

Effects of Palm Oil Colorant on the Renal Functions and Body Weights of Albino Rats

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Abstract

Background/Objectives: Colorants help in enhancing the aesthetic appeal of food. However, the contents of the colorant could adversely affect the health of consumers. The aim of this study was to investigate the effects of palm oil colorant (Sudan dye) on the renal functions and body weights of the albino rats.

Method: The prospective experimental study was conducted with male albino rats in Owerri, South East Nigeria for a period of 4 weeks. Twenty (20) male albino rats divided into 4 groups (Group I, II, III and IV), each with 5 rats were used. Groups I, II and III were the test group while group IV formed the control group. Groups I, II and III received 50 mg/kg, 100 mg/kg and 150 mg/kg of colorants respectively in addition to the standard feeds and water while Group IV (control group) received only the standard feeds and water for a period of 28 days. Their body weights were measured on days 0, 7, 14, 21 and 28 while serum Urea and Creatinine 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 ($P < 0.05$) weight gain for rats in groups I and II compared to the control group. There was also a significant ($P < 0.05$) increase in serum Urea and Creatinine levels when compared with the control and the increase was more at higher doses of the colorant.

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

Keywords: *Palm Oil Colorant; Renal Functions; Body Weight; Adverse Effects; Nigeria*

Introduction

Colorants play a significant role in enhancing the aesthetic appeal of food as foods that are aesthetically pleasing are more likely to be consumed [1]. Food dyes are of two categories; natural and artificial. Natural dyes are extracted from plants or animal source. So, they are quite expensive and not available in large quantity. Artificial dyes (synthetic dyes) are petroleum based products and constitutes aromatic rings [2]. They are less expensive and easy to get.

The oil Palm (*Elaeis guineensis*) is the most important oil producing plant in West Africa [3]. 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 [3]. Crude palm oil (CPO) is the richest natural source of carotenoids and tocotrienols. The red palm oil is a common ingredient in the cooking of almost every type of dish prepared in Nigeria. In recent years there has been rising production (supply) and demand of palm oil in Nigeria, with demand growing faster than the supply. This widening gap between demand and production has also been accompanied by increasing reports of adulteration of the product [3].

Adulteration of food is a global phenomenon that has serious consequences on public health and safety [4]. It is an unacceptable practice that is designated as illegal in food safety regulations globally. Adulteration is believed to be practiced by producers in order to improve the colour of CPO, for the sole purpose of profit maximization. Unfortunately, it is normally done without considering its possible effect on the quality of palm oil and the health of consumers [3]. The adulterants reportedly used include carrot, papaya, natural potash and red dye [3], with potash and red dye being the preferred and most widely used adulterants due to their abundance and low cost (Authors' communication with Local Retailers). The red dye reportedly used is Sudan dyes (I II III and IV) [4]. As food dye, Sudan dyes are considered illegal, mainly because of its harmful effect over a long period of time [5].

Sudan dyes are Synthetic chemical dyes of similar chemical structure [6]. They are oil-soluble, aromatic compounds containing azo group (-N=N-) [6]. Sudan dyes are not authorized food colours which are added to foods to enhance the natural colour lost due to processing [4]. According to a study, these azo dyes are metabolized to possible carcinogenic colourless amines, both in the liver of mammals and by the micro flora present in human skin and the gastrointestinal tract [7]. The International Agency for Research on Cancer (IARC), classified Sudan I, Sudan II, Sudan III and Sudan IV as category 3 carcinogens [8]. However, in some developing countries of the world (including Nigeria), the legislature banning the use of this dye is not properly enforced. One major problem associated with the use of adulterants is that most of these compounds have not undergone stringent studies and the level of threat they may pose to human health when consumed is not well established [3].

Objective of the Study

Therefore, the objective of this study was to investigate the effects of palm oil colorant (Sudan dye) on the renal functions and body weights of the albino rats.

Methods and Materials

Study design

This was a prospective experimental study conducted with male albino rats in Owerri, South East Nigeria for a period of 2 months.

Colorant sample

The colorant used in this work was bought from retailers in an oil mill at Ngor-Okpala, a community near Owerri, Imo State, Nigeria. The colorant (Sudan dye, locally called 'metu') is generally not soluble in distilled water. Solubility was achieved by adding few drops of DMSO and Tween 20 (about 4 drops). 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 (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 observed daily for general clinical conditions.

Experimental grouping/treatment of animals

The experimental animals (20 male albino rats) were weighed before they were assigned into four groups (I, II, III and IV) consisting of five animals in each cage. They were acclimatized to their environment and diet for one week before experimentation. Groups I, II and III

were the test groups while group IV was the control Group. Groups I, II, and III received 50 mg/kg, 100 mg/kg and 150 mg/kg of colorants respectively while the Group IV (Control Group) received only the standard feeds and distilled water for a period of 28 days.

Weighing of animals

The animals were weighed on Days 0, 7, 14, 21 and 28 of the study period. The weighing balance was zeroed and kept on a flat surface. Each of the animals was weighed by placing it in a circular container placed on the balanced scale and measurement (weight) taken and recorded.

The percentage body weight change was determined using the formula:

$$\text{Percentage body weight change} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

Sample collection for renal function test

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 experimental 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

Renal function tests

The serum urea and creatinine levels were determined using Randox Diagnostic Kits following strictly the Standard Guideline [9]. Serum Urea and Creatinine levels were calculated as shown below:

$$\begin{aligned} \text{Creatinine Umol/l} &= \frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{absorbance of standard}} \\ &= \frac{\text{Absorbance of test}}{\text{absorbance of standard}} \times \frac{500\text{mg}}{\text{dl}} \\ \text{Urea concentration } \left(\frac{\text{mg}}{\text{dl}}\right) &= \frac{\text{Absorbance of sample}}{\text{absorbance of standard}} \times 40 \end{aligned}$$

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 \pm STD. A P-value of < 0.05 was considered statistically significant.

Results

The weights gain (g) observed in group I, II, III and IV were 50.8 ± 22.82 , 34.05 ± 5.01 , 29.93 ± 12.3 and 67.7 ± 4.69 respectively. There was significant ($P < 0.05$) weight gain in the rats in Groups 1 and II but weight gain in Group III rats was not significant ($P > 0.05$) when compared to group IV, the control group (Table 1).

| Groups | Dose of colorant (mg/kg) | Initial body weight (g) | Final body weight (g) | Weight change (g) | P-Value |
|--------------|--------------------------|-------------------------|-----------------------|-------------------|----------|
| I | 50 | 190.95 4.89 | 241.75 | 50.8 | P < 0.05 |
| II | 100 | 210.55 | 244.60 | 34.05 | P < 0.05 |
| III | 150 | 232.10 | 262.03 | 29.93 | P > 0.05 |
| IV (Control) | None | 177.6 23.54 | 245.30 | 67.7 | |

Table 1: Mean and standard deviation values of body weight and weight gains of experimental rats before and after treatment with colorant.

The level of urea in group III (59.05 ± 2.80) increased significantly ($P < 0.05$) when compared to the control group (21.77 ± 0.81). Also, a significant ($P < 0.05$) increase of urea was observed in Group II rats (38.36 ± 1.62) when compared to the control group (21.77 ± 0.81). But there was no significant ($P > 0.05$) increase of urea in Group I rats (24.89 ± 1.08) when compared to the control group (21.77 ± 0.81) (Table 2).

| Groups | Dose of colorant (mg/kg) | Urea (mg/dl) | Creatinine (mg/dl) | P- Value |
|--------------|--------------------------|--------------|--------------------|----------|
| I | 50 | 24.89 1.08 | 0.33 | P > 0.05 |
| II | 100 | 38.36 1.62 | 0.46 | P < 0.05 |
| III | 150 | 59.05 | 0.60 | P < 0.05 |
| IV (Control) | None | 21.77 | 0.29 | |

Table 2: The effects of the colorant used in colouring palm oil on urea and creatinine in rats administered with different concentrations.

Creatinine level was observed to be significantly ($P < 0.05$) increased in Group III (0.60 ± 0.15) and Group II (0.46 ± 0.53) when compared to the Control Group (0.29 ± 0.01). However, no significant ($P > 0.05$) increase of creatinine was recorded in Group I rats (0.33 ± 0.3) when compared to control group (0.29 ± 0.01) (Table 2).

Discussion

Colourants are recently added to palm oil to increase the aesthetic appeal of the oil without consideration of its effect to human health. In this study, the effect of the colourant (Sudan dye) used in palm oil at different concentrations was investigated. There was a significant weight gain in the rats in group 1 and II. The result is in line with the work of Ikechukwu., *et al.* who recorded a significant increase in body weight of the animals when the initial weights of all the animals were compared with their final weights in all groups [10]. There was no significant weight gain in group 3 rats showing that at a higher dose of the colorant, there was no significant weight gain. This result in a way collaborates with the findings of Imafidon and Okunrobo [11], who observed that there was no significant weight gain associated with test rats fed with high dose of dye when compared with the control group.

It was observed that the level of serum urea in rats administered with high doses of the colourant increased significantly when compared with the control rats. This is in line with the work of Abdellah., *et al.* [1] and Nagy., *et al.* [12], who observed significant increase in serum urea level of experimental animals treated with azo dyes. This increase in serum urea is associated with oxidative stress caused by the high dose of the colourant. Acute or chronic renal failure is the most common cause of high urea levels [13].

High increase of creatinine was also observed in group II and III rats treated with 100 mg/kg and 150 mg/kg of the colourant respectively, when compared with the control group. This is also in line with the work of Abdellah., *et al.* [1] and Mohamed., *et al.* [14], who observed that synthetic colourants adversely affect renal parameters. Serum creatinine levels are elevated in all diseases of the kidney in which 50% or more of the nephrons are destroyed. Normal causes of elevation of serum creatinine levels are few, making this a more specific test of renal failure [13]. These increases were observed to be dose dependent as a lower dose (50 mg/kg) of the colourant caused no significant increase in creatinine and urea while the higher doses group (group 2 and 3) showed significant increase in creatinine and urea level compared to the control group. The study thus agreed with the work of Ikechukwu., *et al.* who observed that increase in serum urea and creatinine level is dose dependent [10].

Conclusion/Recommendations

Sudan dye used as palm oil colorant has adverse effects on the renal functions and body weight of albino rats and could be harmful to human consumers. There is need for public enlightenment and appropriate enactment and implementation of laws on the public consumption of palm oil with Sudan dye colorant in the populous country of Nigeria. Further studies on other possible adverse effects such as hepatic and carcinogenic effects using experimental animals and possibly human subjects after proper ethical considerations is recommended.

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