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# Evaluation of Anti-oxidant Enzymes, Lipid Peroxidation, Lipid Profile and Liver Function in Albino Rats Orally Administered Tartrazine

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author IE designed the study, performed the statistical analysis and wrote the protocol. Author UAA wrote the first draft of the manuscript. Authors GI, ONB and HAW managed the analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To evaluate the anti-oxidant enzymes, lipid peroxidation, lipid profile and liver function in albino rats orally administered tartrazine.
**Study Design:** A total number of 63 female albino rats weighing approximately 0.2 kg were used for this study. The study was divided into two phases, phase 1 which lasted for the first 30 days, comprised of 35 rats, 20 rats were used as test group while 15 rats served as the control group. Phase 2 of the study was for 60 days and 28 rats were used with 16 as test group and 12 as the control. The test groups were orally administered with 7.5 mg/kg of tartrazine (ADI) daily over the specified periods while the control groups were not treated with tartrazine but given only food and water.
**Place and Duration of Study:** The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria within a period of 12 months (Feb., 2019 – Jan., 2020).

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**Methodology:** At the end of the study, 5 mls of whole blood specimens was collected by means of cardiac puncture into plain bottles. To obtain the serum, the whole blood samples were allowed to clot and later dislodged and spun at 3500 rpm for 10 minutes. The collected serum specimens were used to analyze SOD, MDA, GPX, ALT, GGT, ALP, TG, TCHOL, and HDL-C, while LDL-C was calculated using Friedwald equation.
**Results:** The chronic treatment of rats with tartrazine azo food dye at the ADI dose caused an increase in MDA levels after 30 and 60 days test rats compared to the control, while TCHOL and HDL-C showed significant decrease after 30 and 60 days of treatment in the test group compared to the control group. In addition, ALT indicated significant increase in test group after 60 days of treatment compared to control group. ALP, GGT, TG, LDL-C, SOD and GPX showed no significant difference after 30, and 60 days of treatment at ADI doses. Histologic examination of the liver indicated hydropic dilation, degenerating hepatocyes and infiltration of central vein with parenchymal materials alongside kupffer cells.
**Conclusion:** The results from this study revealed that orally administered tartrazine at the recommended ADI dose increased lipid peroxidation as seen in the elevated MDA levels. Hepatic derangements were also seen as revealed by increased ALT and histologic distortions as well fall in TCHOL and HDL-C lipid fractions.

*Keywords: Albino rats; anti-oxidant enzymes; lipid peroxidation; lipid profile; liver enzymes; oxidative stress; tartrazine food dyes.*

## ABBREVIATIONS

*ADI : Acceptable Daily Intake*
*TCHOL : Total Cholesterol*
*HDL-C : High Density Lipoprotein- Cholesterol*
*LDL-C : Low Density Lipoprotein- Cholesterol*
*TG : Triglyceride*
*SOD : Superoxide Dismutase*
*MDA : Malondialdehyde*
*GPX : Glutathione Peroxidase*
*ROS : Reactive Oxygen Species*

## 1. INTRODUCTION

Food colours are widely utilized to enhance the visual attributes of food, increase consumers interest and appeal of certain food and food products in the food and beverage industries. Food colours are generally grouped into two, synthetic/ artificial azo food dyes and natural food dyes or pigments [1]. Synthetic food dyes have been discovered to give food more intense colouring effects, they are more stable when exposed to extreme temperatures and are cheaper to use than natural food dyes [2]. The use of synthetic food dyes is associated with clinical decline in the liver, kidney and nervous system [3-6]. Recent [4,5] studies have also shown that these azo dyes are able to induce oxidative stress.

Tartrazine (E102) is a lemon-yellow coloured synthetic azo dye. It is mostly used to give the colour yellow to foods like sauce, jellies, cakes, chewing gums, beverages and drinks. Other products besides food are cosmetics (brown powders, foundations, nail polish, soaps and shampoos) and pharmaceuticals (vitamin capsules, tablets, and syrups), also make use of this azo dye [7]. The acceptable daily intake (ADI) of tartrazine ranges between 0 - 7.5 mg/kg body weight of tartrazine per day which was established by the joint Food and Agriculture Organization (FAO)/World Health organization (WHO). Due to health problems associated with the use of tartrazine in food products, many countries have placed strict measures on the use of tartrazine and other azo food dyes in food and food products [8]. Data regarding synthetic food dyes have proven that many synthetic food dyes are produced from petroleum-based products such as coal tar and have been reported to be toxic and carcinogenic to human health [9]. The harmful effects of synthetic food dyes are attributed to the metabolic biotransformation of the azo bond in the intestine and liver yielding reactive products like aryl amines, amines and free radicals [10].

Lipids are generally grouped into apolar and polar lipids. Apolar lipids are the triglycerides and are usually stored in adipose tissues and other cells of the body where they function as energy reserves in mammals [11,12]. Polar lipids act as part of the components of the structure of cell membrane forming lipid bilayers which enables the permeability barrier of the cell. Tartrazine has been reported to alter lipid metabolism and lipid fraction in rats fed with azo dyes [6].

Lipid peroxidation are series of events instigated by the harm done by oxidants such as free radicals and reactive oxygen species (ROS) on lipids made up of carbon-carbon double bonds mostly the polyunsaturated fatty acids PUFA's. Lipid peroxidation is the oxidative degradation of lipids. ROS are the major initiators of lipid peroxidation and membrane bound polyunsaturated fatty acids (PUFAs) are their major targets. This phenomenon arises when the balance between the levels of antioxidants in cells and tissues and the levels of pro-oxidants is compromised, favouring increased activity of the pro-oxidants. In the body, reactive oxygen species (ROS) are generated following enzymatic reactions from constituents of cell membranes such as the plasma membrane, Endoplasmic reticulum and mitochondria [13]. These reactive oxygen species are also formed after exposure to ionizing radiation, and toxins from environmental pollution. Lipids are mostly attacked by two main ROS; hydroxyl radical and hydroperoxyl. Initiation, propagation, and termination are three phases of lipid peroxidation [14-16]. In the initiation phase, allylic hydrogen is extracted by the pro-oxidant, followed by the propagation phase involving the reaction of lipid radical with oxygen creating a lipid peroxyl radical (LOO), this radical extract a hydrogen molecule from another lipid molecule. In the termination phase, antioxidants donate a hydrogen atom to lipid peroxyl radical species to generate vitamin E radical which reacts with LOO to form non radicals. Many oxidation products are generated after the process of lipid peroxidation. Lipid hydroperoxides are the primary products of lipid peroxidation while malondialdehyde (MDA), propanal, 4-hydroxynonenal and hexanal are secondary products of lipid peroxidation [17-21]. MDA is a reliable marker of oxidative stress [22].

Oxidative stress is a pathological state encompassing an imbalance between the levels of free radicals and antioxidants, either due to an over production of these free radicals or a decline in the body system to generate antioxidants that readily detoxify this reactive oxygen species. In this state, these reactive oxygen species or free radicals attack macromolecules in the body such as proteins, lipids, carbohydrates and DNA thereby altering their normal structure and function [23,24]. Oxidation of these macromolecules can lead to genotoxicity, cytotoxicity and carcinogenesis. The internal defence mechanism against these reactive oxygen species and free radicals includes glutathione, vitamin E, ascorbic acid or enzymes such as catalase, glutathione peroxidase and superoxide dismutase (SOD) that scavenge oxygen radicals. Oxidative stress is a major cause of many pathological conditions such as cardiovascular disorders, atherosclerosis, Alzheimers disease [25]. In this study, we will evaluate the effect of azo dye such as tartrazine orally given to albino rats at ADI dose on anti-oxidant enzymes, lipid peroxidation, lipid profile and liver enzymes.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Materials used in this study includes plain bottles, polypropylene gavage tubes (Intech Laboratory Incorporated, Plymouth Meeting, USA), Bucket centrifuge Model 351 (MPW Medical Instruments, Warsaw, Poland), Biotechnica Spectrophotometer BT 224 (MedWrench), Haier thermocool refrigerator (China),using Microplate Reader Stat-Fax 4500 (Awareness incorporated, California, USA), Shandon AS325 rotary microtome (Fisher Scientific, United Kingdom), digital Olympus microscope with camera (Olympus, Tokyo, Japan), Tartrazine dyes (CI. 19140, CAS No 1934-21-0, MW 534,37, E102, FD& C NO 5) with serial no. of FI19371 purchased in a granular form from Fiorio Colori Spa, Gessete, Italy, with purity of 86.7%. Superoxide Dismutase (SOD) and Malondialdehyde (MDA) were purchased from Bioassay technology Laboratory (Shangai, China), Glutathione Peroxidase (GPX) was purchased from Elascience (Houston, Texas, USA), while Alanine aminotransferase (ALT), Gamma glutamyl transferase (GGT), Triglyceride (TG), Total cholesterol (TCHOL), and High density lipoprotein (HDL-C) were purchased form Atlas Medicals (Cambridge, United Kingdom), while ALP reagent that was purchased from Teco Diagnostics. Other materials used include automatic pipettes, glass tubes, mixer, and chloroform.

### 2.2 Experimental Animals

A total of 63 female albino rats weighing approximately 0.2 kg were used for the experiment. All the rats used for the experiment were purchased and housed in the animal house of Rivers state University, Port Harcourt, Nigeria. The rats were kept in well-ventilated cages, at regulated room temperature of 23°C-26°C, with

controlled 12 hour light-dark cycles and allowed access to feed and water *ad libitum*. The rats were allowed to acclimatize for 10 days prior to the commencement of the study.

## 2.3 Preparation of Tartrazine Dye

The tartrazine dye used for this study was purchased in granular form, therefore it was dissolved to allow easy oral administration in rats. 1.50 grams of tartrazine was weighed and dissolved in 1.0 liter of distilled water in a sterile container. This indicated that, 1.0 ml of the tartrazine solution contained 0.00015 g, which was equivalent to 7.5 mg/kg when administered into a 0.2 kg rat.

## 2.4 Experimental Design and Administration of Dye

The method of treatment was oral, gavage tubes were used to administer the dyes to the rats daily. This was a chronic study toxicity, and was performed over a period of 30 and 60 days, comprising two phases. 35 experimental rats weighing approximately 0.2 kg were used in the 30 days treatment while 28 rats were used in the 60 days treatment. In each phase, the rats were divided into two groups assigned $T_{Tg}$ (tartrazine treated group), and C (control, untreated group). Rats in each of these groups were further grouped randomly into 10 cages with 3 to 4 rats per cage with labels indicating the duration and treatment pattern. In the treatment pattern, the acceptable daily intake (ADI) of 7.5 mg/kg of tartrazine was administered orally. The control groups were given food and water only.

## 2.5 Study Area

The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria within a period of 12 months (Feb., 2019 – Jan., 2020).

## 2.6 Specimen Collection, Preparation and Analysis

At the end of the study, the animals were anaesthetized with chloroform and 5mls of whole blood samples was collected by means of cardiac puncture into plain bottles. The collected whole blood samples were allowed to clot in order to obtain the serum, the clotted samples were dislodged and spun at 3500 rpm for 10 minutes. The collected serum specimens were used to analyze Superoxide Dismutase (SOD), Malondialdehyde (MDA), Glutathione Peroxidase (GPX), Alanine aminotransferase (ALT), Gamma glutamyl transferase (GGT), Alkaline phosphatase (ALP), Triglyceride (TG), Total cholesterol (TCHOL), and High density lipoprotein (HDL-C). The laboratory analysis of ALP was determined using spectrophotometer as described by Kind and King [26]. Plasma ALT and GGT were also measured with spectrophotometer as described by Reitman and Frankel [27] and de Gruyter [28] respectively. Determination of SOD, MDA, and GPX were based on ELISA technique as described by Engvall [29]. The method of assay for TC and TG were based on enzymatic methods as described by Stavropoulous et al. [30] and Flegg et al. [31] respectively. HDL-C was assayed by precipitating out Very low density lipoprotein VLDL-C and Low density lipoprotein LDL-C using phosphotungstic acid and magnesium ions, and enzymatic evaluation of HDL-C in the supernatant as described by Flegg et al. [31]. LDL-C was computed as described by Friedwald et al. [32] using the equation: LDL-C (mmol/l) = TC - (TG/2.2 + HDL-C).

### 2.6.1 Histological preparation and examination

Liver tissues were obtained from the sacrificed animals. The tissues were washed in normal saline and fixed in 10% formalin saline. The tissues were passed through ascending grades of alcohol, cleared and embedded in paraffin wax. Leica automatic tissue processor (Leica Biosystems, USA) was used to process the tissues. Rotary microtome was used to obtain 5µm thick sections, the sections were stained using Haematoxylin and Eosin staining technique and examined using the digital Olympus microscope with camera.

## 2.7 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5.03 (San Diego, California, USA). Results were presented as Mean ± Standard deviation (SD). Inferential statistics using Students' statistical t-test was employed to compare values of the treated rats and control rats. Statistical significance was set at $P$=.05.

## 3. RESULTS

### 3.1 Results on Lipid and Oxidative Parameters in Rats Treated with Tartrazine Orally

Significantly higher values in MDA were observed in rats treated with tartrazine over a period of 30 days and 60 days as compared to the control. However, there was no significant difference seen in SOD, GPX, TCHOL, TG, HDL-C and LDL-C in rats treated with tartrazine when compared with the control group (Table 1 and Table 2). More so, when treated rats of 30 and 60 days were compared, significantly higher and lower values were seen in MDA and HDL-C respectively in the 60 days treated compared with the 30 days treated rats. However, there was no significant difference in SOD, GPX, TCHOL, TG, and LDL-C (Table 3).

**Table 1. Results of lipid and oxidative parameters in rats treated with tartrazine (7.5 mg/kg bw) over a period of 30 days against controls**

| Parameters | Control rats (n=15) | Treated rats (n=20) | P value | T value | Remark |
|---|---|---|---|---|---|
| SOD (ng/ml) | 256.3±195.6 | 173.7±81.39 | 0.097 | 1.710 | NS |
| GPX (pg/ml) | 10.69±5.99 | 9.89±7.47 | 0.737 | 0.339 | NS |
| MDA (ng/ml) | 55.28±29.03 | 104.2±87.90 | 0.047 | 2.067 | S |
| TCHOL (mmol/L) | 2.40±0.62 | 2.64±0.56 | 0.237 | 1.204 | NS |
| TG (mmol/L) | 1.34±0.35 | 1.22±0.42 | 0.357 | 0.934 | NS |
| HDL-C (mmol/L) | 1.61±0.47 | 1.28±0.62 | 0.090 | 1.744 | NS |
| LDL-C (mmol/L) | 0.47±0.45 | 0.94±0.60 | 0.015 | 2.574 | NS |

*S=Significant at p<0.05, NS=Not significant at p<0.05, GPX= Glutathione peroxidase, SOD= Superoxide dismutase, MDA=Malondialdehyde, C TCHOL=total cholesterol, TG= triglycerides, HDL-C=High Density Lipoprotein-Cholesterol, LDL-C=Low Density Lipoprotein-Cholesterol, n= No of rats, Control Rats (n=15), 30 days Treated Rats (n=20). Results were expressed as Mean±SD*

**Table 2. Results of lipid and oxidative parameters in rats treated with tartrazine (7.5 mg/kg bw) over a period of 60 days against controls**

| Parameters | Control rats (n=12) | Treated rats (n=16) | P value | T value | Remark |
|---|---|---|---|---|---|
| SOD (ng/ml) | 188.9±116.4 | 168.0±70.18 | 0.514 | 0.659 | NS |
| GPX (pg/ml) | 10.91±7.68 | 9.02±4.41 | 0.365 | 0.918 | NS |
| MDA (ng/ml) | 53.25±14.79 | 114.1±40.31 | <0.0001 | 5.551 | S |
| TCHOL (mmol/L) | 2.09±0.41 | 2.04±0.73 | 0.807 | 0.246 | NS |
| TG (mmol/L) | 1.08±0.50 | 1.25±0.51 | 0.330 | 0.988 | NS |
| HDL-C (mmol/L) | 1.00±0.35 | 0.86±0.26 | 0.187 | 1.349 | NS |
| LDL-C (mmol/L) | 0.67±0.40 | 0.89±0.61 | 0.251 | 1.169 | NS |

*S=Significant at P=.05, NS=Not significant at P=.05, GPX= Glutathione peroxidase, SOD= Superoxide dismutase, MDA=Malondialdehyde, C TCHOL=total cholesterol, TG= triglycerides, HDL-C=High Density Lipoprotein-Cholesterol, LDL-C=Low Density Lipoprotein-Cholesterol, n= No of rats, Control Rats (n=12), Treated Rats (n=16). Results were expressed as Mean±SD*

**Table 3. Results of 30 and 60 days comparative analysis of lipid and oxidative parameters in rats treated with tartrazine (7.5 mg/kg bw)**

| Parameters | Treated rats (30 Days) | Treated rats (60 Days) | P value | T value | Remark |
|---|---|---|---|---|---|
| SOD (ng/ml) | 173.7±61.39 | 168.0±70.18 | 0.816 | 0.235 | NS |
| GPX (pg/ml) | 9.89±7.46 | 9.02±4.41 | 0.656 | 0.449 | NS |
| MDA (ng/ml) | 104.2±87.90 | 114.1±40.31 | 0.996 | 0.004 | NS |
| TCHOL (mmol/L) | 2.64±0.56 | 2.04±0.73 | 0.005 | 2.951 | S |
| TG (mmol/L) | 1.34±0.35 | 1.25±0.51 | 0.079 | 1.807 | NS |
| HDL-C (mmol/L) | 1.61±0.47 | 0.86±0.26 | 0.008 | 2.792 | S |
| LDL-C (mmol/L) | 0.47±0.45 | 0.89±0.61 | 0.774 | 0.289 | NS |

*S=significant at P=.05, NS=Not significant at P=.05, GPX= Glutathione peroxidase, SOD= Superoxide dismutase, MDA=Malondialdehyde, TCHOL=Total cholesterol, TG= Triglycerides, HDL-C=High Density Lipoprotein-Cholesterol, LDL-C=Low Density Lipoprotein-Cholesterol, n= No of rats, 30 days treated Rats (n=20), 60 days Treated Rats (n=16). Results were expressed as Mean±SD*

Furthermore, when liver enzymes were considered, no significant differences were seen in hepatic enzymes ALT, ALP, and GGT in rats treated over a period of 30 days as well as 60 days when compared with their respective control rats (Table 4 and Table 5). However, ALT indicated significant increase after 60 days in treated rats compared to control rats (Table 5). In addition, when treated rats of 30 and 60 days were compared, no significant difference in hepatic enzymes ALT, ALP, and GGT were seen (Table 6).

### 3.2 Histological Examination of the Liver

The histological examinations of the liver tissues are seen in Fig. 1.

## 4. DISCUSSION

This study is intended to evaluate the effects of tartrazine at ADI dose on lipid peroxidation and oxidative capacity, using oxidative stress markers such as GPX, SOD and MDA as well as lipid parameters such as TCHOL, LDL, HDL, and TG, over a period of 30 and 60 days in albino rats.

The result of this study regarding oxidative stress showed elevated levels of MDA in tartrazine treated rats after 30 and 60 days compared to control rats. However, there was no significant difference in SOD values when treated and control rats were compared. MDA is a secondary product of lipid peroxidation associated with oxidative damages. The significant increase observed in the treated rats in our study is in line with the results reported by [6,33]. Amin et al. [6] and Omca et al. [33] recorded a significant increase in MDA levels in rats treated with tartrazine at high and low doses when compared to their control group. Amin et al. [6] also reported significant reduction in GPX and SOD in rats treated with tartrazine though at low and high dose of 15 mg/kg and 500 mg/kg respectively for 30 days orally. Again, our finding with respect to SOD is contrary to the reports of [34,35]. Moutinho et al. [34] and Bansal [35] in their respective studies reported increase in oxidative parameters such as SOD in rats treated with azo food dyes. The non-significant decreases seen in SOD and GPX could be as a result of SOD and GPX been consumed in an attempt to prevent or mitigate cell death due to oxidative stress when ROS were generated in the system in course of tartrazine metabolism. The increase in MDA levels might be also attributed to the metabolic transformation of tartrazine in the gastrointestinal tract (GIT) to sulfanilic acid which possibly interacts with nitrate or nitrite containing food in the stomach, thereby yielding reactive oxygen species (ROS) as a metabolic product. Additionally, the elevated levels of MDA can also be due to the harmful effects of reactive oxygen species on cellular membrane lipids. The toxic activities of these free radicals or reactive oxygen species on lipids of cellular membranes could have accounted for more of the increase seen in MDA. Therefore, the significant increases seen in MDA after 30 and 60 days suggest the presence of lipid peroxidation in the tartrazine treated rats.

**Table 4. Results of hepatic enzymes in rats treated with tartrazine (7.5 mg/kg bw) over a period of 30 days with their control rats**

| Parameters | Control rats (n=15) | Treated rats (n=20) | P value | T value | Remark |
|---|---|---|---|---|---|
| ALT(U/L) | 19.20±10.11 | 23.16±6.12 | 0.096 | 1.697 | NS |
| ALP(U/L) | 23.63±12.09 | 22.10±14.81 | 0.864 | 0.139 | NS |
| GGT (U/L) | 10.43±5.55 | 11.66±2.28 | 0.721 | 0.359 | NS |

*NS=Not significant at P=.05, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, GGT=Gamma glutamyl transferase, n= No of Rats: 30 days Control Rats (n=14), 30 days Treated Rats (n=20), Results were expressed as Mean±SD*

**Table 5. Results of hepatic enzymes in rats treated with tartrazine (7.5 mg/kg bw) over a period of 60 days with their control rats**

| Parameters | Control rats (n=12) | Treated rats (n=16) | P value | T value | Remark |
|---|---|---|---|---|---|
| ALT (U/L) | 21.12±3.83 | 29.94±8.66 | 0.032 | 2.988 | S |
| ALP (U/L) | 23.45±8.90 | 22.08±9.80 | 0.864 | 0.173 | NS |
| GGT (U/L) | 10.02±1.98 | 10.98±2.28 | 0.831 | 0.215 | NS |

*NS=Not significant at P=.05, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, GGT=Gamma glutamyl transferase, n= No of Rats: 60 days Control Rats (n=11), 60 days Treated Rats (n=17), Results were expressed as Mean±SD*

**Table 6. Results of 30 and 60 days comparative analysis of hepatic enzymes in rats treated with tartrazine (7.5 mg/kg bw)**

| Parameters | Treated rats 30 days | Treated rats 60 days | P value | T value | Remark |
|---|---|---|---|---|---|
| ALT (U/L) | 23.16±6.12 | 29.94±8.66 | 0.722 | 1.508 | NS |
| ALP (U/L) | 22.10±14.81 | 22.08±9.80 | 0.980 | 1.086 | NS |
| GGT (U/L) | 11.66±2.28 | 10.98±2.28 | 0.862 | 0.175 | NS |

*NS=Not significant at P=.05, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, GGT=Gamma glutamyl transferase, n= No of Rats: 60 days Control Rats (n=11), 60 days Treated Rats (n=17), Results were expressed as Mean±SD*

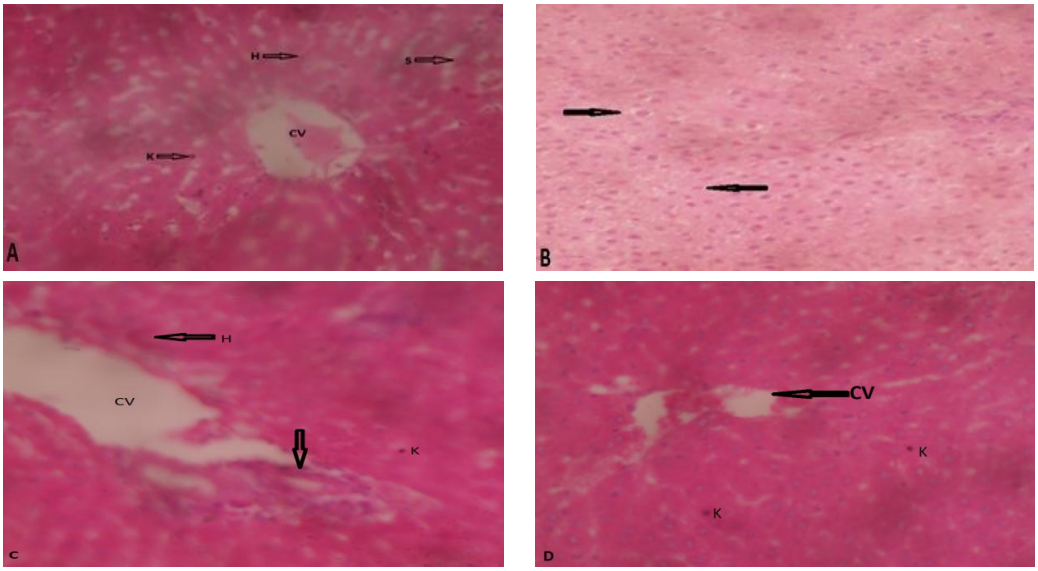


**Fig. 1. A. 30 days tartrazine treatment. Section showed normal central vein (CV) with minute infiltration. The sinusoids (S), hepatocytes (H) and hepatic plate arrangements were not distorted. B. 30 days control. The section showed normal hepatic tissue. The sinusoid, hepatic plates, and hepatocytes appear intact (arrows). The cells are well dispersed and stained appropriately. C. 60 days of tartrazine treatment: The section showed hydropic dilation of hepatic central vein (CV) with infiltrations (arrow). Degenerating hepatocytes (H) and hepatic plates alongside kupffer cells (K) were seen. D. 60 days of tartrazine treatment: The section showed central vein (CV) with infiltration of parenchymal materials. Kupfer cells (K) were seen. H&E stain. X400**

In addition, the decrease in the levels of Total cholesterol (TCHOL) in the serum of tartrazine treated rats after 60 days compared to 30 days treated rats may be related to tartrazine induced liver (hepatocyte) damage and it is considered as a good clinical marker of decline in hepatic function, owing to the fact that the liver is the main site of cholesterol production. The decreased level of TCHOL agrees with the reports of [6,36]. Amin et al. [6] reported decrease in TCHOL concentration in rats administered with 15 mg/kg and 500 mg/kg bodyweight. However, the findings of [37] were contrary to that of our study. Himri et al. [37] recorded a significant increase in total cholesterol levels in rats administered with 10mg/kg bodyweight of tartrazine. The non-significant increase seen in LDL-C and other lipid parameters recorded in treated rats agrees with the findings of [6]. More so, the significant decrease in HDL-C concentration in treated rats is in accordance with the work of [38]. Mehedi et al. [38] reported significant reduction in HDL-C values when rats were fed with tartrazine azo dyes. The fall in HDL-C level in the treated rats could be as a result of hepatocyte destruction by the metabolic products of tartrazine which are associated with increase rate at which HDL-C are broken down. Mehedi et al. [38] further reported non-significant decrease in total

cholesterol in male and female rats treated with 0.1% of tartrazine as compared to the control. The variations in the normal concentration of lipids in the serum of treated rats are indications of liver damage and are associated with lipid peroxidative alterations.

Furthermore, when liver enzymes were considered, GGT and ALP indicated no significant difference after 30 and 60 days of treatment which is also in agreement with the reports of [39]. Elekima et al. [39] documented non-significant increases in ALP in rats treated with tartrazine at ADI dose over a period of 60 days. However, Mehedi et al. [38] reported significant increase in ALT and GGT after 13 weeks of tartrazine administration at a dose of 0.1% in female rats but such increase was not reported in male rats. However, significant increase was seen in ALT after 60 days of treatment. The result of ALT is in line with the report of [6,39]. Amin et al. [6] reported increase in ALT concentration in rats administered with 15 mg/kg and 500 mg/kg bodyweight. More so, Elekima et al. [39] also reported significant increase in ALT in male albino rats treated with tartrazine at ADI dose of 7.5 mg/kg body weight. However, Himri et al. [37] documented no significant difference in rats treated with tartrazine at ADI dose of 7.5 mg/kg for 90 days as compared to the control. The increase in ALT observed in our study indicates hepatocellular inflammatory response or derangements which could be as a result of ROS generated during the metabolism of tartrazine azo dye. The Increase in ALT over a periof of 60 days further suggest damage to both the hepatic cells and the membrane of the hepatic mitochondria in the tartrazine treated rats. The non-significant increases seen in GGT and ALP could be attributed to the fact that these enzymes are mostly situated within the hepato-biliary duct thus associated with obstruction. However, contrary to our reports, El-Desoky et al. [4] documented significant increases in liver enzymes of tartrazine treated rats which were ameliorated by oral administration of Curcumin.

Our histopathological findings of the liver section after 30 days of treatment with tartrazine showed normal central vein with minute infiltration. The sinusoids, hepatocytes and hepatic plate arrangements were not distorted (Fig. 1A). However, after 60 days of treatment, we recorded infiltration of parenchymal materials alongside Kupfer cells, hydropic dilation as well as degenerating hepatocytes (Fig. 1C & D). These findings are also in accordance with the reports of [39,40,41]. Elekima et al. [39] reported vacuolation accompanied with degenerating hepatic cells after 60 days of treatment with tartrazine at ADI doses given orally over a period of 60 and 90 days. They further reported the presence of fatty cyst due to lipid peroxidation after 90 days of tartrazine treatment at ADI doses. In addition, Khayyat et al. [40] recorded several ultrastructural and histopathological alterations in the liver of rats treated with tartrazine. They reported infiltration, activated Kupffer cells, congestion of blood sinusoids and necrosis of hepatocytes. More so, Saxena and Sharma [41] also recorded that tartrazine induced damage to the normal structure of the liver. In addition, Ali et al. [42] also documented diffuse degeneration in the hepatic parenchymal structures, hepatoportal blood vessels and bile ducts when tartrazine at 220 mg/kg were orally given to rats for 60 days. The presence of Van kupffer cells, central vein congestion or infiltration, parenchymal loss due to vacuolation, and hydropic dilation of the hepatic portal (central vein) of the liver tissue in rats treated with tartrazine over a period of 60 days are indications of oxidation of hepatocytes. These structural alterations and presence of Van kupffer cells which are suggestive of oxidation of hepatic cells may have occurred following the rise in formation of reactive oxygen species or free radicals in course of tartrazine metabolism.

## 5. CONCLUSION

The results from this study revealed that orally administered tartrazine at the recommended ADI dose increased lipid peroxidation as seen in the elevated MDA levels. Hepatic derangements were also seen as revealed by increased ALT and histologic distortions as well as fall in TCHOL and HDL-C lipid fractions after 60 days of treatment. However, there were no significant increase in the levels of antioxidants SOD and GPX as well as ALP, GGT, LDL-C and TG after 30 and 60 days of chronic treatment with tartrazine.

## 6. RECOMMENDATION

It is advised that the consumption of tartrazine in foods or food products should be controlled and not too frequent. Moreover, because of the slight changes seen in the chronic study, further research extending the 60 days period should be considered.

## 7. LIMITATION OF THE STUDY

The duration of the chronic aspect of this study was not more than 60 days. Moreover, our present findings were in rats and therefore cannot be directly interpreted that these effects observed in rats will be exactly and/or physiologically the same in humans. Therefore, our findings are subject to further research and verification especially in humans.

## ETHICAL APPROVAL

We hereby declare that the Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Rivers State University research/ ethics committee with file No: RSU/CV/APU/74/ VOL.VIII/104.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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