

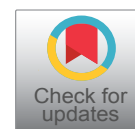


ORIGINAL ARTICLE

Biochemical Changes in Hematological and Liver Parameters in Albino Rats Exposed to Azo Dye Adulterated Palm Oil

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Abstract

In West Africa, the manufacturing and processing of palm oil are done on a small, medium, and large scale, 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 hematological and liver parameters in albino rats. Twenty-five albino rats were divided into five groups and treated as thus; group I (control), groups II and III (1 ml/kg of unadulterated and adulterated palm oil respectively), groups IV and V (50 mg/kg Sudan III and IV respectively) for 28 days. Hematological parameters were determined using an automated analyzer and liver function tests were determined using analytical test kits. The result showed a significant increase ($p < 0.05$) in the activity of liver enzymes aminotransferases (ALT, AST), alkaline phosphatase (ALP), and bilirubin in groups III, IV, and V when compared to control groups (I and II), while higher values of albumin concentration were observed in control groups when compared to other groups. A non-significant ($p > 0.05$) decrease in red and white blood cell indices were observed in groups III, IV, and V in comparison to control groups. This research suggests that exposure to adulterated palm oil can predispose an individual to hepatocellular damage/malfunction and blood cells lysis, thus, not safe for consumption.

Keywords

Azo dye, Palm oil, Adulteration, Hematological parameters, Liver parameters

Introduction

Oil palm (*Elaeisguineensis*) is generally believed to have originated from Africa, it produces two types of oil: Orange-red crude palm oil which is extracted from the mesocarp, and brownish-yellow crude palm kernel oil extracted from the seeds (kernel) [1]. In West Africa, the manufacturing and processing of palm oil are done on a small, medium, and large scale, it is therefore almost impossible to detect fraud in the system [2,3]. It has however been observed that most edible palm oil in Nigeria fall short of the recommended quality standards that are considered safe for consumption [4,5]. The low quality could be a result of the presence of some inclusion that has been added intentionally by the producers or marketers to enhance quantity, appearance, and viscosity. The most widely used adulterant of palm oil are Sudan III and IV (which are a sub-type of azo dye), they are being added to palm oil to improve the color, making it more attractive to buyers [2,4,6]. Sudan dyes are considered illegal dyes, mainly because of their probable harmful effect over a long period of time [6].

Hepatotoxics are exogenous compounds that may include overdose of certain medicinal drugs, synthetic chemicals, herbal remedies, and dietary supplements [7]. The liver is the largest organ in the body and it is the center for the metabolism of nutrients and excretion of wastes [8], it also maintains a central and striking position within the circulatory system. Virtually all of the absorbed doses of xenobiotics and drugs pass through the liver before being distributed through the

bloodstream to the rest of the body. Consequently, it is difficult for any drug or xenobiotic to escape contact with the liver, an important factor in the role of the liver in removing foreign chemicals. The liver's prominence causes it to have increased vulnerability to toxic attacks. Blood is a viscous fluid that consists of cellular elements suspended in plasma. Plasma is a viscous, translucent, yellowish fluid composed of water (90%), proteins (7%), organic salts (1%), and organic compounds (2%) such as amino acids, lipids, and vitamins [9]. The bioavailability of the chemical compounds at toxic levels in biological media causes alteration in various haematological indices. Hematological constituents have been elucidated as pathological indices of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic intervention and hence change in relation to the physiological status of an individual [10]. It has become important to study the effect of azo dye on hematological parameters irrespective of the target organs since the dye is conveyed to their respective sites of action via the bloodstream.

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 is not well established [11,12].

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, [13] was employed to check for the presence of 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 the 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.

Experimental grouping/treatment of animals

25 male albino rats were weighing 111 g-198 g 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 [13]. Groups IV and V were given 50 mg/kg Sudan III and Sudan IV respectively [14].

Collection of blood samples and extraction of tissues

Animals were sacrificed using chloroform anesthesia. About 2 ml each of blood from arterial puncture of the albino rats were collected into anticoagulant tubes (EDTA bottles) and plain bottles for determination of hematological indices, liver function tests. Tissues were also kept in normal saline in plain bottles for homogenization. Hematological blood indices were done using an automated analyzer while liver function tests were done using analytical test kits.

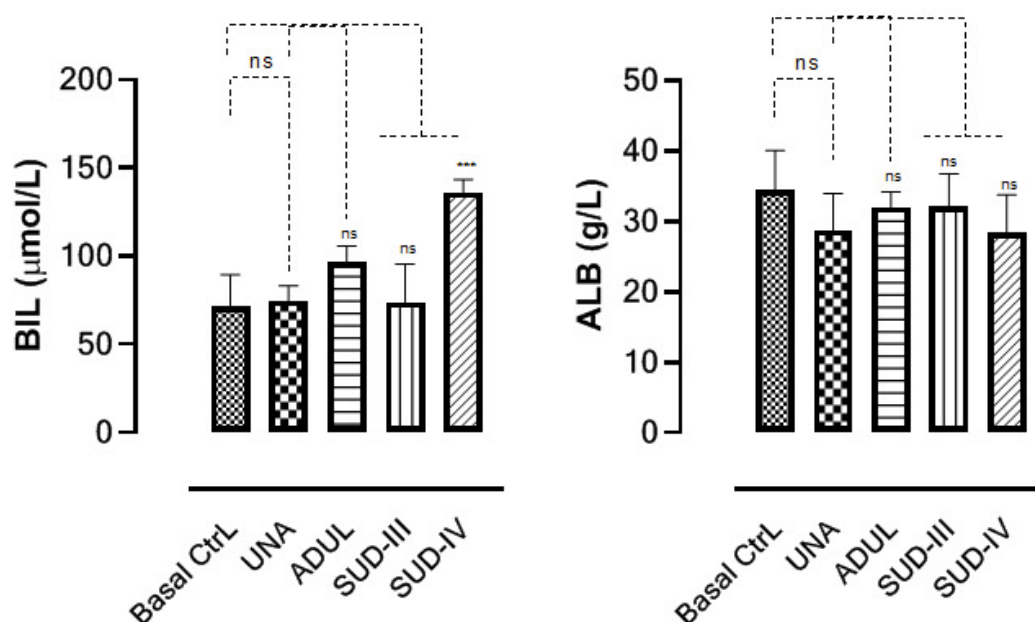


Figure 1: Bilirubin (BIL) and Albumin (ALB) concentration of Rats administered dye and adulterated palm oil ($p < 0.05$): Mean \pm SEM; ($n = 5$). UNA (Unadulterated); ADUL (Adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

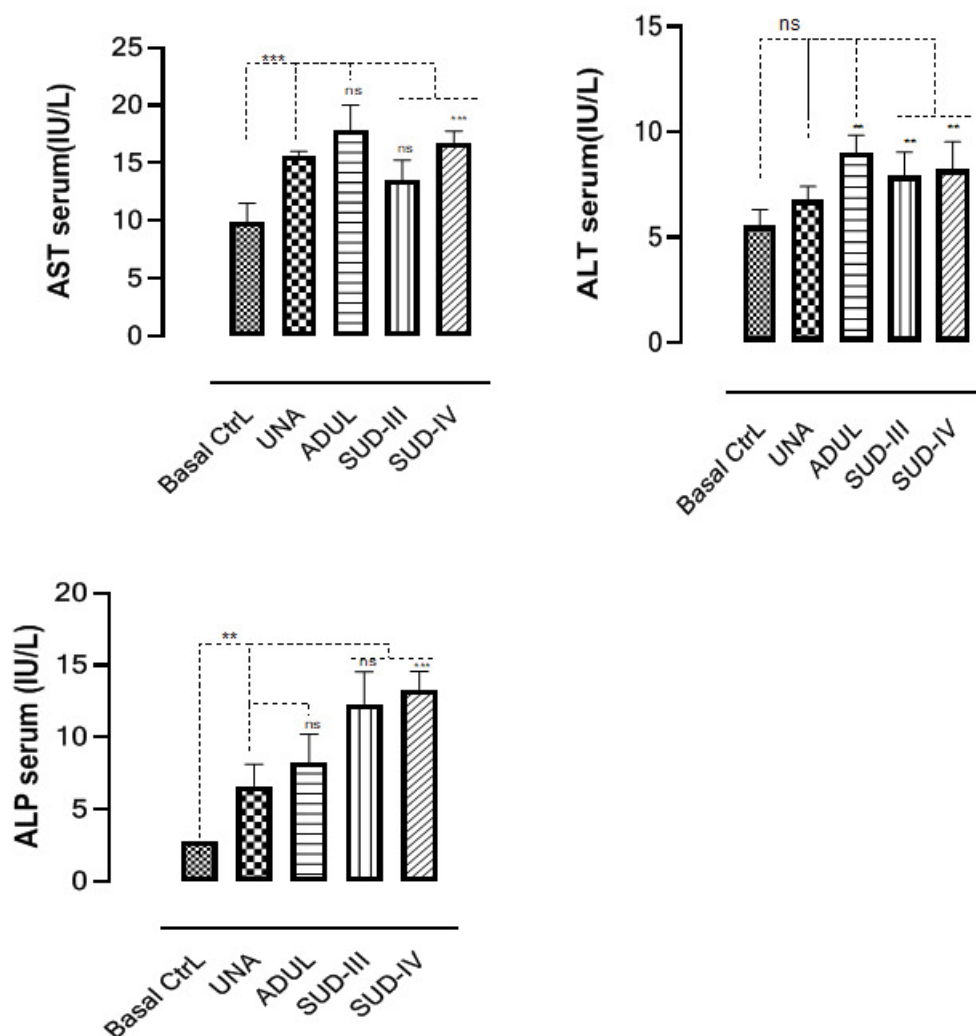


Figure 2: Liver Enzymes in the Serum. UNA (Unadulterated); ADUL (Adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

Results

Figure 1, Figure 2 and Figure 3.

Discussion

The significant increase ($p < 0.05$) in bilirubin observed in treated groups from this study agrees with Oparaocha, et al. [14] who reported elevated serum bilirubin in a dose-dependent manner in albino rats exposed to Sudan dyes. Abdel-Rahim, et al. [15] reported elevated bilirubin levels in rats administered with tartrazine and chocolate brown. It also agrees with Imafidon and Okunrobo [16], who reported significant increase in serum bilirubin in rats treated with Sudan dyes. Ikechukwu, et al. [17] also reported significant elevation in serum bilirubin in rats administered amaranth dye.

The significant increase ($p < 0.05$) in the activity of liver enzymes aminotransferases and alkaline phosphatase (ALT, AST and ALP) respectively in the serum and tissue homogenate of treated animals is coincident with a study by Nwachoko, et al. [12] who reported a significant increase in liver enzymes of rats treated with Sudan III dye when compared with the

untreated group. Abdel-Rahim, et al. [15] reported a significant increase in the level of ALT, AST and ALP in rats treated with tartrazine (azo dye) and chocolate brown when compared to control group. Elhan, et al. [18] also reported significant elevation in the activity of liver enzymes. In tandem with this study, Oparaocha, et al. [14] reported elevated ALT, AST and ALP in albino rats administered colored dye in a dose-dependent manner. Sahar and Manal [19] also reported significant increase in ALT, AST, ALP in albino rats administered with artificially colored fruit juice.

The liver has been observed to be more susceptible to chemical attack because it is the primary organ responsible for the biotransformation of xenobiotics [20]. In a case of hepatocellular damage (alteration of membrane permeability) resulting from chemical toxicity, liver enzymes ALT, AST, ALP (they are cytoplasmic enzymes) leak into the blood thus increasing their serum levels [17,21]. A possible mechanism for azo dye-induced hepatocellular damage is the reactive radicals produced from the metabolism of the dye. Azo dyes are metabolized in the liver to form chemically reactive toxic metabolites (semiquinone radicals and aromatic amines) which covalently bind to crucial cellular

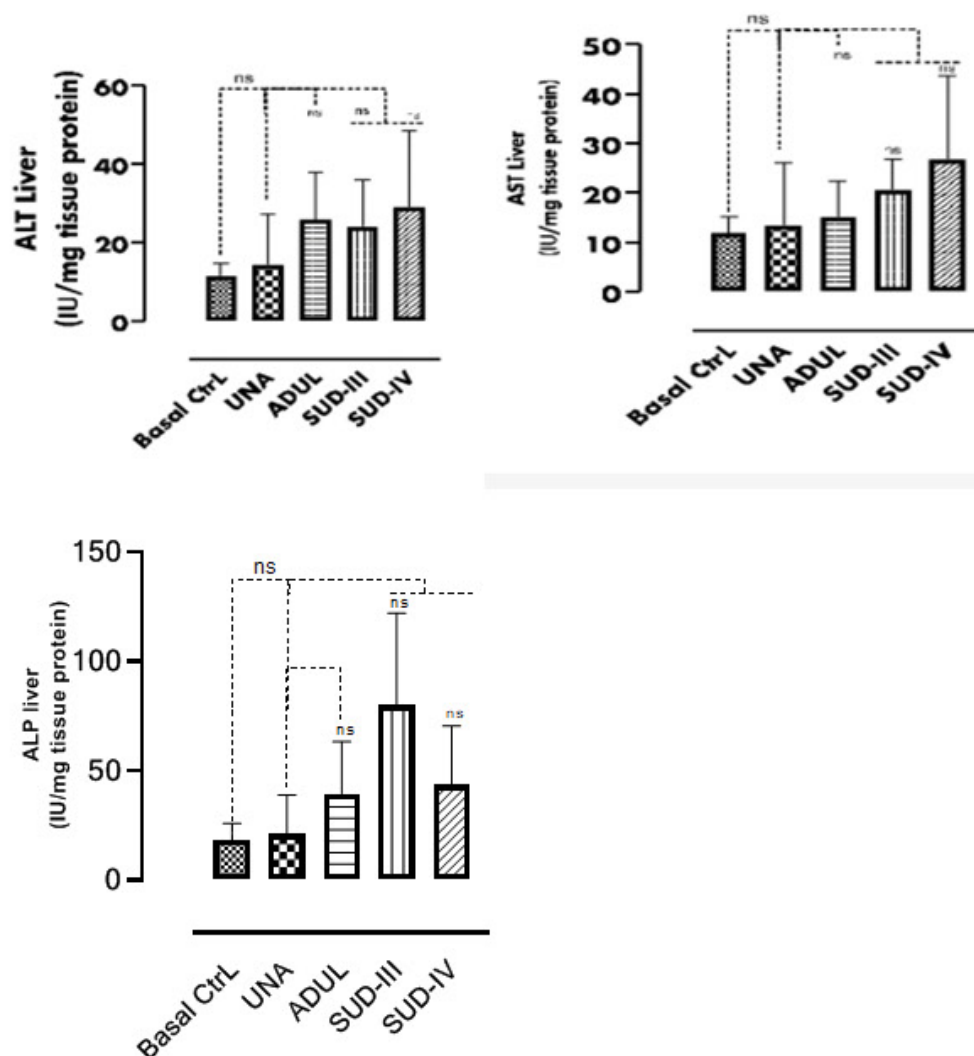


Figure 3: Liver Enzymes in the tissue homogenate. UNA (Unadulterated); ADUL (Adulterated); SUD-III (Sudan III); SUD IV (Sudan IV).

macromolecules. The semiquinone radicals produce superoxide, hydroxyl radicals, and Hydrogen peroxide (H_2O_2). These toxic reactive and electrophilic metabolites generated in the liver through biotransformation can cause hepatic damage and thus unchain a variety of chemical reactions [22]. An elevated aminotransferase (ALT and AST) with accompanying increased bilirubin is an indicative marker for hepatotoxicity [20]. Bilirubin accumulates from the breakdown of hemoglobin present in red blood cells with elevated bilirubin being attributed to the rapid destruction of red blood cells (RBCs). During normal function, the liver removes bilirubin from the blood and excretes it through the bile [12]. Increased bilirubin observed in Groups III, IV, and V when compared with the control groups indicates a compromise in the normal function of the liver in the treated rats. A reduced concentration in albumin level in groups III, IV, and V is indicative of a malfunctioned liver. It can however be said that Sudan dyes in adulterated palm oil are able to trigger hepatocellular damage in albino rats.

The result from (Table 1) shows a non-significant

decrease ($p > 0.05$) in white blood cell count (WBC) in rats treated with adulterated palm oil, Sudan III and Sudan IV (that is groups III, IV, and V respectively) when compared with control groups (I and II). A significant decrease ($p < 0.05$) in Granulocyte count, Granulocyte percentage, lymphocyte count was observed in groups III, IV, and V when compared with the control groups. A non-significant decrease ($p > 0.05$) in red blood cell count (RBC), hematocrit (HCT) and mean corpuscular volume (MCV) with a significant decrease ($p < 0.05$) in hemoglobin count (HGB), mean corpuscular hemoglobin concentration (MCHC) were observed in groups III, IV and V when compared with control groups (I and II). This may be suggestive of blood cells lysis induced by exposure to Sudan dyes.

In agreement with this study, Arika, et al. [10] reported that the bioavailability of chemical compounds at toxic levels in biological media causes alteration in various hematological indices. A significant reduction in hematological parameters (WBC, RBC, HCT and HGB) in rabbits exposed to azo dyes was reported by Elhan, et al. [18] which is in line with this study. Exposure to Sudan

Table 1: Hematological indices of Rats Administered adulterated palm oil.

	Basal control	Unadulterated Palm oil	Adulterated Palm oil	Sudan III	Sudan IV
WBC ($\times 10^9/L$)	4.132 \pm 0.368	3.996 \pm 0.396	2.914 \pm 0.236	3.212 \pm 0.276	3.090 \pm 0.325
GRA count ($\times 10^9/L$)	0.096 \pm 0.069	0.190 \pm 0.019	0.016 \pm 0.009 ^b	0.014 \pm 0.003 ^b	0.012 \pm 0.002 ^b
LYM ($\times 10^9/L$)	9.004 \pm 0.550	7.900 \pm 0.636	2.838 \pm 0.239 ^{ab}	3.184 \pm 0.321 ^{ab}	3.256 \pm 0.328 ^{ab}
% GRA (%)	7.896 \pm 0.738	5.062 \pm 0.360 ^a	2.820 \pm 0.118 ^{ab}	1.538 \pm 0.179 ^{ab}	1.224 \pm 0.248 ^{ab}
RBC ($\times 10^{12}/L$)	7.984 \pm 0.366	8.062 \pm 0.231	7.668 \pm 0.200	7.626 \pm 0.187	7.500 \pm 0.078
HGB (g/dL)	16.760 \pm 4.38	16.560 \pm 2.11	15.380 \pm 1.46 ^{ab}	15.080 \pm 1.80 ^{ab}	14.700 \pm 2.17 ^{ab}
HCT (%)	41.589 \pm 1.581	42.827 \pm 1.676	39.012 \pm 1.101	38.624 \pm 0.851	37.139 \pm 0.525
MCV (fL)	63.202 \pm 0.654	62.178 \pm 0.819	64.328 \pm 0.440	61.730 \pm 0.581	62.710 \pm 0.715
MCH (pg)	21.294 \pm 0.437	21.100 \pm 0.611	19.840 \pm 0.347	18.284 \pm 0.367 ^{ab}	19.436 \pm 0.176 ^a
MCHC (g/dL)	33.154 \pm 3.67	31.202 \pm 3.77 ^a	31.153 \pm 1.91 ^a	29.695 \pm 4.91 ^{abc}	29.741 \pm 1.91 ^{ab}
PLT ($\times 10^9/L$)	10.119 \pm 2.283	10.001 \pm 1.068	9.727 \pm 0.377	10.138 \pm 2.117	9.667 \pm 0.272
PCT (%)	1.036 \pm 0.053	0.916 \pm 0.036	0.934 \pm 0.017	0.880 \pm 0.027 ^a	0.826 \pm 0.017 ^a

Significant values at ^a(P < 0.05): Significant compared to control; ^b(P < 0.05): Significant compared to unadulterated; ^c(P < 0.05): Significant compared to adulterated. Mean \pm SEM; (n = 5). Group 1: control; Group II: Unadulterated palm oil; Group III: adulterated palm oil; Group IV: Sudan III only; Group V: Sudan IV only.

dyes has also been implicated in hemolytic anemia, methemoglobinemia and aplastic anemia [23]. When azo dyes are degraded, in the presence of azo reductase, they produce aromatic amines, semiquinone radicals which on further metabolism produce superoxide, hydroxyl radicals and hydrogen peroxide. These reactive species have been reported by many studies to have adverse effect on blood leading to lysis of existing blood cells, they are thus regarded as hemotoxic agents [24,25]. The reactive metabolites from the degradation of azo dyes formed via cytochrome P450 metabolism are capable of causing hemolytic anemia and methemoglobinemia [26]. The redox cycle results in the formation of reactive oxygen species (ROS i.e. H₂O₂) in RBC. The ROS oxidize proteins in the cytoskeleton of RBC altering the normal RBC discoid morphology and damaging the RBC membranes by crosslinking adjacent proteins leading to RBC lysis. Another serious concern with exposure to aromatic amines is their potential to induce hemorrhagic cystitis (bleeding from bladder damage) and bladder cancer [26].

In summary, it can be said that palm oil adulterated

with Sudan dyes has a toxicological effect on hematological parameters.

Conclusion

This study was able to establish blood cell lysis and hepatocellular damage induced by reactive species form the breakdown of Sudan dyes and adulterated palm oil in albino rats. It can be advised that consumers patronize palm oil from Oil mills which appears to be the genuine source rather than the open markets.

Declarations

Funding

Not applicable.

Consent to participate

Not applicable.

Ethics approval

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

Code availability

Not applicable.

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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