Effect of Sudan II adulteration of palm oil on the serum enzyme, bilirubin concentration and renal function biomarkers of albino wistar rats.

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Abstract

Aim: This study investigated the impact of Sudan II adulteration of palm oil on serum enzymes (ALT, AST, ALP), bilirubin and renal function biomarkers (creatinine, urea) 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). Animals were sacrificed and blood was collected *via* cardiac puncture for biochemical analysis. Calorimetric methods were used to determine the bilirubin, urea and creatinine concentrations while standard methods were used for the kinetic determination of ALT, AST, ALP. Data analysis was carried out with SPSS using one-way analysis of variance (ANOVA).

Key findings: Result showed that the serum enzymes activities and functional biomarkers increased significantly ($P<0.05$) in both short-term and long-term feeding conditions. Intentional addition of Sudan II dye to palm oil had adverse effects.

Significance: The significant increase of the parameters in this study is indicative of an adverse effect of the dye on health and therefore a major public health concern. It is pertinent to create awareness and the need for enforcement of regulatory acts and food safety procedures.

Keywords: Colour, Palm oil, Sudan II dye, Serum enzymes, Renal biomarkers, Adulteration.

Introduction

Adulteration of food is a global phenomenon that has serious consequences on health and safety. It is an unacceptable practice that is designated as illegal in food safety regulations [1]. The spectra of food products being adulterated varies from nation to nation and may include fruit juices, palm oil, flour and meat products [2]. Food is adulterated mainly to make it attractive and mask the effect of the use of unwholesome ingredients. Thus, food adulteration brings economic benefits to those who engage in it. In Nigeria, palm oils are being adulterated through the use of prohibited food dyes or colourants by food fraudsters.

The oil palm (*Elaeis guineensis*), is believed to originate from West Africa. The commercial value of this crop lies mainly in its oils. The crop is unique in that it produces two types of oil. The fleshy mesocarp produces red palm oil and the kernel produces palm kernel oil. Both of which are edible but with different chemical composition, physical properties and applications [3]. The composition of red palm oil, together with its natural consistency, appearance and pleasant aroma make it an ideal ingredient in the development and production of a variety of edible oils, in particular, margarines and fats and also ideal when making products like biscuits, cakes, sauces [4]. The term "quality" denotes the degree of excellence of a product. The quality of red palm oil is virtually determined by its colour, which is a brilliant red colour. Hence, any palm oil without the bright red colour would not be acceptable to consumers. Thus consequently, Sudan II dye is added to enhance the colour appeal. The palm fruit has carotenoids which are responsible for the bright/orange colour of the red palm oil. According to [5], any factor which affects the carotene content of the fruit invariably influences the colour of the palm oil. During lipid oxidation, hydroperoxides generated accelerate carotene oxidation which results in bleaching and discolouration of the red palm which then breaks down the carotenoids and consequently deteriorate the bright orange red colour of the red palm oil. Hence, the drive and demand by individuals for brightly coloured red palm oil has exacerbated the increasing use of this dye.

Sudan dyes (I, II, III and IV) are synthetic chemical dyes of similar chemical structure. They are oil-soluble, aromatic

compounds containing azo group (-N=N-). Sudan dyes are used in colouring hydrocarbon solvents, plastics and floor polishes, but they are unauthorized food colours. Sudan dyes are fraudulently used in order to maintain the colour of the product. The International Agency Research on Cancer (IARC) classified Sudan II as a suspected carcinogen (group 3) in the technical rules for dangerous substances (TRGS 905). The amines that may be formed during the azo splitting of the dye after oral intake in the body may be carcinogenic. The inclusion of this adulterating colourant in red palm oil may attain toxicopathological concentrations in humans which may further induce histopathological responses. With the demand for edible oils increasing due to growing population,and changing diets, it is essential that policies and advocacy to stop this trend of adulteration is required. The impact of the addition of this dye *via* red palm oil on human health has not been evaluated.

This study therefore aims to assess the toxicological effects of the Sudan II dye inclusion in red palm oil on the serum enzymes and renal function biomarkers for short (30 days) and long (90 days) term administration in male albino rats.

Methods and Materials

Animal experimental protocols

The sixty (60) mature albino rats, weighing between 150 g-180 g were obtained from the animal house of the Physiology Department, University of Calabar and used for the 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°c ± 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 thirty (30) and ninety (90) days along with water ad libitum. The levels of the Sudan II dye (0.025%, 0.03% and 0.04%) was based on the result of the Sudan II dye content (250-350 ppm or 0.025-0.035%) in the red palm oil (from a previous study) to provide low (0.025%), medium (0.03%) and high (0.04%). The LD_{50} 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 ten minutes and the supernatant (serum) collected using a 5 ml syringe and needle and then was used for biochemical and toxicological assays.

Estimation of biochemical parameters

Urea and creatinine concentrations were determined based on calorimetric method of [6]. Bilirubin concentration was determined based on the method as described by [7]. Liver enzymes; Alanine Transferase (ALT) and Aspartate Aminotransferase (AST) were determined by the methods described by [8] and Alkaline Phosphatase (ALP) by standard method described by [9].

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 results of the biochemical and toxicological parameters assessed in the experimental animals that were fed on the different proportion of palm oil adulterated with Sudan II dye on short term and long term are as follows;

Serum enzymes

The serum activities of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) is presented in Table 1 (short term effect and long term effect). In the short term study, the mean AST activities in the animals showed that there was a significant ($P<0.05$) increase in group 3 (12.09 ± 0.41 iu/L), group 4 (12.97 ± 0.41 iu/L), and group 5 (16.82 ± 1.26 iu/L) when compared to group 1 (9.86 ± 0.23 iu/L) (normal control). Group 4 and 5 were significantly ($P<0.05$) higher than group 1 (normal control) and group 2 (11.00 ± 0.22 iu/L). A non-significant ($P>0.05$) increase was observed in group 3 (12.09 ± 0.41 iu/L) when compared to group 2 (11.00 ± 0.22 iu/L).

The mean ALT activities in the animals showed that group 5 (53.66 ± 1.86 iu/L) was significantly ($P<0.05$) higher than the group 1 (45.09 ± 1.51 iu/L) (normal control) and group 2 (47.97 ± 2.05 iu/L). A non-significant increase ($P<0.05$) was observed in group 3 (49.13 ± 1.58 iu/L) and group 4 (49.36 ± 1.43 iu/L) when compared to group 1 (45.09 ± 1.51 iu/L) (normal control) and group 2 (47.97 ± 2.05 iu/L). The mean ALP activities in the animals showed that groups 4 (17.64 ± 0.32 iu/L) and 5 (18.58 ± 0.41 iu/L) were significantly ($P<0.05$) higher than group 1 (14.58 ± 0.62 iu/L) (normal control), group 2 (15.61 ± 0.34 iu/L) and group 3 (15.78 ± 0.46) respectively. However, Group 3 showered a non-

significant (P>0.05) increase when compared to group 1 (normal control).

In the long term study (Table 1), the mean AST activities in the animals that showed that group 3 (13.14 ± 0.54 iu/L), group 4 (14.02 ± 0.83 iu/L) and group 5 (18.98 ± 0.55 iu/L) respectively were significantly (P<0.05) higher than group 1 (9.89 ± 0.29 iu/L) (normal control) and group 2 (10.61 ± 0.30 iu/L). Equally, Group 5 (18.98 ± 0.55 iu/L) was significantly (P<0.05) higher than group 1 (normal control), groups 2, 3, 4. The mean ALT activities in the animals showed that group 5 (53.9 5 ± 1.85 iu/L) was significantly (P<0.05) higher than group 1 (46.80 ± 1.19 iu/L) (normal control) and group 2 (46.44 ± 2.20 iu/L). A non-significant (P>0.05) increase was observed in group 3 (49.63 ± 1.20 iu/L) and group 4 (50.31 ± 0.89 iu/L) when compared to group 1 (normal control) and group 2. The mean ALP activities in the animals showed that group 5 (18.92 ± 0.39 iu/L) was significantly (P<0.05) higher than group 1 (14.09 ± 0.48 iu/L) (normal control) and group 2 (15.91 ± 0.37 iu/L). There was a non-significant (P>0.05) decrease in group 3 (14.50 ± 0.51 iu/L) when compared to group 2 (15.91 ± 0.37) and group 4 (17.48 ± 0.44 iu/L). Group 4 showed a non-significant (P>0.05) increase when compared to group 1 (normal control), group 2 and group 3 (Table 1).

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

GROUPS	Short term			Long term		
	AST (iu/L)	ALT (iu/L)	ALP (iu/L)	AST (iu/L)	ALT (iu/L)	ALP (iu/L)
Group 1 (normal control)	9.86 ± 0.23	45.09 ± 1.51	14.58 ± 0.62	9.89 ± 2.29	46.80 ± 1.19	14.09 ± 0.48
Group 2 (RPO +Feed)	11.00 ± 0.22	47.09 ± 2.05	15.61 ± 0.34	10.61 ± 0.30	46.44 ± 2.20	15.91 ± 0.37
Group 3 (RPO +0.025% dye)	12.09 ± 0.41*	49.13 ± 1.58	15.78 ± 0.46	13.14 ± 0.54*,a	49.63 ± 1.20	14.50 ± 0.51
Group 4 (RPO +0.003% dye)	12.97 ± 0.41*,a	49.36 ± 1.43	17.64 ± 0.32*,a,b	14.02 ± 0.83*,a	50.31 ± 0.89	17.48 ± 0.44
Group 5 (RPO +0.004% dye)	16.82 ± 1.26*,a,b ,c	53.66 ± 1.86*,a	18.58 ± 0.41*,a,b	18.98 ± 0.55*,a,b ,c	53.95 ± 1.85*,a	18.92 ± 0.39*,a

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: AST=aspartate aminotransferase; ALT=alanine aminotransferase; ALP=alkaline phosphatase.

Serum total bilirubin and direct bilirubin

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

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

GROUPS	SHORT TERM		LONG TERM	
	Total Bil. (mg/dl)	Direct Bil. (mg/dl)	Total Bil. (mg/dl)	Direct Bil. (mg/dl)
Group 1 (normal control)	9.36 ± 0.79	3.43 ± 0.46	9.33 ± 0.86	3.86 ± 0.41
Group 2 (RPO+Feed)	10.04 ± 0.61	3.96 ± 0.35	9.87 ± 0.68	4.14 ± 0.29
Group 3 (RPO +0.025% dye)	10.93 ± 1.17	4.17 ± 0.37	12.79 ± 0.77*,a	5.54 ± 0.48*,a
Group 4 (RPO +0.003% dye)	10.98 ± 0.61	4.99 ± 0.34*	13.21 ± 1.31*,a	5.28 ± 0.24*,a
Group 5 (RPO +0.004% dye)	13.07 ± 0.44*,a	5.20 ± 0.43*,a	15.59 ± 0.91*,a,b	6.05 ± 0.33*,a

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: Total Bil = total bilirubin; Direct Bil = Direct bilirubin

Serum creatinine and urea

Table 3 shows the results of short term and long term effect of feeding experimental animals with Sudan II adulterated red palm oil on serum creatinine and urea (Table 3).

Table 3. *Effect of short and long term feeding of experimental animals with Sudan II adulterated red palm oil on serum Creatinine and urea concentration.*

GROUPS	Short term		Long term	
	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Group 1 (normal control)	63.31 ± 1.01	1.20 ± 0.05	62.47 ± 0.61	1.27 ± 0.03
Group 2 (RPO+Feed)	65.09 ± 0.83	1.49 ± 0.03*	64.27 ± 0.87	1.49 ± 0.03
Group 3 (RPO +0.025% dye)	66.80 ± 2.35	1.96 ± 0.41*	65.11 ± 1.32	1.94 ± 0.23*,a
Group 4 (RPO +0.003% dye)	67.18 ± 3.44	2.45 ± 0.19*,a	68.41 ± 3.80*	2.63 ± 0.17*,a
Group 5 (RPO +0.004% dye)	68.25 ± 1.14	2.30 ± 0.17*,a	69.09 ± 0.90*	2.31 ± 0.14*,a

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

Discussion

Effect on serum total and direct bilirubin

Total serum bilirubin (indirect or unconjugated) is created from red blood cell breakdown in the recticuloendothelial system. It travels into the blood to the liver while the direct (unconjugated) bilirubin reaches the liver, undergoes a chemical change and is moved to the intestines before being removed through the stool.

The serum total bilirubin levels in this study showed a significant (P<0.05) increase in group 5 when compared to the control (Table 2). In the long term study (90 days), a significant (P<0.05) increase was also observed within the groups 3, 4 and 5 respectively when compared to the control. Additionally, within the test groups, group 5 was significantly (P<0.05) higher when compared to group 3 (Table 2). The observed increase in total bilirubin in the test groups may suggest that the Sudan II dye in the red palm oil diet caused liver damage leading to the leakage of bilirubin into circulation. The result is consistent with the findings of [17], who reported elevated levels in total bilirubin in test rats administered with different levels of the Sudan IV dye. In addition, it is also consistent with the findings of, who reported elevated level of total bilirubin in rats administered with high dose of amaranth dye.

A significant (P<0.05) increase was observed in serum direct or conjugated bilirubin in groups 4 and 5 when compared to the control. A further increase (P<0.05) was observed on the long term study (90 days) in groups 3, 4 and 5 respectively when compared to the control. This marked increase in direct or conjugated bilirubin level in both the short (30 days) and long term (90 days) is consistent with the findings of [18], who reported elevated level in direct bilirubin in test rats administered with different levels of Sudan IVs dye.

Effect on serum creatinine and urea

The results of serum creatinine and urea are presented in Table 3. The serum urea level increased insignificantly (P<0.05) in the test groups when compared to the control. However, in the long term study (90 days), a significant (P<0.05) increase was observed for only test groups 4 and 5 when compared to the control (Table 3) in a dose-response manner. The increase in serum urea level might suggest renal function impairment caused by the toxicity of the Sudan II dye in the diet. The changes in serum urea suggest that there was serious protein breakdown in the animals' tissue and the liver's inability to adequately synthesize and secrete the urea shows poor liver's secretory ability. The results of the serum urea are in agreement with the findings of [18], who observed a significant elevation in serum urea level when rats were administered with high dose (500 mg/kgb.w) of tartrazine dye. The finding is also in agreement a significant increase in serum urea level of rats dosed with organic azo dye orally for 35 days. Decreased Blood Urea Nitrogen (BUN) is associated with renal failure, negative nitrogen balance, impaired absorption, nephritic syndrome and over hydration. Increased BUN is associated with reduced blood flow to kidney, increased protein catabolism, acute renal failure, chronic renal diseases and urethral destruction by stones [19]. Consequently, intentional addition of Sudan II dye to palm oil would have adverse health concerns.

The serum creatinine levels increased significantly (P<0.05) in Test groups 3, 4 and 5 when compared to the control and group 2 (Table 3). Significant increases (P<0.05) were also observed in the long term study (90 days) in groups 3, 4 and 5 respectively when compared to the control and group 2 (Table 3). Creatinine, being a normal metabolic product in the dephosphorylation of creatinine phosphate by creatine kinase, is a metabolite present in the serum in normal condition and it is cleared normally by the liver. Creatinine serves as a good marker for kidney's filtration and clearance ability, and thus a better analyte than urea. The increased serum creatinine level observed in this study could have resulted from kidney function impairment. The result is in agreement with the findings of [16] and [20], who reported an increase in creatinine levels in rats treated with tatrazine and brilliant blue mixture. The result of the creatinine level indicated that red palm oil adulterated with sudan II dye diet may cause harm to kidney function. These findings of significant elevation in both urea and creatinine levels may indicate that the dye could impair kidney function due to the effect of the dye metabolite on kidney tissues.

Conclusion

Adulteration of red palm oil samples with Sudan II dye was observed in all the samples in varying levels. This possibly suggests the endemic nature of this unwholesome practice. It could therefore be inferred that Sudan II dye was added deliberately to improve the colour of the red palm oil samples.

The detection of Sudan II dye in the red palm oil samples is an indication of inadequate surveillance and testing protocols by the Nigerian Regulatory Institutions. The toxicological effects indicates that the red palm oil adulterated with Sudan II dye could impair vital organs (hepatic and renal functions) especially at 0.04% of the dye administration in both short term and long term exposure. Hence, the use of this dye to enhance the red palm oil colour 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. In addition, the Standard Organization of Nigeria (SON) and the CODEX Alimentarius Commission specifications for edible red palm oil do not permit the use of this dye on any food products.

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 analysed 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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