml of oil sample in four (4) different test tubes, 15ml of petroleum ether was added followed by the 5ml hydrochloric acid of different concentrations to different test tubes. Different shades of yellow were observed indicating the absence of adulterants.

Animal Grouping/Administration of the Sample

The albino rats were grouped into five (5) (1, 2, 3, 4 and 5) of four (4) rats in each group. Animals in group 1 served as control, group 2 animals were given sample A, while groups 3, 4 and 5 were administered with samples B, C and D respectively after seven (7) days of acclimatisation for fourteen (14) days at 1ml/kg.

Histological Examination Method

The kidney of both the control and experimental groups were removed immediately after sacrifice and fixed in formalin solution for 24hrs and then dehydrated with ascending grade of alcohol (80% ethanol), cleared in xylene and embedded in paraffin wax. Thin sections of 7 microns thick were sectioned using a rotatory microtome. The sections were then deparaffinised and stained using the routine with haematoxylin and eosin (H & E). The sections were then examined under bright field light microscopy, and Photomicrographs of the desired results were obtained using a digital research photographic microscope.

RESULTS

Result of Histological Examination of the Kidney (H&EX 100)

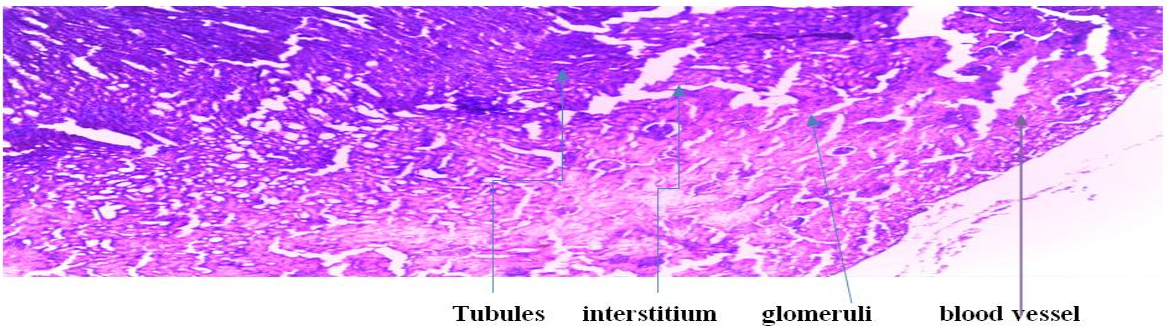


Fig. 1:

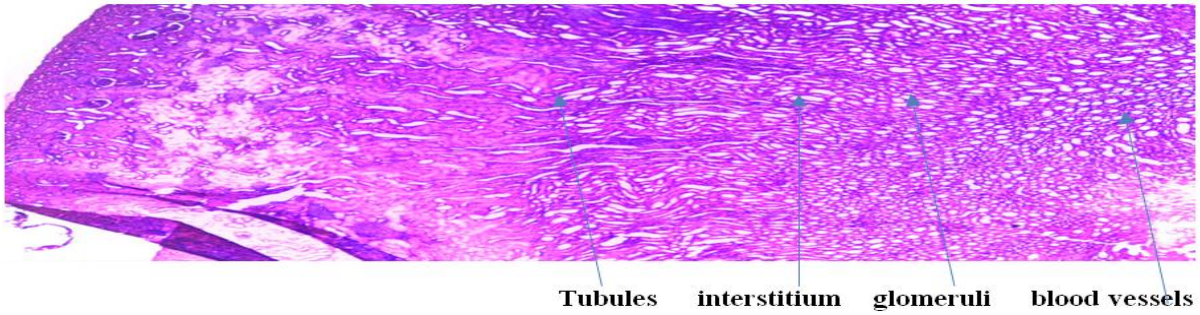


Fig. 2:

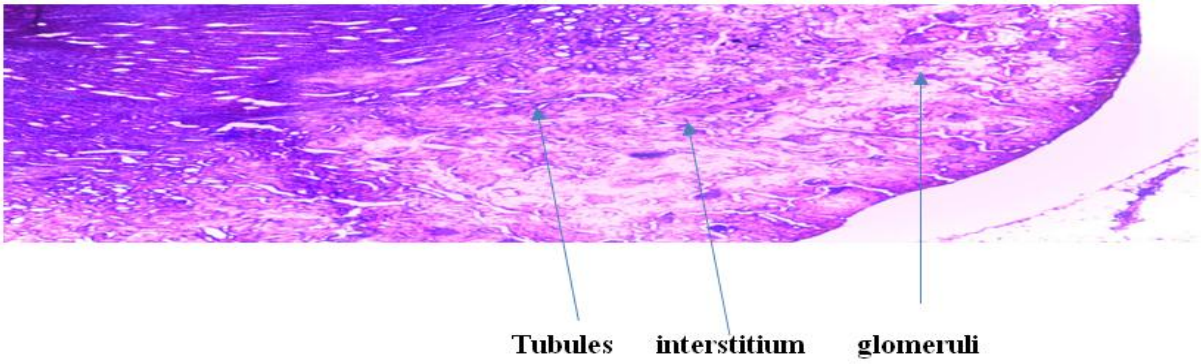


Fig. 3:

Figs. 1-3. Photomicrograph of the kidney of animals in group 1 (control). The slides showed normal histological features of the kidney.

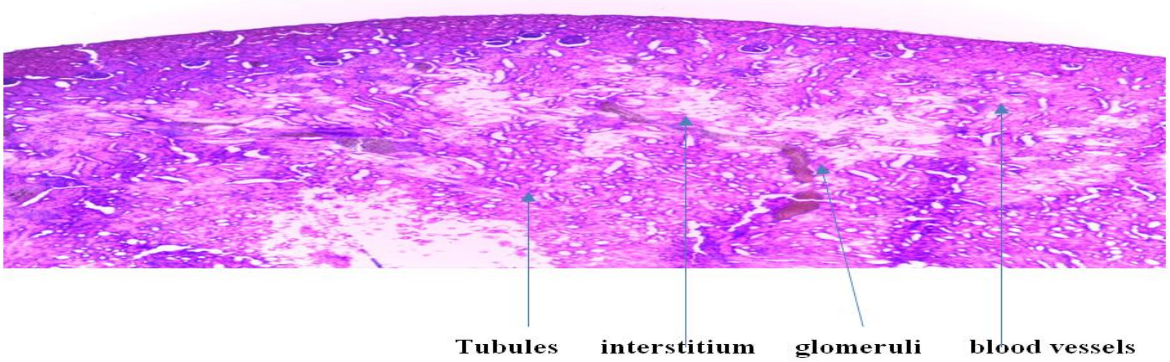
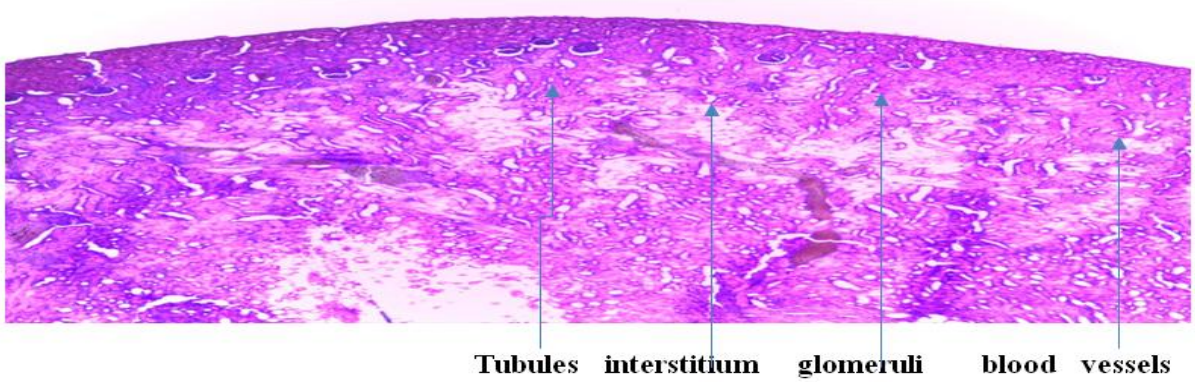


Fig. 4:



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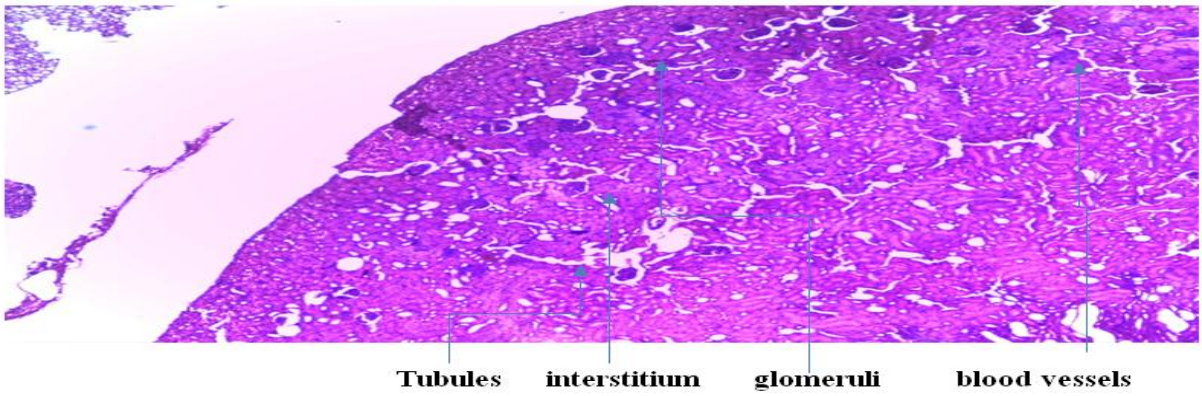
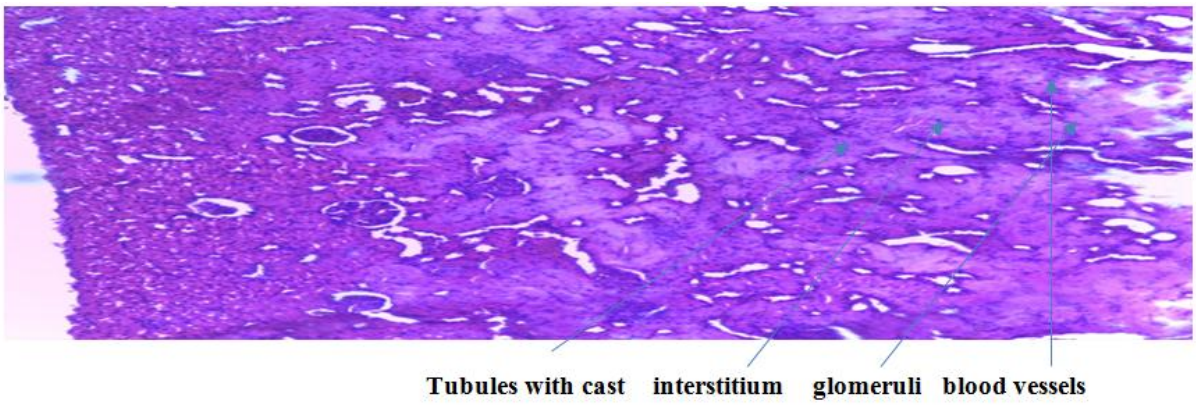


Fig. 6:

Figs. 4-6. Photomicrograph of the kidney of animals in group 2 administered with unadulterated palm oil. The slides showed normal histological features of the kidney.



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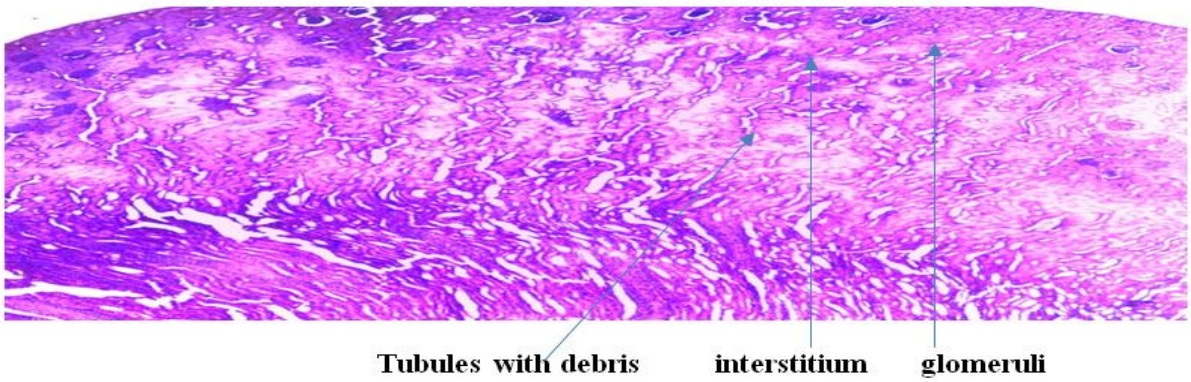

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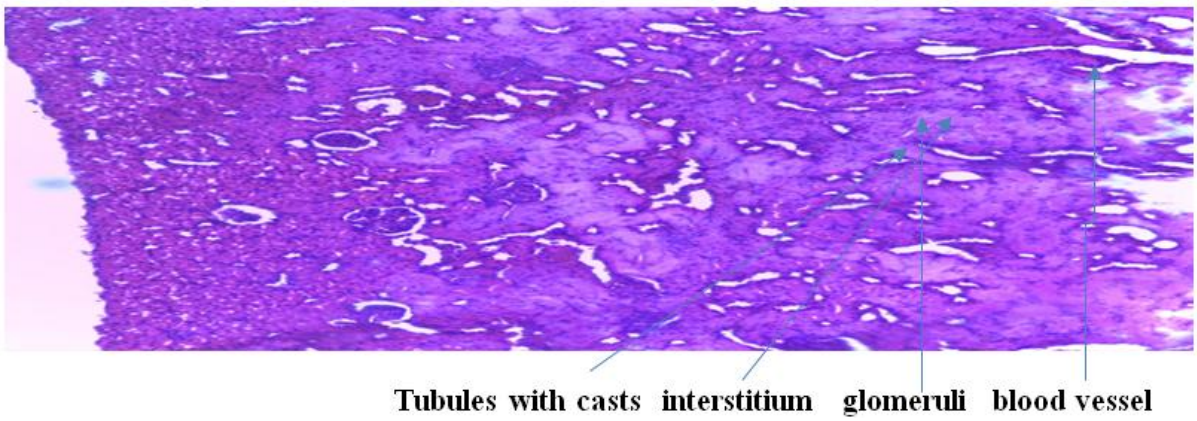
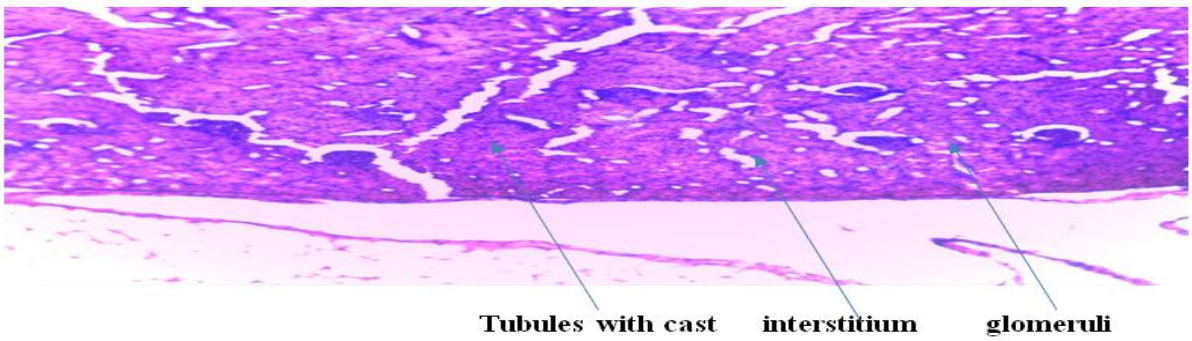


Fig. 9:

Figs. 7 – 9: Photomicrograph of the kidney of animals in group 3 administered with 0.1g/20ml of adulterated palm oil. The slides showed casts and debris in some proximal tubules.



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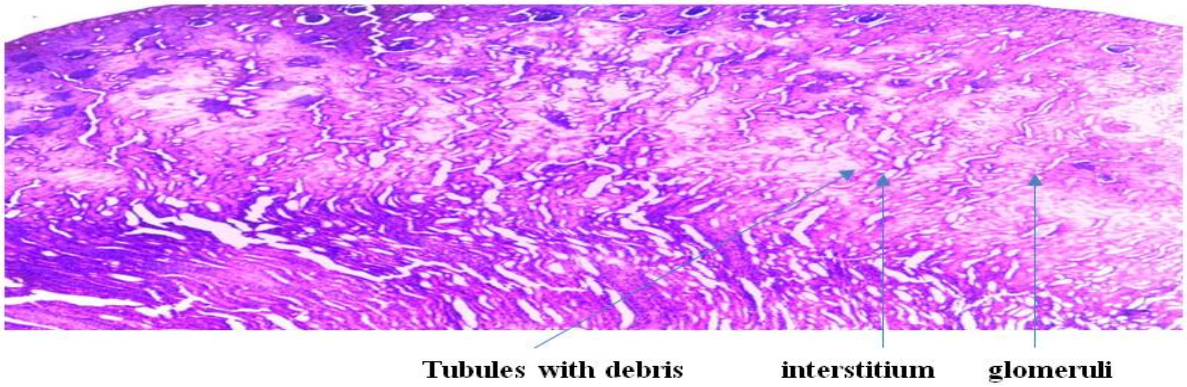

Fig. 11:

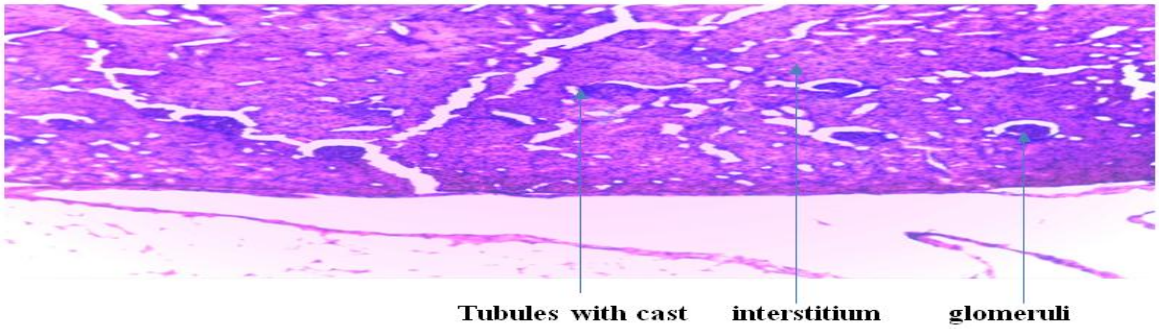

Fig. 12.

Figs. 10 – 12: Photomicrograph of the kidney of animals in group 4 administered with 0.2g/20ml of adulterated palm oil. The slides showed casts and debris in some proximal tubules.

DISCUSSION

Urea, creatinine, sodium ion, chloride, bicarbonate and potassium ion are parameters elevated in an event of kidney damage, they serve as markers of renal function (jozef *et al.*,2002). Table 1.0 showed the concentration of kidney parameters analysed. The result showed changes in sodium ion, potassium ion, urea, creatinine, chloride and bicarbonate in the groups administered with adulterated palm oil following daily administration of unadulterated and adulterated palm oil for a period of fourteen (14) days. The result showed no significant difference ($p<0.5$) in the values of the parameters between group 1 (control) and group 2 (group administered with unadulterated palm oil). This showed that unadulterated palm oil (1ml/kg) has no toxicological effect on the kidney parameters of an albino rat. Also comparing the values of the parameters for group 2 and groups administered with adulterated palm oil, there was a significant ($p<0.5$) increase in the values of these parameters in the groups administered with adulterated palm oil. The significant ($p<0.5$) increase in the values showed that adulterated palm oil may have a toxicological effect on the kidney parameters of an albino rat thus the elevated values. Since the rats used were male rats, an increase in the concentration of urea and creatinine could have led to an increase in muscle mass degradation (verma *et al.*, 2006).

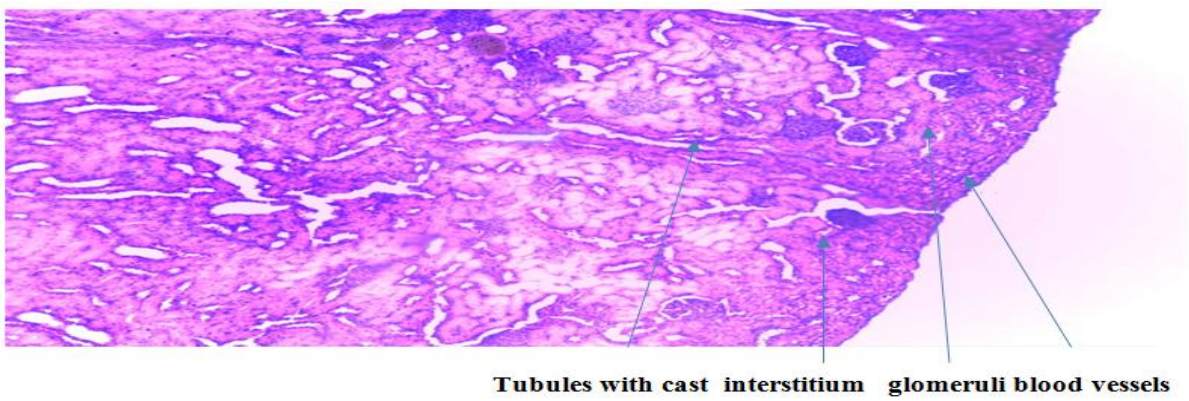

Fig. 13:

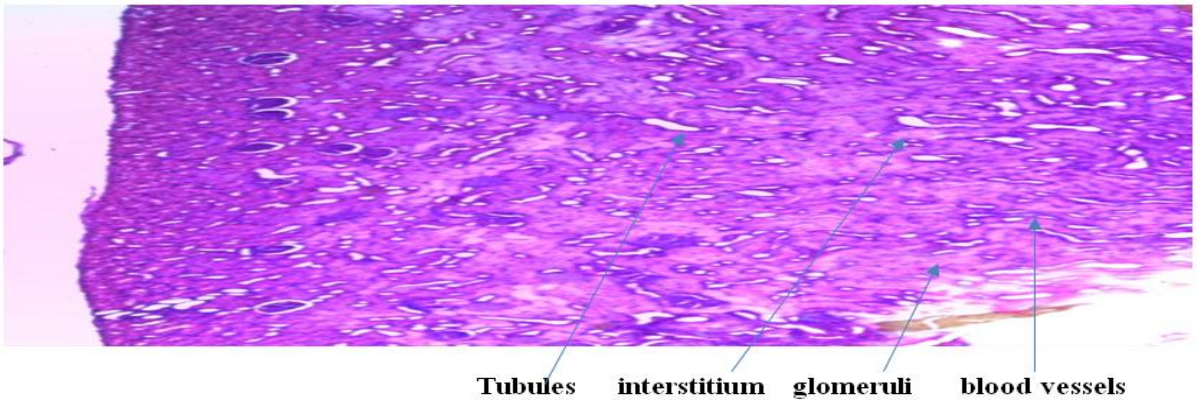

Fig. 14:

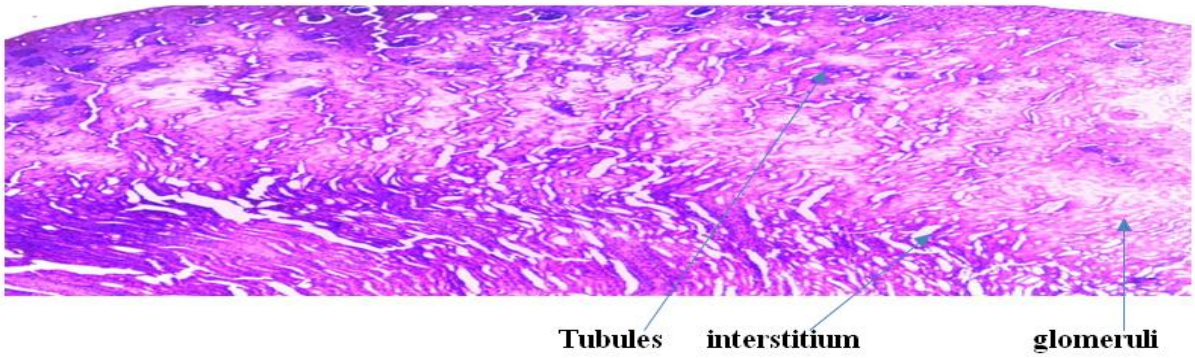

Fig. 15:

Figs. 13 – 15: Photomicrograph of the kidney of animals in group 5 administered with 0.5g/20ml of adulterated palm oil. The slides showed casts and debris in some proximal tubules.

Also, the increase in sodium ion and potassium ion in the blood could be attributed to an effect on the transporter (aldosterone) as reported by Omeh *et al* (2014). Thus from the study, the adulterated palm oil could have had an effect on the transporter leading to an increase in sodium ion and potassium ion concentration in the blood.

Results of the histological examination showed differences between the treated groups (groups 3, 4, and 5) when compared to group 2 and control. Slides 1- 3 which is the control group showed normal histological features of the kidney which includes the tubules, interstitium, glomeruli and blood vessels. Slides 4-6 showed the histological result of animals in group 2. The result revealed normal histological features of the kidney. Slides 7-15 showed the result of the animals treated with adulterated palm oil. The result revealed histological section from kidney showing debris (scattered remains) and casts (moulds) in some proximal tubules.

Table 1. Concentrations of kidney parameters.

Albino rats	Na (µ/L)	K (µ/L)	Ur (µ/L)	Cr (µ/L)	CL^{---} (µ/L)	HCO_3 (µ/L)
GROUP 1 (Control)	142.25 ± 0.63^{bfhj}	4.5 ± 0.09^{bfhi}	2.175 ± 0.14^{afhj}	125 ± 0.77^{bfhj}	31.75 ± 0.75^{bfhj}	23.25 ± 0.48^{bfhj}
GROUP 2 (Non adulterated)	145.83 ± 0.92^{afhj}	5.475 ± 0.08^{afhj}	2.55 ± 0.03^{afhj}	132 ± 1.82^{afhj}	36.5 ± 0.64^{afhj}	25.75 ± 0.25^{afhj}
GROUP 3 (0.1g/20ml)	$150 \pm 1.29^{*bchj}$	$6.55 \pm 0.09^{*bchj}$	$3.5 \pm 0.08^{*bchj}$	$141.5 \pm 3.23^{*bchj}$	$44 \pm 0.91^{*bchj}$	$27.75 \pm 0.25^{*bchj}$
GROUP 4 (0.2g/20ml)	$155.25 \pm 0.85^{*bfgj}$	$7.275 \pm 0.05^{*bfgj}$	$4.4 \pm 0.07^{*bfgj}$	$149.5 \pm 2.96^{*bfgj}$	$49.25 \pm 0.85^{*bfgj}$	$29.75 \pm 0.48^{*bfgj}$
GROUP 5 (0.5g/20ml)	$160.5 \pm 0.64^{*bfhi}$	$8.125 \pm 0.05^{*bfhj}$	$5.125 \pm 0.05^{*bfhi}$	$160.5 \pm 1.32^{*bfhi}$	$54 \pm 1.08^{*bfhi}$	$32.75 \pm 0.48^{*bfhi}$

Key: Na^+ =sodium, K^+=potassium, Ur = urea, Cr = creatinine, CL^- = chloride, HCO_3^- = bicarbonate.

Values are expressed as mean ± standard error.

Values in each column with superscript (*) differ significantly when comparing control with a group (1, 2, 3 and 4). Values with different superscript (a, b) differ significantly when compared with other groups (1, 3, 4and 5).values with different superscript (c, f) differ significantly when comparing group 3 with other groups (2, 4, and 5). Values with different superscript (g, h) differ significantly when compared with groups 1, 2, 3 and 5.values with different superscript (i, j) differ significantly when comparing group 5 with groups 1, 2, 3and 5 at $p< 0.05$.

CONCLUSION

The study has indicated that the consumption of sudan iii adulterated palm oil may have an effect on the sodium ion, creatinine, urea, potassium ion and bicarbonate level in the blood of albino rats and thus should be discouraged. In this work, it was also observed that the adulterated palm oil had effects on the adipose tissues making them soft and reddish in colour.

Ethical Approval

As per international standard or university standard was written ethical approval has been collected and preserved by the authors.

Competing Interests

Authors have declared that no competing interests exist.

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