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ASSESSMENT OF KIDNEY FUNCTION AND LIPID PROFILE IN ALBINO RATS EXPOSED TO AZO-DYE ADULTERATED PALM OIL

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ABSTRACT: *Food is an important factor in human existence which makes exposure to adulterants in food an important environmental factor challenging the biological system. In West Africa, the manufacturing and processing of palm oil are done without proper hygienic monitoring; it is therefore almost impossible to detect fraud in the system. A major disadvantage associated with the use of adulterants in palm oil is that the adulterants have not undergone adequate research and the degree of health hazards they can pose to humans when consumed. This study was designed to evaluate the toxic effects of Azo dye adulterated palm oil on kidney function and Lipid profile in albino rats exposed to Azo-dye adulterated palm oil. Twenty-five albino rats were divided into five groups and treated as thus: Group I (control), Groups II and III were treated with only 1 ml/kg of unadulterated and adulterated palm oil respectively, while Groups IV and V were treated with only 50 mg/kg of Sudan III and IV dyes respectively for 28 days. Renal function tests, and lipid profiles were determined using analytical test kits. Data obtained were statistically analyzed with one-way analysis of variance (ANOVA) followed by tukey test using Graph Pad prism 9.0.0. The results were presented as mean ± standard error of mean (SEM). Differences between means of treated and control groups values at $P \leq 0.05$ at 95% confidence interval were considered significant. The results showed a significant increase ($p < 0.05$) in creatinine, urea concentrations, Sodium ion (Na+) and Potassium ion (K+) in groups treated with adulterated palm oil, Sudan III, Sudan IV (groups III, IV and V respectively) when compared with control groups. A significant increase ($p < 0.05$) in low density lipoprotein (LDL), cholesterol, very low-density lipoprotein (VLDL) and Triacylglycerol (TAG) was observed in treated groups when compared with control groups while a significant decrease ($p < 0.05$) in high density lipoprotein level (HDL) observed in treated groups when compared with the control group. This suggests that adulterated palm oil can induce renal damage and alter lipid profile.*

KEYWORDS: Azo dye, lipids, renal function, palm oil.

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INTRODUCTION

The global issue of food adulteration has grave implications for both health and safety in humans. This reprehensible practice is unequivocally prohibited by food safety regulations worldwide. The range of food items subjected to adulteration varies from one country to another, encompassing products like fruit juices, palm oil, flour, and meat. The primary purpose of food adulteration is to improve the product's visual appeal while concealing the presence of subpar ingredients. Consequently, those who engage in this unethical practice stand to gain economically. In Nigeria, palm oil is particularly susceptible to adulteration through the illicit use of prohibited food dyes or colorants by unscrupulous individuals (Peters *et al.*, 2022). Food additives, on the other hand, predominantly consist of chemical substances incorporated into processed foods to enhance or preserve their quality attributes. These attributes encompass but are not limited to texture, physical properties, color, taste, and flavors. They are also employed to extend the shelf life of processed foods and prevent spoilage. Food adulterants, conversely, refer to additives that are not permitted for use in a given food product (Vasireddi, 2013).

Palm oil serves as the most commonly utilized ingredient in food preparation throughout Nigeria and the broader West African region. However, it has come to light that in Nigeria, instances of palm oil adulteration with Sudan Azo dye have been identified. This situation raises concerns regarding the safety of the red palm oil found in local markets and consumed by the Nigerian population, making it difficult to guarantee its safety (Eshalomi, 2010; Kola-Ajibade *et al.*, 2021; Peters *et al.*, 2022). 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 research works have linked them with deleterious effects on individuals such as cancers, damage to the central nervous system, liver and kidney damage, etc. (Lakshmi, 2012).

The liver is the central organ that plays a role in metabolism of macromolecules in the body. Fat is synthesized from carbohydrates and protein, primarily in the liver. Fat absorbed by lacteals in the intestinal villi enters the liver through the lymphatics, primarily as triglycerides. In the liver, the triglycerides can be hydrolyzed to glycerol and free fatty acids and used to produce metabolic energy adenosine triphosphate (ATP), or they can be released into the bloodstream as lipoprotein. The lipoproteins are carried by the blood to adipose cells for storage. The liver also synthesizes phospholipids and cholesterol, which are needed for the hepatic production of bile salts, steroid hormones, components of plasma membranes and other special molecules (Martin, 2012).

The kidney also plays an essential role in maintaining a number of vital body functions; it is the principal excretory organ in all vertebrates. Therefore, a disruption of normal kidney function by the action of toxic agents can result in serious sequelae asides a disruption in blood waste elimination. However, for clinical purposes, alterations in the excretion of wastes are the principal endpoints for determining the action of nephrotoxicants (Philip *et al.*, 2015). The kidney is one of the important tissues because of its role in filtration, metabolism and excretion of compounds. The kidney also plays a key role in regulating total body homeostasis. These homeostatic functions include the regulation of extracellular volume, the regulation of calcium metabolism, the control of electrolyte balance, and the control of acid–base balance (Rayner *et al.*, 2016).

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The kidney functions majorly in filtration and excretion, homeostatic regulation and endocrine role. Filtration occurs through the glomerular filtration barrier (Pollak *et al*., 2014). About 22 % of cardiac output goes to the kidneys and about 20 % of the plasma is filtered, producing about 170 L of glomerular filtrate per day. The filtrate contains diffusible constituents at almost the same concentrations as in the plasma. About 30 000 mmol of sodium, 800 mmol of potassium, 800 mmol of urea, 300 mmol of free ionized calcium and 1000 mmol of glucose are filtered daily. Proteins (mainly low-molecular-weight proteins) and protein bound substances are filtered in only small amounts by normal glomeruli and most are reabsorbed. Ninety-nine percent (99%) of this is reabsorbed as it flows along the nephrons, so only about 1.5 L of urine is produced per day (Arkill *et al.*, 2014).

The kidney is also central in the homeostatic regulation of acid–base balance in the biological system. Hydrogen ions are secreted from renal tubular cells into the lumina, where they are buffered by constituents of the glomerular filtrate. Most of the excess H^+ can only be eliminated from the body by the renal route. The kidneys are of major importance in compensating for chronic acidosis; without them, the hemoglobin (Hb) buffering capacity would soon become saturated. The kidney can be called a primary endocrine organ and secondary. They are sites of degradation of hormones, for example, insulin and aldosterone. The kidneys produce *erythropoietin*, a glycoprotein hormone secreted majorly in the kidneys of adults and liver of fetus that acts on the bone marrow cells to stimulate *erythropoiesis* (Philip *et al.*, 2015).

In evaluating kidney function, the biochemical findings and urine output in renal disease depends on the relative contributions of glomerular and tubular dysfunction. When the GFR falls, substances that are little affected by tubular action (such as urea and creatinine) are retained. Plasma concentrations of urea and creatinine depend largely on glomerular function.

This study was carried out in order to assess the kidney function and lipid profile in albino rats exposed to Azo-dye adulterated palm oil and to determine probable health implications in humans.

MATERIALS AND METHODS

Test for Adulteration of Palm Oil

Palm oil samples were left to stand on the bench for six (6) months in transparent pet bottles; red dyes were seen settled at the base of palm oil containing adulterants.

Chemical Test

The method used by Nwachoko and Fortune (2019) was employed to check for the presence of the adulterant (Sudan dye) in samples of palm oil. This analysis was done using petroleum spirit and concentrations of hydrochloric acid/water (1:1). To 5 ml of each oil sample in different test tubes, 15 ml of petroleum ether was added followed by 5 ml hydrochloric acid to different test tubes. Different shades of yellow were observed at the top and a clear/colorless base indicating the absence of adulterants while samples containing adulterants showed a reddish yellow top and a reddish clear base.

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Experimental Grouping/Treatment of Animals

Twenty-five (25) male albino rats weighing 120g - 148g were assigned into 5 groups (I, II, III, IV, V), five animals in each cage; they were acclimatized to their environment and diet for 7 days. Groups II, III, IV, and V were the test groups. Group I was the control group. Groups II and III were given 1 ml/kg of unadulterated palm oil and adulterated respectively (Nwachoko & Fortune, 2019). Groups IV and V were given 50 mg/kg Sudan III and Sudan IV respectively (Oparaocha *et al.,* 2019).

Collection of Blood Samples

Animals were sacrificed using chloroform anesthesia with about 2 ml each of blood from arterial puncture of the albino rats. Lipid profiles (HDL, TAG, CHOL, VLDL) were determined using analytical test kits while serum LDL (LDL-C) was estimated according to protocol of Friedewald *et al.* (1972). Urea and creatinine concentration in serum and tissue homogenate were determined using colorimetric methods by Weatherburn [1967], and Bartel and Bohmer (1972) respectively, as outlined in the Randox test kits. Electrolyte balance was assayed for: Sodium ions and Bicarbonate ions; Potassium ions in the serum were determined using a method by Tietz (1967), Chloride ions in the serum of samples were determined using a method by Tietz (1964) and Calcium ions concentration was determined using colorimetric method (Ray *et al.,* 1967). All reagents used were of analytical grade.

STATISTICAL ANALYSIS

Data obtained were statistically analyzed with one-way analysis of variance (ANOVA) followed by tukey test using Graph Pad prism 9.0.0. The results were presented as mean $\pm$ standard error of mean (SEM). Differences between means of treated and control groups values at $P \leq 0.05$ at 95% confidence interval were considered significant.

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RESULTS

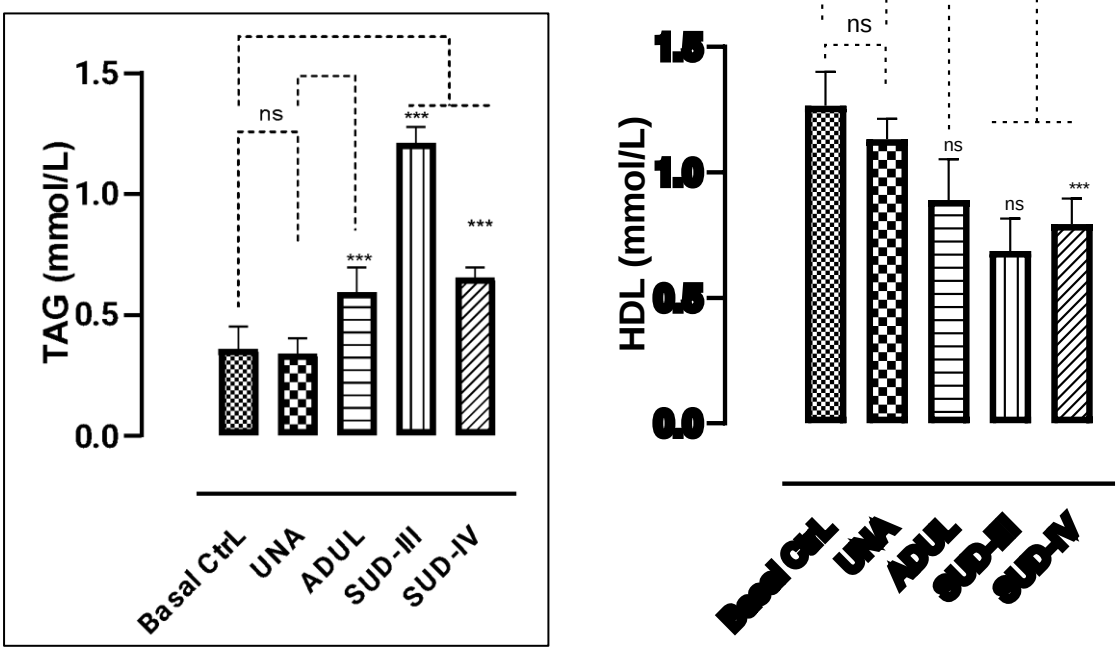

Figure 1a: Triacylglycerol (TAG) in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

Figure 1b: High density lipoprotein (HDL) in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

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UNA (unadulterated); ADUL (adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

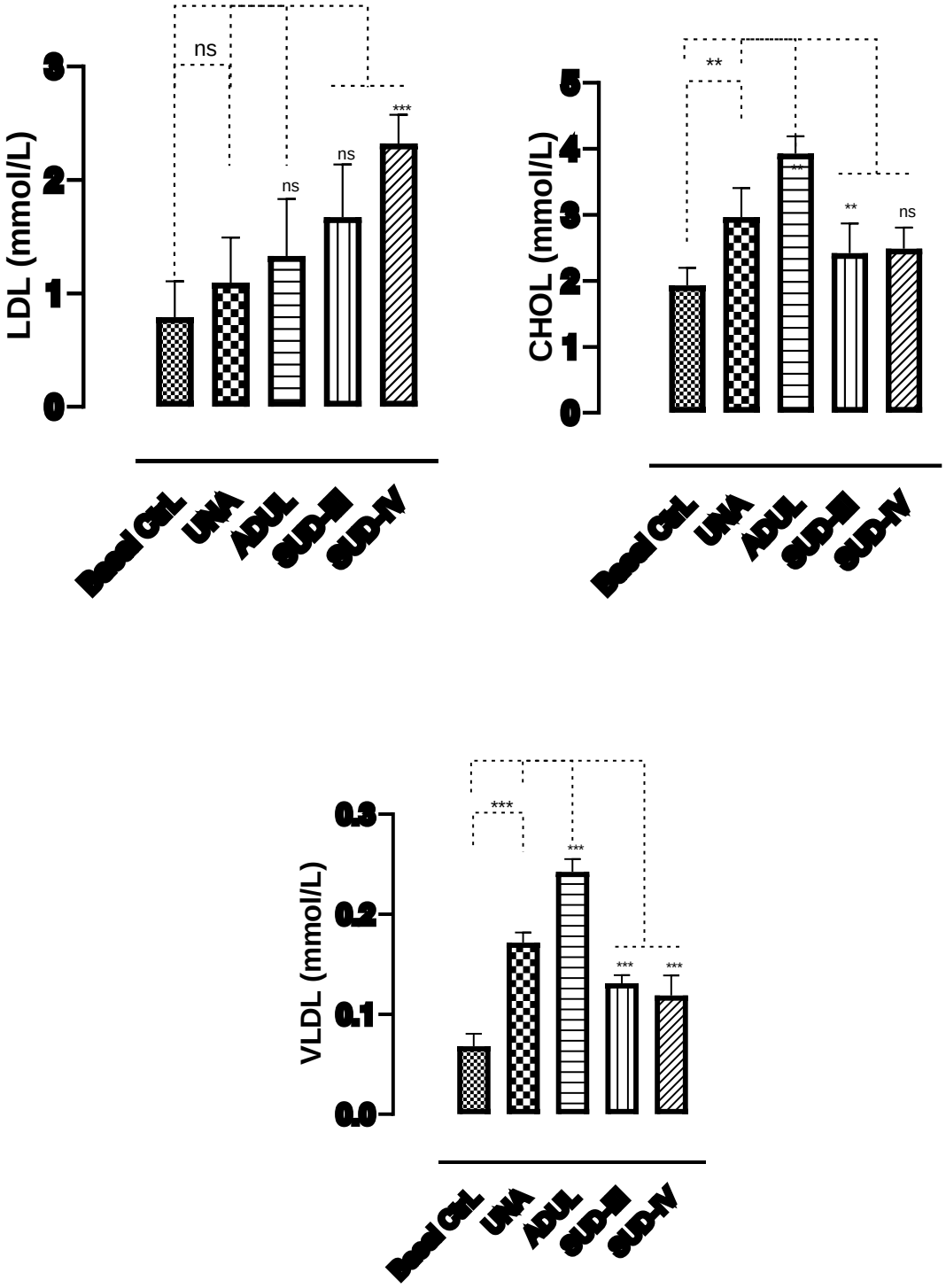

Figure 2a: Low density lipoprotein (LDL) in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

Figure 2b: Cholesterol (CHOL) in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

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Figure 2c: Very low-density lipoprotein (VLDL) in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

UNA (unadulterated); ADUL (adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

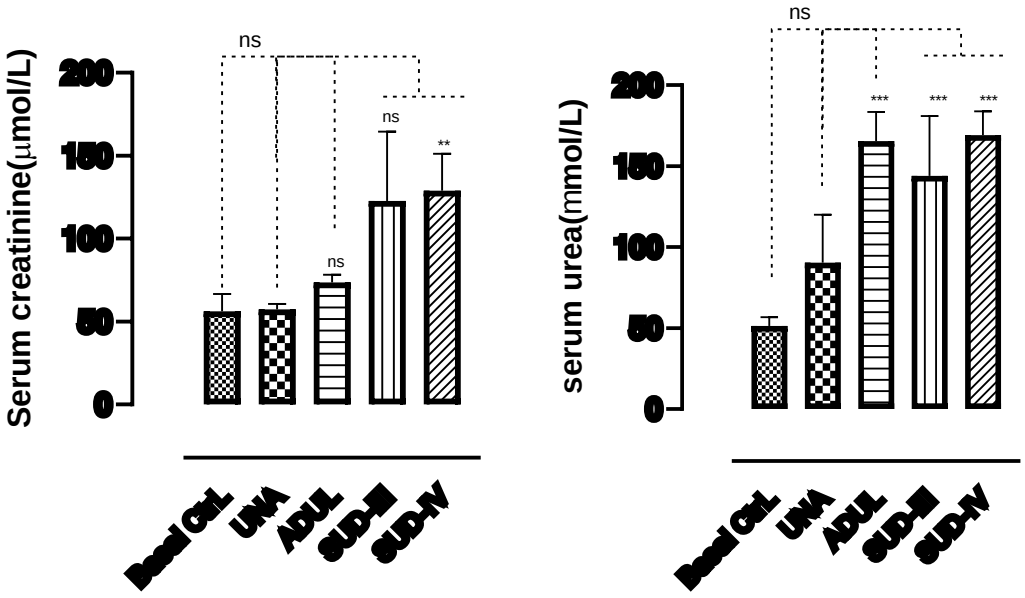

Figure 3a: Creatinine concentration in the serum.

Figure 3b: Urea concentration in the serum.

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UNA (unadulterated); ADUL (adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

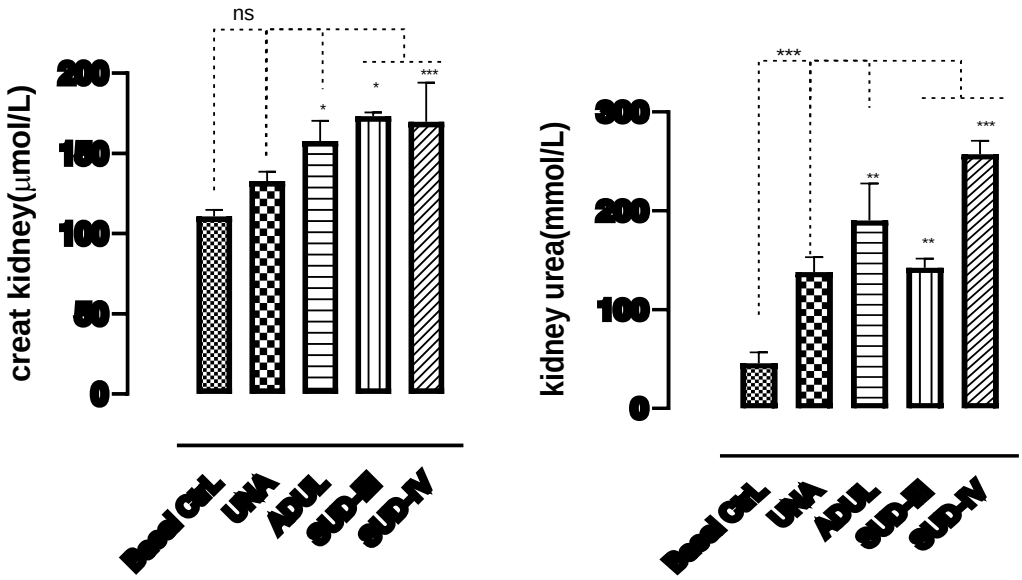

Figure 4a: Creatinine concentration in tissue homogenate.

Figure 4b: Urea concentration in tissue homogenate.

UNA (unadulterated); ADUL (adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

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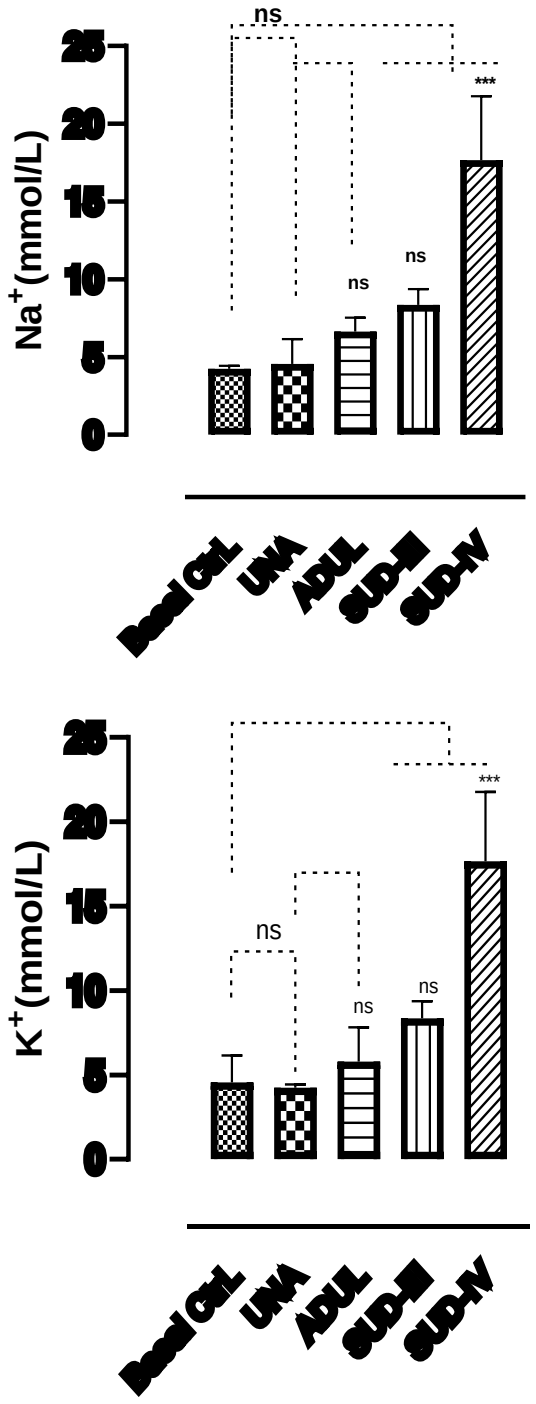

Figure 5a: Na^+ concentration in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

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Figure 5b: K^+ concentration in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

UNA (unadulterated); ADUL (adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

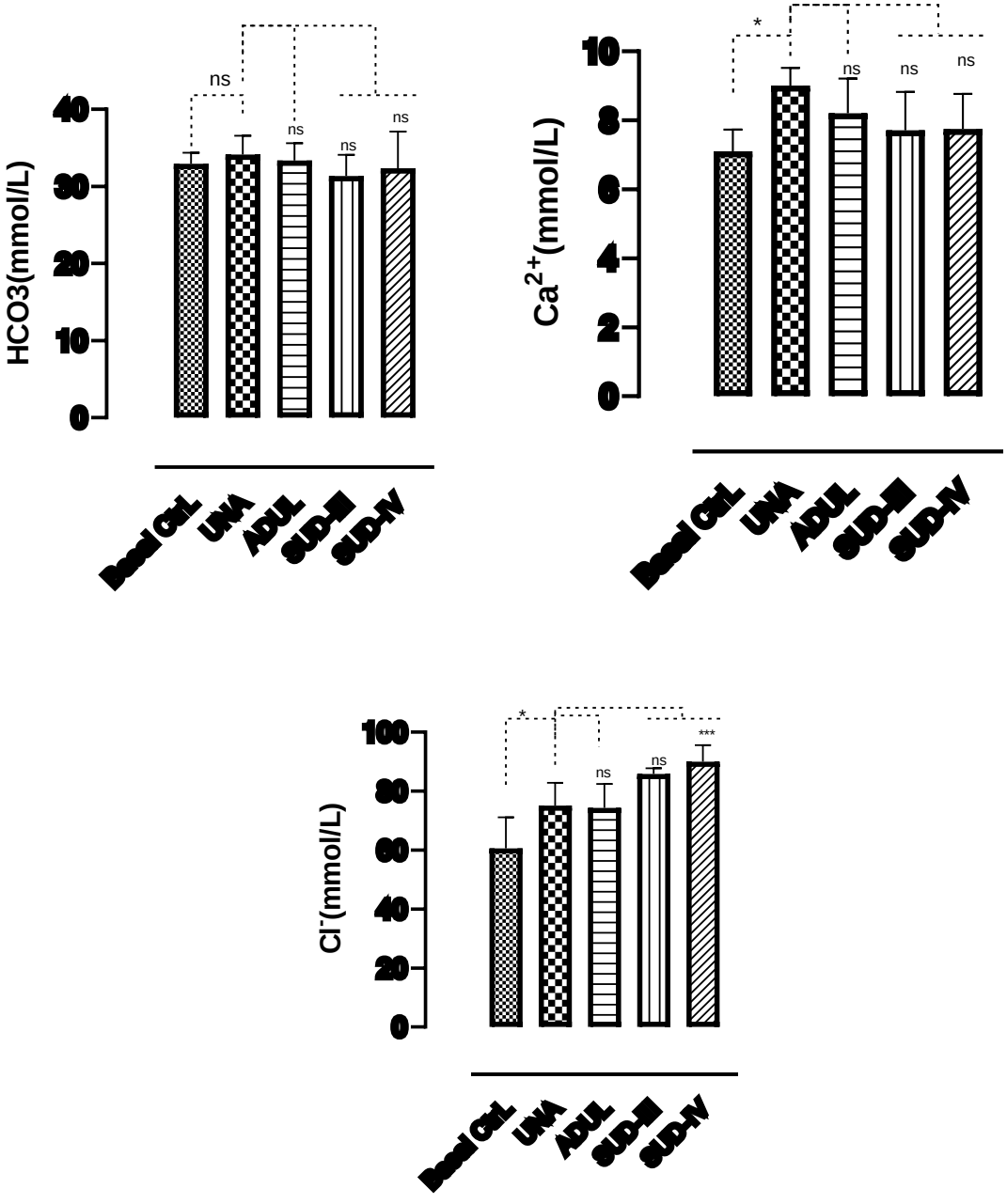

Figure 6a: HCO_3^- concentration in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

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Figure 6b: Ca^{2+} concentration in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

Figure 6c: Cl^{-} concentration in rats administered Sudan dyes, unadulterated and adulterated palm oil and control group.

UNA (unadulterated); ADUL (adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

DISCUSSION

Effect of Azo-dye Adulterated Palm Oil on Lipid Profile

Lipids serve as an important precursor of hormone synthesis, generation of ATPs (energy), aiding in digestion and as structural components of functional biological cells (Elekima *et al.*, 2017). For lipids to be transported properly in aqueous medium, they are bound to complex proteins called lipoproteins. Such lipoproteins are chylomicrons, VLDL, IDL, LDL and HDL.

Exposure to exogenous reactions by chemicals such as Azo dyes in palm oil upon metabolism in the liver leads to generation of free radicals and ROS, which leads to biochemical and physiological lesions that may result in cell death from oxidative damages to lipids, proteins and DNA. Abnormalities in lipids and lipoprotein metabolism are closely associated with cardiovascular, cerebrovascular and peripheral vascular diseases (Chatterjea *et al.*, 2007; Elekima *et al.*, 2017).

As observed from this study (**Figure 1a, 1b, 2a, 2b, 2c)**, a significant increase ($p < 0.05$) in low density lipoprotein (LDL), cholesterol, very low-density lipoprotein (VLDL) and triacylglycerol (TAG) was observed in groups treated with adulterated palm oil, Sudan III and IV (Groups III, IV and V respectively). A significant decrease ($p < 0.05$) in high density lipoprotein level (HDL) was also observed in Groups III, IV and V when compared with the control group and group treated with unadulterated palm oil.

In tandem with this study, Abdel-Rahim *et al.* (2019) reported a significant increase in TAG and a significant decrease in HDL in rats treated with synthetic dyes—tartrazine and chocolate brown. Elekima *et al.* (2017) reported that tartrazine (Azo dye) orally administered at varying concentrations induced increased levels of TAG, TC (total cholesterol) and LDL and decreased HDL cholesterol which is in tandem with this study. Samar *et al.* (2016) also reported an increase in serum level of cholesterol; TAG and LDL were observed at low and high doses of male rats treated with Sudan III dye. Al-Mashhedy (2013) and Amin *et al.* (2010) also reported increased TAG, TC and LDL levels with a decreased HDL level in textile workers exposed to textile dyes and male rats exposed to Tartrazine and Carmoisine and respectively, which is also in line with this study.

It can be said that exposure to adulterated palm oil can increase the risk of developing cardiovascular disorder such as atherosclerosis, hypertension, coronary heart diseases (which are usually linked with increase in the plasma levels of lipid parameters such as TAG, TC and LDL) and decreased level of HDL concentration (Elekima *et al.*, 2017).

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Effect of Azo-dye Adulterated Palm Oil on Kidney Parameters

Renal tubular cells, in particular, proximal tubule cells, are vulnerable to the toxic effects of xenobiotics because of their role in concentrating and reabsorbing glomerular filtrate which exposes them to high levels of circulating toxins. Drugs that cause tubular cell toxicity do so by impairing mitochondrial function, interfering with tubular transport, increasing oxidative stress, or forming free radicals (Cynthia, 2008). From this research work, **Figures 3a and 3b, and 4a and 4b** show creatinine and urea concentrations in the serum and tissue homogenate respectively. A significant increase ($p < 0.05$) in the concentration of creatinine and urea in the serum and tissues of groups administered with adulterated palm oil, Sudan III and IV (III, IV and V respectively) was observed when compared with control groups (basal control and group administered unadulterated palm oil (Groups I and II).

In line with this study, Nwachoko and Fortune (2019) and Oparaocha *et al.* (2019) reported a significant increase in creatinine and urea levels in rats treated with colorant when compared to the control group. Also, in tandem with this study, Imafidon and Okunrobo (2012) reported significant elevation in urea and creatinine levels in albino rats administered Sudan IV dye.

In the event of kidney damage, urea and creatinine are usually elevated (Jozef *et al.,* 2002; Oparaocha *et al.,* 2019). Azo dyes had been reported to pose health risks and exert negative effects on the kidney and nervous system (Bianca *et al.*, 2020).

A possible mechanism of Azo-dye nephrotoxicity is the reactive intermediate from the metabolism of Azo dye which is capable of binding covalently to macromolecules altering their activity, leading to injury (Raju *et al.,* 2011). The toxicant may increase reactive oxygen species (ROS) in the cell after being bio-transformed into a reactive intermediate or through redox cycling. The resulting ROS leads to oxidative damage and cell injury (Raju *et al.,* 2011).

Effect of Azo-dye Adulterated Palm Oil on Electrolyte Concentration

Electrolyte concentrations are usually elevated in an event of kidney damage; they serve as markers of renal function (Nwachoko & Fortune, 2019). As shown in **Figures 5a, 5b, 6a, 6b and 6c** respectively, a significant increase ($p < 0.05$) in the concentrations of Sodium ion (Na^+) and Potassium ion (K^+) was observed in groups treated with adulterated palm oil, Sudan III, Sudan IV (Groups III, IV and V respectively) when compared with the control group (basal control group) treated with unadulterated palm oil (Groups I and II). No significant increase ($p > 0.05$) was observed in the concentration of Bicarbonate ion (HCO_3^-) in the treated groups and control groups, while a significant increase ($p < 0.05$) was also observed in the concentration of Calcium ion (Ca^{2+}) and Chloride ion (Cl^-) in group II when compared with Group I and a significant increase ($p < 0.05$) in treated groups was observed when compared to Group I.

In line with this study, an elevated concentration of Sodium, Chloride, Bicarbonate and Potassium ions was reported by Nwachoko and Fortune (2019). Na^+ and K^+ reabsorption takes place primarily at the renal tubule; however, the collapsed renal tubule of this study may account for malabsorption of Na^+, K^+ and other electrolytes. The result shows that adulterated palm oil has an adverse effect on electrolyte balance.

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DECLARATIONS

Ethics Approval

All animal experiments were approved by the quality control unit of the university.

Competing Interest

The authors declare that there are no conflicts of interest.

Availability of Data and Material

Data generated as part of this study is available upon request from the corresponding author.

Consent for Publication

All authors provide consent for publication.

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