



## **The Effect of Some Synthetic Food Colorants on Selected Biochemical Indices of Male Wistar Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author WI designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Authors IA and CAU managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The aim is to evaluate the effect of tartrazine and erythrosine on serum glucose, cholesterol and triglycerides. A total of 20 adult male Wistar rats were divided into five groups with 4 rats per group. Group 1 served as the control and was given only water and feed. Whereas group 2, 3, 4, 5 were administered 5 mg/kgb.wt, 10 mg/kgb.wt, 20 mg/kgb.wt, 40 mg/kgb.wt of Tartrazine and Erythrosine via orogastric feeding for 21 days. At the end of 21 days, blood samples were collected via ocular puncture and used to measure glucose, cholesterol and triglyceride concentration in the serum of the animals. The results were analyzed using One way ANOVA, followed by post hoc multiple comparisons and level of significance set at  $p < 0.05$ . The result revealed that there was a significant difference  $p < 0.05$  in the mean glucose concentration of Group 4 ( $5.81 \pm 0.40$  mmol/L) and Group 5 ( $5.28 \pm 0.25$  mmol/L) when compared with the control ( $3.27 \pm 0.11$  mmol/L). Although

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Group 4 showed a higher mean cholesterol concentration ( $0.92\pm 0.27$  mmol/L) when compared with the control ( $0.18\pm 0.05$  mmol/L) the difference was not significant  $p>0.05$ . Furthermore, Group 5 showed no significant mean triglyceride concentration ( $2.01\pm 0.15$  mmol/L) when compared with the control ( $2.95\pm 0.04$  mmol/L). This result revealed that the colourants increased the glucose and cholesterol levels in the test group in a dose-dependent manner. The increase in serum glucose concentration may be an indication of pancreatic cancer caused by the hydrocarbons contained in the colourants.

**Keywords:** Tartrazine; erythrosine; triglyceride; cholesterol; glucose.

## 1. INTRODUCTION

A wide range of food colouring or additives, running into more than 2,500 items used to preserve or enhance foods are a consequence of industrialization and the development of food processing technology, [1]. They come in many forms consisting of liquids, powders, gels, and pastes. Food colouring is used both in commercial food production and in domestic cooking. Food colourants are also used in a variety of non-food applications including cosmetics, pharmaceuticals, home craft projects, and medical devices [2]. A food additive is only approved for human consumption after studying its acute, sub-acute and chronic toxicity. Several metabolites of these substances, such as nitrous compounds, are carcinogens. Toxicity or benefit depends on to what extent food components affect absorption, excretion or the metabolism as a whole. Because there may be interaction among different substances, the definition of adequate safety limits for human consumption is further compounded [3,4]. A study of Montaser et al. [5] designed to assess the effects of two colouring agents, carmoisine (synthetic) and curcumin (natural) on the expression of some genes with fertility impact. They recorded that carmoisine induced hazardous effects on fertility at different levels when consumed in concentrations higher than the acceptable daily-authorized level and however, curcumin as a natural food colour is safer than carmoisine up to certain levels.

Tartrazine is a synthetic lemon yellow azo dye primarily used as a food colouring [6]. It is also known as E number E102, C.I. 19140, FD&C Yellow 5, Acid Yellow 23, Food Yellow 4, and trisodium-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-5-pyrazolone-3-carboxylate. Products containing tartrazine commonly include processed commercial foods that have an artificial yellow or green colour, or that consumers expect to be brown or creamy looking [7,8]. It has been frequently used in the

bright yellow colouring of imitation "lemon" filling in baked goods. Types of pharmaceutical products that may contain tartrazine include vitamins, antacids, cold medications (including cough drops and throat lozenges), lotions and prescription drugs.

Erythrosine, also known as Red No. 3, is an organoiodine compound, specifically a derivative of fluorone. It is cherry-pink synthetic, primarily used for food colouring [9]. It is the disodium salt of 2, 4, 5, 7-tetraiodofluorescein. Its maximum absorbance is at 530 nm [10] in an aqueous solution, and it is subject to photodegradation. Erythrosine (E127) is commonly used in sweets such as some candies and popsicles, and even more widely used in cake-decorating gels. These synthetic food colourants have been implicated with a high concentration of serum glucose which could lead to diabetes [11]. The study aimed to evaluate the effect of tartrazine and erythrosine on serum glucose, cholesterol and triglycerides.

## 2. MATERIALS AND METHODS

### 2.1 Laboratory Animals

Twenty (20) male albino Wistar rats aged 8 weeks and weighing 128–244 g were used for this study. The animals were housed in the Department of Biochemistry Animal House, Madonna University, Nigeria. Standard animal cages with wood dust as bedding were used in keeping the animals. They were allowed *ad libitum* access to rat chow and clean water and exposed to 12/12-hr light/dark cycle. The animals were acclimatized for 7 days. The animals were kept in line with laid down principles for animal care as prescribed in Helsinki's 1964 declaration. The Animal Ethics Committee of the Madonna University approved the study protocol.

### 2.2 Sample Preparation

The samples Tartrazine (T0388-100G) and Erythrosine (1159360025) were gotten from

Sigma Company United States of America. 5 mg/kg body weight, 10 mg/kg b.wt, 20 mg/kg b.wt, 40 mg/kg b.wt of each sample were weighed and dissolved in 84 ml of distilled water in a ratio of 1:1.

### **2.3 Experimental Design and Prepared Colourant Administration**

The animals were randomly assigned into five (5) groups of four each. The first group serves as control; second, third, fourth and fifth groups were administered with 5 mg/kg, 10 mg/kg, 20 mg/kg, and 40 mg/kg body weight respectively. Administration of the colourants was done via orogastric feeding once daily for 21 days while the control group received normal saline as vehicle after which the animals were sacrificed under chloroform anaesthesia and the blood sample was collected via ocular puncture using the capillary tube for serum cholesterol, triglyceride and glucose assay.

### **2.4 Serum Assessment of Biochemical Parameters**

The samples of blood were introduced into plain capped EDTA bottles and allowed to stand for 2 hours, after which they were centrifuged at 5,000 rpm for 10 minutes using a bucket centrifuge (B-Bran Scientific and Instrument Company, England). Serum settled on top and was utilized for the assays. The serum concentration of glucose, total cholesterol and triglyceride were assessed using appropriate methods as outlined below.

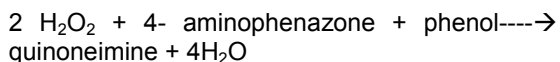
#### **2.4.1 Serum glucose concentration determination**

Serum glucose concentration was assessed by Randox method as used by Julio & Mottola [12].

**Principle:** Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinone imine dye as an indicator.

The reactions are shown below;

GOD



#### **2.4.2 Serum total cholesterol (TC) concentration determination**

Serum total cholesterol (TC) concentration was assessed by the method of Allain, Poon & Chan [13]; Khalil et al. [14].

**Principle:** Cholesterol esters were hydrolyzed by esterase of cholesterol as a catalyst by breaking into free fatty acid and cholesterol. Cholesterol-3-one and hydrogen peroxide were obtained by oxidizing cholesterol using cholesterol oxidase as a catalyst. Colourimetric red quinonimine was obtained via a combination of 4-amino-antipyrine and phenol with hydrogen peroxide using a peroxidase enzyme as a catalyst. Colour concentration obtained was relative total cholesterol present.

#### **2.4.3 Estimation of serum triacylglyceride concentration (TG)**

Serum triglyceride concentrations in the samples were assessed using the method of Negele, Dotson, Liu & Putkey [15].

**Principle:** A lipoprotein lipase hydrolyses triglycerides to glycerol and fatty acid. The glycerol obtained was done by transferring a phosphate group of glycerol-3-phosphate catalyzed by glycerol kinase enzyme. Hydrogen peroxide and dihydroxyacetone phosphate were obtained by enzymatic oxidation of glycerol-3-phosphate. Chromogen consist of n-ethyl-n-sulphohydroxypropyl-n-foludine is then oxidized. A quinoneimine dye (purple coloured) formed as a result of these reactions were read at 540 nm colourimetrically.

### **2.5 Statistical Analysis**

All results are presented as mean  $\pm$  standard error of the mean (SEM). One way analysis of variance (ANOVA) was utilized in comparing the difference within groups, followed by post hoc multiple comparisons. The level of significance was placed at  $p < 0.05$ .

## **3. RESULTS**

### **3.1 Effect of Tartrazine and Erythrosine Administration on the Concentration of Serum Glucose**

This Fig. 1 showed that the mean serum glucose concentrations were significantly ( $p < 0.05$ ) elevated in rats fed with 20 mg/kg and 40 mg/kg bodyweight of colourants compared to control. Furthermore, rats fed with 5 mg/kg and 10 mg/kg

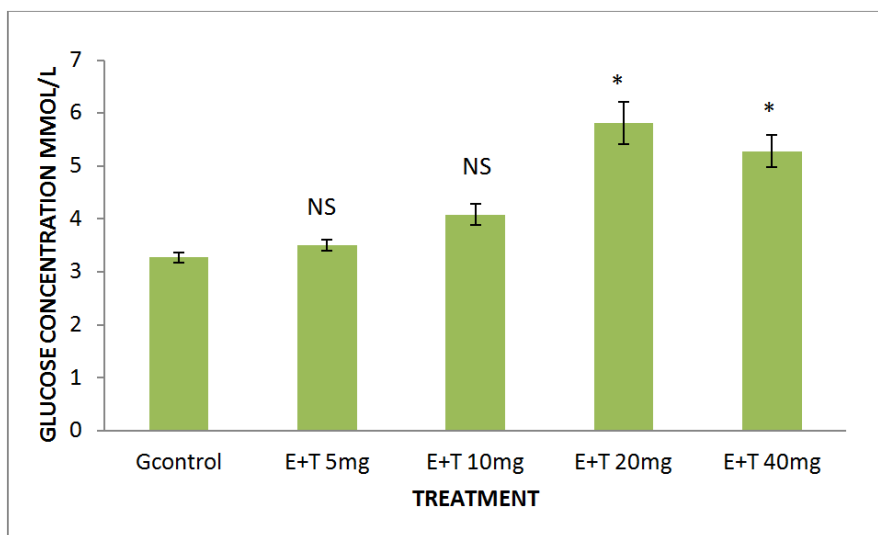
body weight did not show any significant increase when compared with the control.

### 3.2 Effect of Tartrazine and Erythrosine Administration on Triglyceride Concentration

As shown in Fig. 2, rats fed with 40 mg/kg bodyweight of the colourants (group 5) showed the lowest average triglyceride concentration when compared with the control, though the mean difference was not statistically significant ( $p > 0.05$ ).

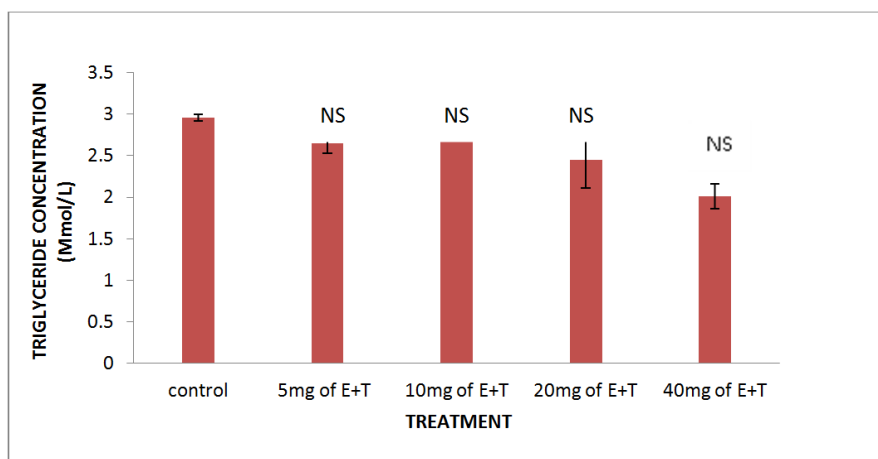
### 3.3 Effect of Tartrazine and Erythrosine Administration on the Concentration of Cholesterol

As shown in Fig. 3, rats the experimental groups (colourant treated) indicated raised mean total cholesterol concentration (TC) when compared with the control. However, the elevated TC was higher in rats fed with 20 mg/kg bodyweight of colourants compared to the control. However, the difference was not statistically significant ( $p < 0.05$ ).



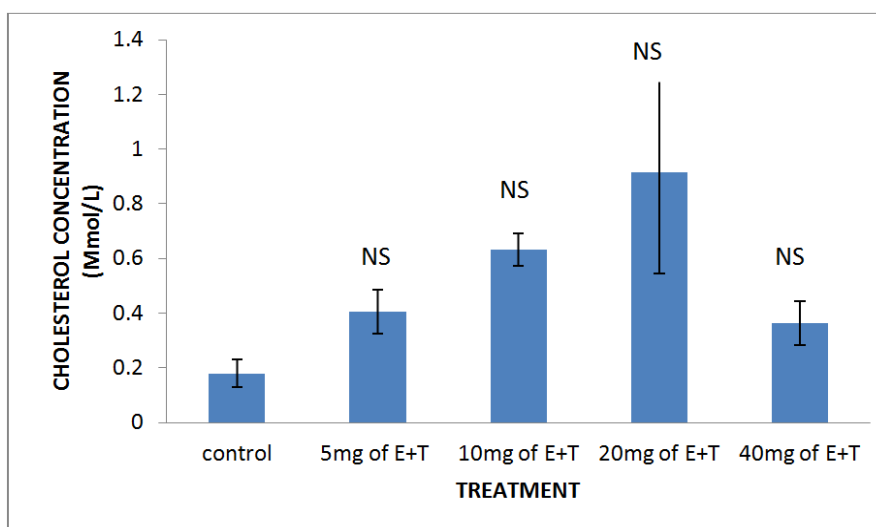
**Fig. 1. Effect of tartrazine and erythrosine administration on the concentration of serum glucose**

Values are presented in mean  $\pm$  SEM,  $n = 4$ . NS = not significant versus control group



**Fig. 2. Effect of tartrazine and erythrosine administration on the concentration of triglyceride**

Values are mean  $\pm$  SEM,  $n = 4$ . NS = not significant versus the control group



**Fig. 3. Effect of tartrazine and erythrosine administration on the concentration of cholesterol**  
 Values are mean  $\pm$  SEM, n = 4. NS = not significant versus the control group

#### 4. DISCUSSION

Food additives are used to protect food, increase quality and extend shelf life in many stages, from production to consumption of food. Additives used in the food industry are added to the food during preparation, production, and packaging and storage stages. Colourants added by food producers to colour food or to adjust the colour to the desired level are among the commonly used food additives. Considering today's developing production technologies, foods fade or discolour at various stages of processing, storage, and sale due to physical and chemical conditions such as heat, light, pH and oxygen. Colourants are used to regain these colour losses, to enhance weak colours, to give colour to the colourless food, and to win back the favour of customers by hiding low quality. Colourants are used in the production of soft drinks, candies, bakery products, canned and vegetable products, dairy products, and meat and fish products [1,16,17].

Tartrazine and erythrosine are commonly used synthetic food colourants all over the world due to its good colouration and enticing to food [6]. These food colourants have shown to cause increased serum levels of glucose which may subsequently lead to diabetes mellitus [18].

In this present study, the serum concentration of biochemical parameters (TC, TG and glucose mmol/L) were assessed after three weeks (21

days) ingestion of synthetic food colourants (Tartrazine and erythrosine). There was significant ( $P < 0.05$ ) increase in serum glucose concentration on the rats fed with 20 mg/kg and 40 mg/kg bodyweight of the prepared colourants compared with the control. However, rats fed with 5 mg/kg and 10 mg/kg bodyweight of the prepared colourants did not show any significant increase with the control. Furthermore, the serum total cholesterol and triglyceride concentrations on colourants fed rats did not show any significant difference when compared with the control.

Glucose is a simple sugar which is the most abundant monosaccharide subcategory of carbohydrates. Glucose is produced by plants during photosynthesis from water and carbon dioxide using ATP from sunlight. During energy metabolism, glucose is an essential source of energy. Glucose is stored in the animals as glycogen and circulates in the body as blood sugar [19]. The inability of the body's control mechanism to regulate blood glucose is as a result of the inability of insulin-secreting cells in the pancreas to produce the hormone which will eventually lead to diabetes mellitus [20]. The significant increase in serum glucose concentrations in 20 mg/kg and 40 mg/kg bodyweight of colourants fed rats indicates hyperglycemic effects of these food additives and may likely be attributed to pancreatic cancer caused by the hydrocarbons contained in the colourants, [3,4]. This result is in agreement with

the report of Aboel-Zahab, et al. [11] and Amin et al. [21]. The increase in cholesterol concentration in colourants fed rats corresponds to the report of Aboel-Zahab, et al. [11]. High cholesterol concentration in blood could lead to the accumulation of cholesterol on the walls of the arteries leading to atherosclerosis and subsequent reduction of blood flow through the arteries [22].

## 5. CONCLUSION

Ingestion of rats with tartrazine and erythrosine at different doses caused significant alteration of serum glucose and cholesterol concentrations in male rats. There was a significant increase in glucose in 20 mg/kg and 40 mg/kg bodyweight of colourants fed rats. If these results apply to humans it is advised that synthetic food colourants application in food and consumption should be drastically minimized as it poses detrimental physiological adverse effects.

## ETHICAL APPROVAL

As per international standard written ethical permission has been collected from the Madonna University ethical committee and preserved by the author(s).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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