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Research Article

Effects of Palm Oil Colorant on the Hepatic Functions of Albino Rats

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Abstract

Background/Objectives: Colorants enhance the visual appeal of food products. However, their contents could affect the health of the consumers adversely. The aim of this study was to investigate the effects of palm oil colorant (Sudan dye) on the hepatic functions of experimental albino rats.

Methods: This was a prospective experimental study conducted with 20 male albino rats in Owerri, South East Nigeria for a period of 2 months. The albino rats were divided into 4 groups (Groups 1, 2, 3, 4) each with 5 rats. Groups 1, 2 and 3 were the test groups while Group 4 was the control group. Groups 1, 2, and 3 received 50 mg/kg, 100 mg/kg and 150 mg/kg of colorants respectively while Group 4 serving as control group received only the standard feeds and water for a period of 28 days. The serum bilirubin and liver enzymes levels were measured at the end of study (Day 28). Statistical analysis of the data was done with IBM SPSS version 22.0 using Independent sample T-test.

Results/Discussion: The results showed that the colorant (Sudan dye) induced a significant increase ($P < 0.05$) in the levels of serum total and conjugated bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALT) for rats in test groups (1, 2 and 3) compared to the control group. This effect was more pronounced at higher doses of the colorant.

Conclusion: Sudan dye used as palm oil colorant has adverse effects on the hepatic functions of albino rats and could as such be harmful to human consumers. This calls for more studies and appropriate enactment of restrictive laws on the public consumption of palm oil with Sudan dye colorant in the Nigeria.

Keywords: Palm Oil Colorant; Hepatic Functions; Adverse Effects; Nigeria

Introduction

Many colour additives had never been tested for toxicity or other adverse effects. Historical records show that injuries, even deaths, resulted from tainted colorants [1]. It has been estimated that about 75% of the Western diet is made up of various processed foods, with an individual consuming an average of 8 - 10 pounds of food additives per year [2].

A researcher noted that many synthesized dyes were easier and less costly to produce and were superior in colouring properties when compared to naturally derived alternatives [3]. According to the Center for Science in the Public Interest (CSPI), the amount of dyes used in foods increased 500 percent since the 1950s [4]. In Africa, there has been a sharp increase in the use of synthetic food colorants during the past few years and this use of synthetic colorants particularly in food items is not controlled by any regulatory body [5].

The oil Palm (*Elaeis guineensis*) is West Africa's most important oil producing plant [6]. The fruit produces two distinct 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). The former consists of mainly palmitic and oleic acids and the latter of mainly lauric acid and both oils are important in the world trade [6]. Crude palm oil (CPO) is the richest natural source of carotenoids and tocotrienols. The carotenoids (500 - 700 ppm) are responsible for the characteristic deep orange-red colour [7], while it's semi-solid consistency at tropical room temperature is mainly due to the presence of triacylglycerols of palmitic and oleic acids [8].

The red palm oil (commonly called red oil) is a common ingredient in the cooking of almost every type of dish prepared in Nigeria. Otu noted that in recent years there has been rising production (supply) and consumption (demand) of palm oil in

Nigeria, with demand growing faster than the supply [6]. As a result, the trend has been that of increasing domestic consumption unequally matched by a rather slow growth in production. This widening gap between demand and production has also been accompanied by increasing reports (from the media) of adulteration.

Adulteration of food is a global phenomenon that can have serious consequences on public health and safety and it is an unacceptable practice that is designated as illegal in food safety regulations globally [9]. This fraudulent practice is economically motivated without considering the effect of the unsafe ingredient on consumer's health [10].

The adulteration of palm oil is believed to be practiced by producers in order to make the oil appear more reddish in colour, for the sole purpose of profit maximization. The adulterants reportedly used in CPO include carrot, papaya, natural potash and red dye; with potash [6] and red dye being the preferred and most widely used adulterants due to their abundance and low cost (Authors' communication with local retailers). Crude palm oil can be successfully adulterated with red dye and the adulterated oil can remain undetected by consumers.

There is need to identify the renal, hepatic, carcinogenic effects of palm oil colorant (Sudan dye). Therefore, the aim of this study was to investigate the effects of palm oil colorant (Sudan dye) on the hepatic functions of albino rats.

Materials and Methods

Study design

This was a prospective experimental study conducted with male albino rats in Owerri, South East Nigeria for a period of 28 days.

Colorant sample

The colorant used in this work was obtained from retailers in an oil mill at Ngor- Okpala, a community close to Owerri, Imo state, South East Nigeria. The colorant (Sudan dye, locally called 'metu') was dissolved in few drops of DMSO and Tween 20 (about 4 drops). DMSO is a known hepatoprotective agent [11]. The solution obtained was administered orally to the rats using an intra-gastric feeding syringe.

Experimental animals

Twenty (20) healthy male albino rats weighing 150 - 283g were used for this study. The animals were purchased from the animal farm of the University of Nigeria, Nsukka, Enugu State, Nigeria. The rats were properly housed in well ventilated cages and fed with standard growers feed obtained from Super Starter Vital Feeds Ltd, and water *ad libitum* throughout the duration of their housing. The cages were cleaned daily and the water and feed changed regularly. The animals were meticulously observed daily for general clinical conditions.

Experimental grouping/treatment of animals

The experimental animals (20 male albino rats) were assigned into four groups (1, 2, 3 and 4) consisting of five animals in each cage. They were acclimatized to their environment and diet for one week before experimentation. Groups 1, 2 and 3 were the Test Groups while Group 4 was the Control Group. Groups 1, 2, and 3 received 50 mg/kg, 100 mg/kg and 150 mg/kg of colorants respectively while the group 4 (Control Group) received only the standard feeds and distilled water for the period of 28 days.

Sample collection

The rats were subjected to an overnight fast, after which blood samples were collected via ocular puncture into plain sample bottles to obtain serum. The samples were allowed to clot at room temperature before they were placed in a centrifuge tube and centrifuged at 3000 rpm for 10 minutes to separate serum. Serum from each individual animal was used for biochemical analysis.

Laboratory reagents

All reagents were commercially purchased from a reliable company, Kenton Diagnostics, Okigwe Road, Owerri, Imo State, Nigeria. The manufacturer's Standard Operating Procedures (SOP) were strictly followed.

Laboratory assay/methodology

Using Randox Diagnostic Kits, determination of bilirubin was done by the Diazo Reaction Method as described by Malloy and Evelyn [12], while the liver enzymes (ALT and AST) were determined by the methods described by Reitman and Frankel [13] and ALP by the Standard Method described by Kind and King [14].

The serum bilirubin (mg/dl), ALT, AST and ALP all in (iu/L) were each calculated with the formula below:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{1}$$

Ethical approval

Ethical approval was obtained from the Ethics Committee, School of Health Technology, Federal University of Technology Owerri, Imo State, Nigeria.

Statistical analysis

Statistical analysis of the data was carried out with SPSS version 22.0 using independent sample T-test. The statistically analysed data were reported as Mean ± STD. A P-value of < 0.05 was considered statistically significant.

Results

The level of total bilirubin (TB) in Group 3 (1.28 ± 0.04) increased significantly (P < 0.05) when compared with Control Group 4 (0.76 ± 0.11). Also, a significant (P < 0.05) increase of TB was observed in Group 2 rats (1.07 ± 0.06) when compared with Control Group (0.76 ± 0.11). But no significant increase of TB (P > 0.05) was observed in Group 1 rats (0.89 ± 0.03) when compared to Control Group (0.76 ± 0.11) (Table 1). In addition, there was a significant (P < 0.05) increase in the level of conjugated bilirubin (CB) in Group 3 rats (0.63 ± 0.04) when compared with Control Group (0.40 ± 0.02). Also, a significant increase of CB (P < 0.05) was observed in Group 2 rats (0.50 ± 0.10) when compared with Control Group (0.40 ± 0.02). But no significant increase of CB (P > 0.05) was observed in Group 1 rats (0.43 ± 0.05) when compared to Control Group (0.40 ± 0.02) (Table 1).

On the hepatic enzymes, the levels of ALT were 13.61 ± 0.46 iu/L, 15.47 ± 0.43 iu/L, 21.68 ± 1.06 iu/L and 10.89 ± 0.93 iu/L for groups 1, 2, 3 and 4 respectively. When compared with the control (Group 4), the increase in ALT level was significant (P < 0.05) in Groups 1, 2 and 3 (Table 2).

The levels of AST in iu/L were 14.93 ± 1.02, 18.89 ± 1.08, 26.42 ±1.50 and 12.85 ± 0.73 for Groups 1, 2, 3 and 4 respectively. The increase in AST levels was significant (P < 0.05) in all the test groups (Groups 1,2 and 3) when compared with the Control Group (group 4) (Table 2).

The level of ALP in group 3 (93.05 ± 1.06) increased significantly (P < 0.05) when compared with Control Group (43.66 ± 2.70). Also, a significant (P < 0.05) increase in ALP was observed in Group 2 rats (61.77 ± 7.27) when compared with Control Group (43.66 ± 2.70). However, no significant (P > 0.05) increase of ALP was observed in Group 1 rats (47.39 ± 1.53) when compared to Control Group (43.66 ± 2.70) (Table 2).

Groups	Dose of colorant (mg/kg)	Total Bilirubin (mg/dl)	Conjugated Bilirubin (mg/dl)	P-Value
1	50	0.89 0.03	0.43	P > 0.05
2	100	1.07	0.50	P < 0.05
3	150	1.28	0.63	P < 0.05
4 (Control)	None	0.76	0.40	

Table 1: The effects of the palm oil colorant on serum bilirubin in rats administered with different concentrations of Sudan dye.

Groups	Dose of colorant (mg/kg)	ALT(iu/L)	AST (iu/L)	ALP (iu/L)
1	50	13.61	14.93	47.39
2	100	15.47	18.89	61.77
3	150	21.68	26.42	93.05
4 (Control)	None	10.89	12.85	43.66

Table 2: The effects of the Sudan dye colorant on the liver function tests indexes in rats administered with different concentrations.

Discussion

There was increase in total and conjugated bilirubin in rats administered with high doses of the colorant when compared with the control rats. This increase in serum bilirubin suggests that at high dose, the colorant (Sudan dye) causes liver damage leading to leakage of bilirubin into circulation. The result is in line with Ikechukwu., *et al*. [15] who observed that there was a significant increase in serum bilirubin at high dose of amaranth dye. This finding also agree with that of Imafidon and Okunrobo [16], who found out that serum bilirubin was significantly increased in a dose-dependent manner in rats treated with Sudan dye.

The level of AST, ALT and ALP increased significantly when compared with Control Group. This increase is highly significant in Group 3 rats administered with 150 mg/kg of the colorant. This result is in agreement with the findings of Sahar and Manal who reported significant increase in the level of ALP, ALT and AST of rats administered with coloured fruit juice [17]. Their findings showed that coloured fruit juice containing sunset yellow, tartrazine and carmoisine lead to significant increase in ALP, ALT and AST of rats. Due to the cytoplasmic nature of these enzymes, upon liver injury, they enter into the circulatory system as a result of alteration of membrane permeability [15]. This result is well supported by the data reported by Amin., *et al*. [18], who indicated that rats which consumed high dose of synthetic colour (tartrazine, carmoisine, sunset yellow and fast green) showed a significant increase in serum ALP, ALT and AST when compared to control rats. This result is also in agreement with Sharma., *et al*. (2008) who reported that synthetic colours have adverse effect on vital organs [19]. The result is also in line with the work of Imafidon and Okunrobo (2013) who reported that ALP, ALT and AST activities were significantly increased in a dose - dependent manner.

Conclusion/Recommendation

The rate at which palm oil is being adulterated with colorant is alarming. Sudan dye unarguable affects the liver functions adversely. Further research assessing the effect of this colorant on cancer markers (i.e. prostate specific antigen, gamma glutamyl transferase and alpha fetoprotein) should also be carried out. Also public enlightenment and appropriate enactment and implementation of restrictive laws on the public consumption of palm oil with Sudan dye colorant are urgently needed.

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Volume 3 Issue 4 April 2019