

# Toxicity assay of Sudan II dye adulterated palm oil on the serum proteins, electrolytes and lipid parameters of male albino wistar rats.

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## Abstract

**Aim:** This study was carried out to investigate the effect of Sudan II adulteration of palm oil on the serum proteins, electrolytes and lipid parameters of albino wistar rats.

**Methods:** A total of sixty (60) 750 mL bottles of red palm oil were purchased from random markets in Nigeria. Sixty (60) male albino rats weighing 150-180 g were divided into 5 groups of 12 rats each. Group 1 served as normal control. Groups 2 to 5 were fed 90% rat chow supplemented with 10% red palm oil. The Sudan II dyes were co-administered with the red palm oil with the diet (rat chow) to provide levels of 0.025% (PO/0.025) (group 3), 0.03% (PO/0.03) (group 4) and 0.04% (PO/0.04) (group 5) for a period of 30 days (short term) and 90 days (long term). The animals were sacrificed and blood was collected via cardiac puncture for biochemical analysis. The total serum protein (globulin and albumin) concentrations, electrolyte (sodium ion, chloride ion and potassium ion) concentrations and the lipid parameters (HDL, TChol, LDL-Cholesterol, VLDL-Cholesterol) were determined using standard methods. Data analysis was carried out with SPSS using one-way analysis of variance (ANOVA).

**Result:** The results showed that there was a significant ( $P < 0.05$ ) increase in serum Na and K levels in the test groups. Also, total serum proteins were significantly ( $P < 0.05$ ) elevated in the test groups. Significant ( $P < 0.05$ ) increase in lipid parameters were also observed in the test groups. A rise in serum electrolytes is indicative that the dyes may interfere with these electrolytes in several metabolic pathways.

**Significance:** The findings from this study, indicates that this unethical practice might be hazardous to human health, hence it needs to be discontinued.

**Keywords:** Palm oil, Serum protein, Electrolytes, Lipid parameters, Rats, Sudan II dyes.

## Introduction

Food additives are mainly chemical substances added to processed food to enhance or maintain quality features which include but not limited to texture, physical properties, colour, taste and flavours. They are also added to enhance shelf-life of processed foods and control spoilage. They are not adulterants when used in accordance to maximum limits and Good Manufacturing Practices (GMP). Food adulterants are additives which are not permitted for use in a given food [1].

The European Union (EU) rules that all food additives need to be authorized and listed in the EU positive list for food additives with conditions of use after safety assessment, technological need and the fact that its use should not mislead consumers. Azo dyes are not included in the Codex general standard for food additives (EC, 2015b) and the positive additive list for the EU (Annex II of Regulation (EC) No 1333, 2008). Hence, the use of dyes is not permitted in food.

Food fraud is economically motivated. Perpetuators of food fraud are mainly actors involved in the food chain. The harm associated with food fraud on public health is maybe generally negligible, but sometimes mistakes and unintended health consequences occur [2]. Food fraud can be divided into three broad categories which are; replacement, addition and removal [3].

Food quality is closely related to its colour and the use of colorants has been an age-old practice to enhance the aesthetic appeal of foods. Red Palm Oil (RPO) is widely used for food preparation in Nigeria and across West Africa. It has been found that in Nigeria, palm oil has been adulterated with Sudan II dye, a situation which means that the safety of red palm oil available in the local markets and consumed by Nigerians cannot be ascertained.

Synthetic dyes such as azo (Sudan dyes), nitro, nitroso and quinoid dyes are known to affect the liver and kidney functions and may induce responses in humans. Previous researches

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have linked them with deleterious effects on individuals such as cancers, damage to the central nervous system, liver and kidney damage etc. Virtually every household in Nigeria use palm oil for one dish or the other, hence the use of Sudan dyes for colour enhancement would have implications on public health. Due to lack of stringent protocols to guarantee the quality of red palm oil sold in local markets and with the fact that the inclusion levels (different additions) of this dye in red palm oil may attain toxicopathological concentrations, it is pertinent to carry out this study. The start-up point is the assessment of market samples to determine the toxic effect of adulteration with Sudan II dye in male albino wistar rats. It is anticipated that findings emanating from this research will validate the toxicological claims of this adulterating dye and proffer intervention strategies.

## Materials and Methods

### Sample collection

A total of sixty, 750 milliliters bottle of red palm oil samples were purchased from four randomly selected markets in different Nigerian cities namely; Aba, Calabar, Kano and Lagos. In these cities, five (5) samples of red palm oil were each purchased from three (3) different markets. The markets were Ariaria, Cemetery, Ahia Ohuru (Aba), Watt, Marian, Goldie (Calabar), Sabongari, galadima, kofar wabe (Kano) and Balogun, Alade, Ketu (Lagos). The samples were then stored in a transparent plastic container on ice and taken to the laboratory for analysis. The red palm oil samples for qualitative analysis were coded A<sub>1</sub> (red oil samples from Aba), C<sub>1</sub> (red oil samples from Calabar), K<sub>1</sub> (red oil samples from Kano) and L<sub>1</sub> (red oil samples from Lagos). The red palm oil used as control was purchased from Ibiaye Oil Palm in Biase local government area of Cross River state, Nigeria.

### Animal experimental protocols

Sixty (60) mature male albino rats, weighing between 150 g-180 g were obtained from the animal house of the Department of Physiology, University of Calabar and then used for this study. The animals were allowed two weeks for acclimatization, after which they were reweighed and housed in plastic cages with plastic bottom and wire-mesh top, under controlled environmental conditions of temperature (28 ± 2°C), relative humidity (50 ± 5%) and a 12 hour light/dark cycle. The animal facility was adequately ventilated and the animals maintained regularly on the commercial rat chow. Water was provided ad libitum throughout the experimental period. At the end of the acclimatization period of two weeks (14 days), the experimental animals were divided into five groups of twelve animals each. The groups were given 90% commercial rat chow supplemented with 10% red palm oil. Sudan II dye was co-administered in the diet to provide levels of 0% (normal control), 0% (PO) (group 2), 0.025%, (PO/0.025) (group 3), 0.03% (PO/0.03) (group 4) and 0.04% (PO/0.04) (group 5). The experimental animals were given these diets for ninety days along with water ad libitum. The levels of the Sudan II dye (0.025%, 0.03% and 0.04%) was based on the preliminary result of the Sudan II dye content (250-350 ppm or 0.025-0.035%) in the red palm oil, to provide

low (0.025%), medium (0.03%) and high (0.04%). The LD50 of Sudan II dye is 1000 ppm (0.1%).

### Collection of blood samples

At the end of the treatment period i.e. 30 days (short term) and 90 days (long term), the animals were sacrificed and the blood was collected via cardiac puncture. Part of the blood (1 ml) was collected into heparinized or EDTA tubes, and used for haematological studies, while the remaining part was put into non-heparinized (plain) tubes. The blood in the plain tubes was allowed to stand for about two hours (2 hrs) for proper clotting. Thereafter, the tubes were centrifuged at 3000 rpm for 10 min and the supernatant (serum) collected using a 5 ml syringe and needle and then was used for biochemical and toxicological assays.

### Estimation of total biochemical parameters

**Serum proteins:** Total serum protein was determined using calorimetric method of [4].

**Electrolytes:** Sodium ion concentrations were estimated by calorimetric method [4]. Serum chloride concentration was estimated based on a modification of the colorimetric method of Skeggs and Hochestrasser using AGAPPE diagnostic kits, Potassium ion concentration was determined according described by Abeol-Zahab [4].

**Lipid parameters:** High Density Lipoprotein cholesterol (HDL) and Low Density Lipoprotein cholesterol (LDL) concentrations were determined according to the method of [5]. Total cholesterol (TChol) in blood serum was estimated using the enzymatic colorimetric test (CHOD-DAP) kit method [6]. The triacylglycerol concentrations in the samples were estimated using the Chiron diagnostic triglyceride GPO kit method [7] and the Very Low Density Lipoprotein (VLDL- cholesterol) was obtained by dividing the serum triglyceride concentration by 5. This factor of 5 is based on the understanding that in fasting subjects with triglyceride concentration of 400 mg/dl, the VLDL to total plasma triglyceride ratio is fixed relatively at 1:5.

**Statistical analysis:** All data obtained were collated using standard statistical methods. They were expressed as mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) at 5% level of significance. Statistical analysis was performed using SPSS statistical package.

## Results

The serum proteins of the experimental animals fed on the different proportion of palm oil adulterated with Sudan II dye on short term (30 days) and long term (90 days) are presented in Table 1.

The serum electrolytes of the experimental animals fed on the different proportion of palm oil adulterated with Sudan II dye on short term (30 days) and long term (90 days) are presented in Table 2.

The serum lipid parameters of experimental animals fed on the different proportion of palm oil adulterated with Sudan II dye on short term (30 days) and long term (90 days) are presented on Tables 3a and Table 3b.

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**Table 1.** Effect of short and long term feeding of experimental animals with Sudan II adulterated red palm oil on serum proteins.

Groups	Short term			Long term		
	TP (mg/dL)	ALB (mg/dL)	GLB (mg/dL)	TP (mg/dL)	ALB (mg/dL)	GLB (mg/dL)
Group 1 (normal control)	5.07 ± 0.30	2.92 ± 0.17	1.99 ± 0.09	5.09 ± 0.11	2.69 ± 0.25	1.96 ± 0.26
Group 2 (RPO+Feed)	5.17 ± 0.14	2.93 ± 0.18	2.07 ± 0.20	5.17 ± 0.26	2.76 ± 0.13	1.96 ± 0.08
Group 3 (RPO+0.025% dye)	5.92 ± 0.37	3.30 ± 0.21	2.34 ± 0.23	6.14 ± 0.32 <sup>*</sup>	3.25 ± 0.213 <sup>*</sup>	2.01 ± 0.28
Group 4 (RPO+0.003% dye)	6.11 ± 0.44 <sup>*</sup>	3.44 ± 0.25	2.63 ± 0.20	6.23 ± 0.56 <sup>*a</sup>	3.29 ± 0.24 <sup>*</sup>	2.01 ± 0.09
Group 5 (RPO+0.004% dye)	6.46 ± 0.41 <sup>*a</sup>	3.77 ± 0.44 <sup>*a</sup>	3.02 ± 0.48 <sup>*a</sup>	6.45 ± 0.36 <sup>*a</sup>	3.34 ± 0.15 <sup>*a</sup>	2.03 ± 0.04 <sup>*a</sup>
Values are expressed as mean ± SEM, n=6; *significantly different from group 1 (normal control) at p<0.05; a=significantly different from group 2 (palm oil) at p<0.05; Key: TP=total protein; ALB=albumin; GLB=Globulin						

**Table 2.** Effect of short and long term feeding of experimental animals with Sudan II adulterated red palm oil on serum electrolytes.

Groups	Short term			Long term		
	Na <sup>+</sup> (mg/dl)	Cl <sup>-</sup> (mg/dl)	K <sup>+</sup> (mg/dl)	Na <sup>+</sup> (mg/dl)	Cl <sup>-</sup> (mg/dl)	K <sup>+</sup> (mg/dl)
Group 1 (normal control)	57.66 ± 3.11	44.37 ± 1.03	4.23 ± 0.53	53.01 ± 3.20	45.23 ± 0.64	4.27 ± 0.44
Group 2 (RPO+Feed)	58.59 ± 0.89	44.76 ± 0.86	4.25 ± 0.57	57.64 ± 0.41	45.24 ± 0.36	4.50 ± 0.35
Group 3 (RPO+0.025% dye)	62.39 ± 3.10	46.07 ± 0.62	4.32 ± 0.45	63.12 ± 2.60 <sup>*</sup>	45.98 ± 0.87	4.55 ± 0.36
Group 4 (RPO+0.003% dye)	65.66 ± 3.52 <sup>*</sup>	46.13 ± 0.54	4.57 ± 0.62	64.91 ± 1.09 <sup>*a</sup>	45.67 ± 0.62	5.97 ± 0.40
Group 5 (RPO+0.004% dye)	68.8 ± 1.59	46.62 ± 1.50	4.72 ± 0.56	72.65 ± 0.50 <sup>*a,b,c</sup>	45.82 ± 0.70	6.72 ± 1.10 <sup>*a,b</sup>
Values are expressed as mean ± SEM, n=6; *significantly different from group 1 (normal control) at p<0.05; a=significantly different from group 2 (palm oil) at p<0.05; b=significantly different from group 3 (0.025% dye) at p<0.05; c=significantly different from group 4 (0.03% dye) at p<0.05. Key: Na <sup>+</sup> =sodium; Cl <sup>-</sup> =chloride; K <sup>+</sup> =potassium						

**Table 3a.** Physicochemical properties of red palm oil samples.

Groups	TCHOL (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
Group 1 (normal control)	145.08 ± 8.66	114.37 ± 2.57	90.56 ± 3.07	22.88 ± 0.51	52.83 ± 7.10
Group 2 (RPO+Feed)	191.76 ± 13.58 <sup>*</sup>	123.46 ± 2.74	87.58 ± 3.36	24.69 ± 0.54	86.58 ± 12.39 <sup>*</sup>
Group 3 (RPO+0.025% dye)	196.09 ± 6.02 <sup>*</sup>	125.52 ± 11.12	80.30 ± 3.71 <sup>*</sup>	25.10 ± 2.22	90.74 ± 7.34 <sup>*</sup>
Group 4 (RPO+0.03% dye)	198.79 ± 15.94 <sup>*</sup>	135.78 ± 15.86	73.49 ± 2.32 <sup>*a</sup>	27.16 ± 3.17	88.05 ± 12.85 <sup>*</sup>
Group 5 (RPO+0.04% dye)	206.34 ± 15.71 <sup>*</sup>	136.83 ± 12.11	69.37 ± 2.30 <sup>*a,b</sup>	27.37 ± 2.42	88.40 ± 13.29 <sup>*</sup>
Values are expressed as mean ± SEM, n=6; *significantly different from group 1 (normal control) at p<0.05; a=significantly different from group 2 (palm oil) at p<0.05; b=significantly different from group 3 (0.025% dye) at p<0.05; Key: TCHOL=total cholesterol; TG=triacylglycerol; HDL=high density lipoproteins; LDL=low density lipoproteins; VLDL=Very Low Density Lipoproteins.					

**Table 3b.** Effect of long term feeding of experimental animals with Sudan II dye adulterated sample on serum lipid parameters.

Groups	TCHOL (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
Group 1 (normal control)	169.51 ± 12.08	121.22 ± 4.70	95.26 ± 0.43	24.24 ± 0.94	58.72 ± 13.98
Group 2 (RPO+Feed)	209.70 ± 7.52 <sup>*</sup>	133.08 ± 7.30	93.35 ± 1.67	26.62 ± 1.18	94.71 ± 8.23 <sup>*</sup>
Group 3 (RPO+0.025% dye)	211.03 ± 5.71 <sup>*</sup>	136.79 ± 5.88	89.45 ± 2.31 <sup>*</sup>	27.36 ± 1.26	94.22 ± 4.17 <sup>*</sup>
Group 4 (RPO+0.03% dye)	213.50 ± 7.17 <sup>*</sup>	136.94 ± 6.28	88.36 ± 1.19 <sup>*</sup>	27.38 ± 0.89	96.76 ± 7.34 <sup>*</sup>
Group 5 (RPO+0.04% dye)	225.50 ± 3.49 <sup>*</sup>	139.56 ± 4.44	86.55 ± 3.10 <sup>*a,b</sup>	27.91 ± 0.53 <sup>*</sup>	102.33 ± 3.71 <sup>*</sup>
Values are expressed as mean ± SEM, n=6; *significantly different from group 1 (normal control) at p<0.05; a=significantly different from group 2 (palm oil) at p<0.05; b=significantly different from group 3 (0.025% dye) at p<0.05; Key: TCHOL=total cholesterol; TG=triacylglycerol; HDL=High Density Lipoproteins; LDL=Low Density Lipoproteins; VLDL=Very Low Density Lipoproteins.					

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## Discussion

### *Effect on serum protein*

The result of the serum total protein, albumin and globulin are presented in Table 1 for short term and long term respectively. The total protein level of test animals that received the different proportion of the dye increased significantly ( $P < 0.05$ ) in Test group 4 and group 5 when compared to the control. The increase in total protein can be attributed to the increased release of enzymes by the damaged tissues to counteract the effect of the dye and hence, the increased levels observed in the serum total protein. The result is in harmony with an increase in serum total proteins with rats, whose diets were supplemented with chocolate colours A and B dye. [8,9] Also reported that high dose of tartrazine dye caused a significant increase in serum total protein concentration, when rats consumed high dose of tartrazine (500 mg/kgb.w). In this study, the negative changes in serum total protein levels in the groups suggest that this dye may pose health threat.

The changes in albumin levels of the test animals that received the different proportion of the dye increased significantly ( $P < 0.05$ ) in group 5, when compared to the control and group 2. Additionally, a significant increase ( $P < 0.05$ ) was observed for the long term feeding (90 days) in groups 3, 4, and 5 respectively when compared to the control (Table 1) respectively. The level of serum albumin is an important marker for liver's secretory ability, since albumin is secreted exclusively by the liver [10]. In this study, the increases in the albumin level of the test animals is suggestive of the fact that exposing the animals to red palm oil adulterated with Sudan II dye does pose serious hepatocellular pathophysiology. The increased serum albumin levels in this study are in agreement with an increase in serum albumin levels in male rats that consumed tartrazine at low and high dose for 30 days. However, the result is contrary to a decrease in serum albumin levels [9]. Albumin is essential for the maintenance of the pressure needed for compartments and the body tissues. It also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones [11].

The changes in serum globulin levels of the test animals that received the different proportion of the dye increased significantly ( $P < 0.05$ ) in group 5, when compared to the control and group 2 (Table 1). The increase in globulin level may be attributed to the fact that exposure of the test animals to the red palm oil adulterated with Sudan II dye, triggered the production and secretion of antibodies supposedly meant to fight the toxic dye present in the diet. Consequently, it resulted in high serum globulin concentration. Globulin like albumin is produced in the liver except the gamma-globulin [12].

### *Effect on serum electrolytes*

The serum electrolytes profile is one of the major biochemical/toxicological parameters used to check the body's homeostatic states and kidney functionality [13]. The sodium levels of animals in Test groups 4 and 5 respectively showed significant ( $P < 0.05$ ) increases when compared to the control. On the long term study, there was a significant ( $P < 0.05$ ) increase in the

Test groups when compared to the control also. In addition, significant increases were observed within the test groups (Table 2). A dose dependent increase in sodium levels was observed in this study. The changes in sodium levels in this study may be suggestive or indicative that the integrity of the kidney is not maintained, since sodium is one of the major electrolytes used to evaluate the integrity and functionality of the kidney. The result of sodium level in this study is in harmony with an elevated serum level of sodium in rats administered with carnosine and tartrazine dyes [14]. The metabolism of sodium is regulated by aldosterone, an adrenal cortex hormone which promotes the re-absorption of sodium from the kidney tubules [15]. Sodium maintains pH of body fluids and plays a vital role in nerve function [16]. Sodium as bicarbonate is important in the regulation of acid-base balance. It maintains body fluids viscosity and plays a vital role in the absorption of sugars and amino acids from the digestive tract. In the absence of aldosterone, sodium excretion increases and deficiency ensues [16].

The chloride levels of both the control and test animals as seen in Table 2, which showed that there was no significant ( $P < 0.05$ ) increase in the chloride level of animals in the Test groups when compared to the control. Excessive chloride concentration in serum is often associated with congestive heart failure and decreased renal blood flow [16]. In this study, the non-significant increase in the chloride levels in the test groups may be as a result of the inhibitive effect of the dye on tubular cells re-absorption of the ion, which might be due to extensive damage produced by the Sudan II dye. The result of the chloride level is in agreement with an elevated serum chloride in rats administered with carnosine and tartrazine dyes [14]. This therefore suggests that the dye does pose a significant health threat to the kidney.

Potassium levels of both the control and test groups as seen from the Table 2. The result showed that a non-significant ( $P < 0.05$ ) increase was observed between the Test groups when compared to the control. However, on the long term study, a significant ( $P < 0.05$ ) increase was observed in test group 5 when compared to the control, as well as with groups 2 and 3 (Table 2). Potassium ion is the principal intracellular cation responsible for neuromuscular excitability. Potassium functions in the maintenance of acid-base equilibrium. It also functions in nerve impulse transmissions, heart beat relaxation and activation of certain enzymes. Potassium ions are necessary for carbohydrate and protein metabolism. It helps in the uptake of some amino acids [13]. Potassium maintains intracellular osmotic pressure and depolarizes and contracts the heart [15]. The results of the increased electrolytes in this study are indicative of the toxic effect of the dye on renal function or the dye inhibitory action on electrolytes transport in tissues and thus suggest that the dye may interfere with these electrolytes in several metabolic pathways leading to the increase in their levels.

### *Effect on lipid parameters*

Lipids are heterogeneous group of compounds related more by their physical rather than their chemical properties. Lipids

are transported in the form of High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Very Low Density Lipoproteins (VLDL) and chylomicrons. The levels of serum lipid parameters usually reflect the integrity and synthetic ability of the liver, as the liver is the major organ responsible for repackaging of lipids of both exogenous and endogenous sources before they are released into circulation [16]. In this study, the serum total cholesterol levels (Tables 3a and Table 3b) in the test groups increased significantly ( $P < 0.05$ ) in groups 3, 4 and 5 respectively when compared to the control. The increase in serum total cholesterol level may be an indication of membrane function and structure disruption, thus affecting its fluidity, permeability and transport system and hence, its appearance in the serum. Total cholesterol is usually referred to as being the major component of bad lipid and bad lipids are primary culprits in cardiovascular diseases and other related diseases such as atherosclerosis [17]. The levels of total cholesterol recorded in this study showed that the liver was unable to adequately handle cholesterol synthesis and repackaging.

The serum triacylglycerol levels showed a non-significant ( $P > 0.05$ ) increase in the test groups of animals when compared to the control (Table 3a). However, a significant ( $P < 0.05$ ) increase was observed on the long term study (90 days) in group 5 when compared to the control (Table 3b). The observed increase in serum triacylglycerol levels may be due to an alteration of the activity of hepatic lipase, responsible for triacylglycerol catabolism. Hence, with the compromised liver due to the toxic effect of the dye, the lipase activity would be reduced resulting in hypertriacylglyceridemia. The result of serum triacylglycerol levels in this study is in line with a significant increase in serum triacylglycerol levels, when rats were administered with high dose (500 mg/kg/b.w) and low dose (15 mg/kg/b.w) of tartrazine dye [9].

There was a significant ( $P < 0.05$ ) decrease in the High Density Lipoprotein (HDL) levels in animals in groups 3, 4 and 5 respectively when compared to the control. The decrease in HDL may be due to peroxidation of the cell membrane lipids. The findings are consistent with a decrease in HDL in male albino rats administered with two kinds of food additive mixtures [18]. However, it is contrary that showed increase in rates of HDL in rats administered 15 mg and 500 mg of tartrazine/kg of bw/day [9]. High Density Lipoproteins (HDL) are the most complicated and diverse of the lipoproteins, as they contain many different protein constituents, whose main purpose is to enable secretion of cholesterol from cells, esterification of cholesterol in plasma, transfer of cholesterol to other lipoproteins and the return of cholesterol from peripheral tissues to the liver for excretion—a process that has been termed ‘reverse cholesterol transport’ [19,20]. In addition, HDL have an important function in triacylglycerol transport by facilitating the activation of lipoprotein lipase, in the transfer of triacylglycerides between lipoprotein classes and in the removal of chylomicron remnants and Very Low Density Lipoproteins (VLDL) enriched in triacylglycerols as well as the apolipoproteins.

There was no significant ( $P > 0.05$ ) increase in groups 3, 4

and 5 respectively when compared to the control group. However, a significant ( $P < 0.05$ ) increase, was observed on the long term study (90 days) in group 5 when compared to the control (Table 3b). The significant increase observed in Low Density Lipoproteins (LDL) may be due to activities of reactive oxygen species, and the free radicals generated from the microbial transformation of Sudan II dye which in turn precipitated hepatocellular derangements. The results of the LDL levels are consistent with increased levels of LDL in rats administered with 15 and 500 mg of tartrazine/kg of bw/day [21]. However, the findings from this study are contrary to a significant decrease in LDL of rats administered with tartrazine dye, Low Density Lipoproteins (LDL) is produced by the liver from the Very Low Density Lipoprotein (VLDL). Low density lipoproteins are the main carriers of cholesterol to the adrenals and adipose tissue, where there are receptors requiring apo-B100 that are able to take in the LDL by a similar process to that occurring in liver [21]. Within these tissues, the cholesterol esters are hydrolyzed to yield free cholesterol, which is incorporated into the plasma membranes as required. Any excess cholesterol is re-esterified by an acyl-CoA-cholesterol acyltransferase for intracellular storage (Table 4).

## Conclusion

The toxicity effect of red palm oil adulterated with Sudan II dye in male albino Wistar rat was carried out and the results from this study indicated that the red palm oil adulterated with Sudan II dye led to impaired vital organs (hepatic and renal functions) especially at 0.04% of the dye administration in both short and long term exposure. Hence, the use of this dye to improve the colour of red palm oil is deleterious and should be discontinued. Therefore, it is pertinent to create awareness and the need for enforcement of regulatory acts and food safety procedures.

## Author's contribution

Peter Henry contributed to the methodology, laboratory analyses and data collection. Aniekan Henshaw contributed to the writing of the paper. Christene Ikpeme and Ima-obong Williams contributed to the conceptualization of the research, validation and supervision of the research. All authors read and approved the final manuscript.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability

All data generated or analyzed during this study are included in this published article.

## Ethical Approval

All protocols were conducted in conformity to the standards for laboratory animal use and care as found in the European

Community guidelines (EEC Directives of 1986; 86/609/EEC). Approval was obtained from the Faculty of Basic Medical Sciences Animal Research Ethics Committee.

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