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Assessment of Atherogenic Indices and Markers of Cardiac Injury in Albino Rats Orally Administered with Tartrazine Azo Dye

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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 GI wrote the first draft of the manuscript. Authors UAA, ONB and HAW managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Assess the effect of tartrazine azo dye on atherogenic indices and markers of cardiac injury in albino rats.
Study Design: A total number of 63 rats were used for the study. The study was divided into two phases, 1 and 2, which lasted for 30 and 60 days respectively. Phase 1 had 35 rats, 20 as test and 15 as control, while phase 2 had 28 rats, 16 as test and 12 as control. In each phase the test groups were given 7.5mg/kg of tartrazine orally on daily basis over the stipulated period while the control groups were not treated with tartrazine.
Methodology: At the end of the study, 5ml of whole blood was collected from the jugular veins into Lithium Heparin bottles. The sample was spun, plasma collected and analyzed for cardiac Troponin I (cTn-I) and cardiac Troponin T (cTn-T), Total creatinekinase (CK), creatinekinase MM (CK-MM), and creatinekinase MB (CK-MB). Lipid parameters like total cholesterol (TC), High density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and Triglyceride (TG) which were also analysed. Atherogenic indices such as atherogenic coefficient (AC), atherogenic

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index of plasma (AIP), Non High density lipoprotein-cholesterol (nHDL-C), and castelli risk indices 1 and 2 (CRI-1 and CRI-2) were also calculated. In addition, cardiac tissues were collected, fixed in 10% formol saline and examined histologically using Haematoxylin and Eosin stain. Statistical analysis was performed using GraphPad Prism version 8.02.

Results: The results obtained indicate significant increases in nHDL-C, total CK and cTn-T after 30 and 60 days of treatment with tartrazine at ADI doses against controls. Other atherogenic indices such as AIP, AC, CRI-1 and CRI-2 as well as markers of cardiac injury such as cTn-I and CK-MB indicated non-significant increases.

Conclusion: Orally administered tartrazine over a 60 day period induced cardiac injury as shown by the significant increase in the cTn-T and total CK as well as hypertrophied nuclei of cardiomyocytes. This goes to say chronic administration of tartrazine even at the recommended daily dose could pose the risk of cardiovascular disease. This is also supported by an increase in nHDL cholesterol.

Keywords: Tartrazine; azo dye; cardiac markers; atherogenic indices; lipid profile; anti-oxidant enzymes; oxidative stress; albino rats.

ABBREVIATIONS

AC : Atherogenic Coefficient
ADI : Acceptable Daily Intake
AIP : Atherogenic Index of Plasma
CRI-1 : Castelli Risk Index 1
CRI-2 : Castelli Risk Index 2
CVD : Cardiovascular Disease
HDL-C : High Density Lipoprotein-Cholesterol
LDL-C : Low Density Lipoprotein-Cholesterol
MDA : Malondialdehyde
nHDL-C : Non high Density Lipoprotein-Cholesterol
ROS : Reactive Oxygen Species
SOD : Superoxide Dismutase
TC : Total Cholesterol
TG : Triglyceride

1. INTRODUCTION

Food additives are substances often used mostly in food industries with the aim of improving and enhancing the flavor, taste, colour, texture of food and food products [1,2]. With these food additives, consumers are thereby presented with a wide variety of fresh, appetizing, nutritious and palatable food for their convenience and enjoyment [2,3]. They can be classified as preservatives, colourants, anti-oxidants, sweeteners, flavouring agents, texturizers and so on [3].

Colourants are extensively used for the improvement of the sensory experience and aesthetic value of products in the food industries [2,4]. Colourants can either be natural or synthetic [4]. The natural ones are got mostly from plant pigments, though they are safer to use than synthetic ones, they do not produce bright colours and tend to fade easily as they undergo oxidation reactions [2,3]. However, about 95% of the colourants used these days are synthetic because they are easily produced, produce better colouration and are cost effective [2,4]. Approximately eight million tons of synthetic food colourants are produced yearly [1,2]. The safety concerns for humans have led to the significant considerations of these synthetic dyes because it has been reported that they can pose a risk to human health and have negative effects on the liver, kidney and nervous system [5,6]. They also elicit oxidative stress and pro-inflammatory effects [7,8]. The toxic effect of these azo dyes is thought to be attributed to the aromatic amines produced by their biotransformation by the intestinal microbiota [6,9]. Mutagenic and carcinogenic effects have also been documented of these metabolites of synthetic azo dyes [9,10]. Some of the synthetic azo dyes employed by various industries are tartrazine, erythrosine, carmoisine, fast green, and so on and they are all organic compounds made from coal-tar [6,9,11].

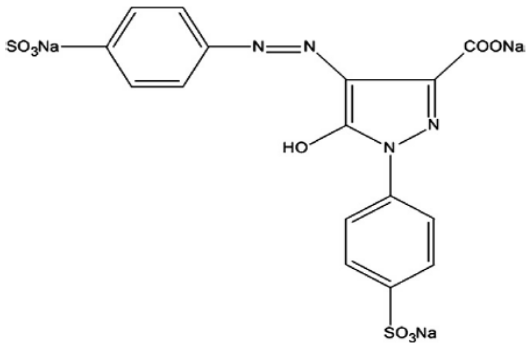

Fig. 1. Structure of Tartrazine Dye

Tartrazine is now predominantly used as colourant in many industries such as food, pharmaceutical, and cosmetic industries to produce yellow colours [1,2]. Some of the products that contain tartrazine are soft drinks, energy drinks, cereals, ice creams, some coloured rice, biscuits, chocolates, yoghurts vitamins, antacids, lotions, cold medications, prescription drugs among others [1,2]. Tartrazine has been reported to have cytotoxic, genotoxic, and mutagenic effects when consumed beyond the recommended daily allowance [9,10]. Also, it has been reported that tartrazine promotes inflammation by increasing the synthesis of leukotriene B4 and F2-isoprostanes thereby posing human health risk [8,9]. Just like other azo dyes, it has also been documented that the toxicity of tartrazine arises from the metabolic reductive biotransformation of the azo dye linkage in the intestine and liver producing reactive amines, aryl amines and other free radicals [12,13].

Due to the reported toxicity of tartrazine, the joint FAO/WHO expert committee established an acceptable daily intake (ADI) of 7.5mg/kg/body weight [13]. The metabolites of tartrazine such as sulfanilic acid and aminopyrazolone have also been shown to generate reactive oxygen species (ROS) leading to oxidative stress which have adverse pathophysiological alterations on several organs [6,14].

Cardiac function is essential for the efficient and effective distribution of blood and oxygen to cells of the body [15]. Therefore, any encumbrances of these processes can induce cardiovascular disease [15,16]. Cardiovascular disease (CVD)is a class of diseases that involve the heart and blood vessels [15,16]. One of the leading causes of morbidity and mortality especially in developing countries is CVD with hypertension and heart failure contributing the highest incidence [17]. Many factors are responsible for the onset of these disease, they include lifestyles, environmental factors, hereditary, and type of food consumed [16]. Since diet is one of the predisposing factors of CVDs, the knowledge of tartrazine food dye on the cardiac function is very important. The toxicity of tartrazine has also been linked to oxidative disturbances [6,9].

Oxidative stress is connected with elevated amount of free radicals in the body which possess the ability to cause cell membrane damage in organs such as those of the heart, renal, hepatic, blood vessels and so on [18]. Cellular membrane damages in oxidative condition ensues as these ROS interacts with macromolecules such as proteins, lipids, carbohydrates and DNA of cells thereby inducing alterations in their normal structural and functional physiology [18,19]. As reported by Leo et al. [18], pathological complications like dyslipidaemia, nephropathy, retinopathy, neuropathy, hepatopathy, and cardiomyopathy are as a result of oxidative induced damages. Most times, oxidative stress induced damages occurs when the amount of generated ROS in a biological system overwhelms the production of antioxidants or antioxidants activities [18,19]. Metabolites of tartrazine such as sulfanilic acid and aminopyrazolone have been documented to generate reactive oxygen species (ROS) leading to oxidative stress induced damages altering normal physiological functioning of associated organs [6,14].

Another area of concern when predicting cardiovascular disease risks is the use of atherogenic indices. Atherogenic indices are very vital in predicting CVD risk especially when

values of atherogenic lipid parameters are not affected or altered [20]. Atherogenic indices considered in this study include Atherogenic index of plasma (AIP), Non-High-density lipoprotein cholesterol (nHDL-C), Atherogenic coefficient (AC), Castelli risk index 1 (CRI-1) and Castelli risk index 2 (CRI-2). AIP is a very useful marker of CVD because it is relating to the size of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) particles and therefore serve as lipoprotein atherogenic indicator [21,22] while nHDL-C gives the cumulative fraction of the atherogenic lipoproteins that make up the total cholesterol [23]. As documented by Devadawson et al. [23], nHDL-C is derived by the removal of HDL-C fraction from total cholesterol (TC). Another atherogenic marker used to assess CVD is the AC. It is a measure of cholesterol in the LDL-C, very low-density lipoprotein cholesterol (VLDL-C) and intermediate density lipoprotein cholesterol (IDL-C) lipoprotein fractions in relation to HDL-C [24]. Therefore, as AC value increases, the risk of developing CVD increases and vice versa [21,24]. Brehm et al. [24], expressed AC mathematically as the ratio of nHDL-C to HDL-C where nHDL-C is the cholesterol fraction without HDL-C as stated above. In addition, CR1-1 and CRI-2 are also used as a predictor of CVD risks based on TG, LDL-C and HDL-C which are independent risk factors for CVD [25]. CRI-1 is expressed as the ratio ofTCtoHDL-CwhileCRI-2 is a molar ratio, expressed as the ratio of LDL-C to HDL-C [24]. As reported by Koleva et al. [25], CRIs are sensitive markers in predicting CVD [20].

In addition, the integrity of the heart or muscle of the heart can also be determined using cardiac markers such as isoenzymes of creatinekinase (CK-MB and to a very small extent CK-MM), cardiac troponin I (cTn-I) and cardiac troponin T (cTn-T) [26,27]. cTn-I and cTn-T have been reported to have increased specificity and sensitivity as indicators of myocardial disorders or cellular (cadiomyocyte) necrosis compared to creatinekinase MB (CK-MB) [26,27]. The troponins are mostly found in striated muscle (myofibrils) of cardiac and skeletal muscles [26]. Though troponin T and I are also increased in other diseases such as renal dysfunction in the absence of myocardial disease, they are more specific in the diagnosis of cardiac disturbance. Increase CK-MB as also been seen as an indicator of myocardial necrosis or disturbance. However, CK-MB has been seen to be less sensitive compared to troponin I and T [26,27]. sOther markers such as CK-MM and total creatineKinase (CK) are also used complementarily alongside CK-MB in the assessment of cardiac integrity. Tartrazine food dye has been reported to alter physiological and biochemical parameters as a result of its toxic effects. Therefore, the aim of this study is to assess the effect of chronic oral administration of tartrazine on the atherogenic indices, and markers of cardiac injury in albino rats.

2. MATERIALS AND METHODS

2.1 Materials

Polypropylene gavage tubes (Intech Laboratory Incorporated, USA), haier thermocool refrigerator (China), microplate reader stat-fax 4500 (Awareness incorporated, USA). shandon AS325 rotary microtome (Fisher Scientific, United Kingdom), digital Olympus microscope with camera (Olympus, Japan) and tartrazine (FiorioColori Spa, Italy). Other materials used include cTn-T, cTn-I, CK-MM, and CK-MB rat specific ELISA kits purchased from Bioassay Technology Laboratory (Shangai, China), while total CK, triglyceride, total cholesterol, and high density lipoprotein commercial kits were purchased from Bridge Biotech Limited, Kwara State, Nigeria.

2.2 Experimental Animals

A total of 63 albino rats weighing approximately 0.2 kg were used for this study. The rats were obtained from the Department of Medical Laboratory Science, Rivers State University, Port Harcourt and housed at the animal house of the same Department. The animals were kept in a well-ventilated environment at optimum temperature, 12 hrs light/dark cycle. They were left for 10 days to be well acclimatized before commencement of the study, and were allowed access to feed and water *ad libitum*.

2.3 Preparation of Tartrazine Food Dye

1.50 g of tartrazine was weighed and dissolved in a sterile container containing 1.0 litre of distilled water. This implies that, 1.0 ml of the tartrazine solution contains 0.0015 g which is equivalent to 7.5 mg/kg when administered into a 0.2 kg rat. The contents of the containers were properly mixed to ensure complete mixture before administration.

2.4 Experimental Design and Administration of Food Dyes

The oral method of treatment was used, and the food dye was administered using the gavage tube which ensured total consumption of the dye. A total number of 63 rats were used for the study. The study was divided into two phases, 1 and 2, which lasted for 30 and 60 days respectively. Phase 1 had 35 rats, 20 as test and 15 as control, while phase 2 had 28 rats, 16 as test and 12 as control. In each phase the test groups were given 7.5mg/kg of tartrazine orally on daily basis over the stipulated period while the control groups were not treated with tartrazine, but given feed 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.

2.6 Sample Collection, Preparation and Analysis

At the end of the study, the animals were allowed to fast for 18 hours and later anaesthetized with chloroform and 5mls of whole blood sample was collected from the jugular vein into lithium heparin during the sacrifice. The blood samples were spun at 3500 rpm for 10 minutes to obtain plasma aliquoted accordingly into labelled plain bottles. Determination of CK-MM, CK-MB, cTn-I and cTn-T were based on ELISA technique as described by Engvall [28]. These parameters were analysed using Stat-Fax 4500microplate reader. The assay of TG and TC were based on enzymatic methods as described by Flegg et al. [29] and Stavropoulous et al. [30] respectively. HDL-C was assayed by precipitating out VLDL-C and LDL-C using phosphotungstic acid and magnesium ions, and enzymatic evaluation of HDL-C in the supernatant as described by Flegg et al. [29]. LDL-C was calculated using the Friedwald equation: LDL-C (mmol/L) = TC - (TG/2.0 + HDLC) as described by Friedwald et al. [31]. Atherogenic indices such as AIP were calculated as Log (TG/HDL-C) as described by Dobiasova [22]. CRI-1 and CRI-2 were calculated as TC/HDL-C and LDL-C/ HDLC respectively as described by Koleva et al. [25], nHDL-C was calculated as TC – HDL-C as described by Devadawson et al. [23] while AC was calculated as (TC – HDL-C)/ HDL-C as described by Brehm et al. [24].

2.6.1 Histological preparation and examination

The heart tissues were collected from the sacrificed animals. The tissues were washed in normal saline and fixed in 10% formalin saline and later processed by passing through ascending grades of alcohol, cleared and embedded in paraffin wax. The processing was done using Leica automatic tissue processor (Leica Biosystems, USA), while rotary microtome was used to obtain 5µm thick sections. The sections were then stained using Haematoxylin and Eosin staining technique.

2.7 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.01 (San Diego, California, USA). Results were presented as Mean (X) ± Standard deviation (SD). Student's t-test was used to compare values of the treatment groups and the control groups. Statistical significance was set at $P<.05$.

3. RESULTS

3.1 Results of Lipid Parameters

The results of lipid parameters obtained from the laboratory analysis were used in calculating the atherogenic indices. The X ± SD lipid results obtained are shown in Table 1.

3.2 Results of Atherogenic Indices in Rats Treated with Tartrazine

The results of ahtherogenic indices over a period of 30 and 60 days showed significantly higher value in nHDL-C in rats treated with tartrazine as against their control group. However, there were non-significant increases in the value of AIP, AC, and CRI-1 and CRI-2 at $P=.05$ (Tables 2 and 3). In addition, when 30 days treated rats were compared with 60 days treated rats, no significant differences were seen in all the atherogenic indices considered at $P=.05$ (Table 4).

3.3 Results of Cardiac Markers in Rats Treated with Tartrazine

When cardiac markers were considered after 30 days, results obtained showednon-significant increase in the level of CK-MM, Total CK, cTnT and cTnI in rats treated with tartrazine while CK-MB indicated non-significant increases in the rats treated for 30days as against their control group

(Table 5). However, when cardiac markers were considered after 60 days, results obtained showed significantly higher values in the level of Total CK and cTn-T in rats treated with tartrazine compared to control rats (Table 6). In addition, the comparison of 30 and 60 days treated rats indicated significant increase in total CK in 60 days treated rats at *P*<.05 (Table 7).

Table 1. Results of lipid parameters in control rats and rats treated with tartrazine over a period of 30 and 60 days

Parameters	Control Rats (30 days, n=15)	Treated rats (30 days, n=20)	Control Rats (60 days, n=12)	Treated rats (60 days, n=16)
TC (mmol/L)	2.40±0.62	2.64±0.56	2.09±0.41	2.56±0.83
TG (mmol/L)	1.34±0.35	1.22±0.42	1.08±0.50	1.25±0.41
HDL-C (mmol/L)	1.61±0.47	1.28±0.62	1.00±0.35	0.97±0.56
LDL-C (mmol/L)	0.47±0.45	0.94±0.60	0.67±0.40	1.01±0.61

TC= Total cholesterol, TG= Triglycerides, HDL-C=High Density Lipoprotein-Cholesterol, LDL-C=Low Density Lipoprotein-Cholesterol, n= No of rats. Results were expressed as Mean ± SD

Table 2. Results of atherogenic indices in rats treated with tartrazine over a period of 30 days

Parameters	Control Rats (n=15)	Treated Rats (n=20)	P-value	t-value	Remark
nHDL (mmol/L)	0.79±0.68	1.36±0.34	0.023	2.382	S
AIP	-0.08±0.04	-0.02±0.01	0.329	0.991	NS
CRI-1	1.49±0.58	2.06±1.41	0.102	1.684	NS
CRI-2	0.29±0.16	0.73±0.63	0.107	1.656	NS
AC	0.49±0.38	1.04±1.41	0.102	1.684	NS

S= Significant, NS=Not significant at P<.05, nHDL=Non-High Density Lipoprotein, AIP, Atherogenic Index of Plasma, CRI-1= Castelli Risk Index 1, CRI-2= Castelli Risk Index 2, AC= Atherogenic Coefficient. n= No of Rats: Results were expressed as Mean ± SD

Table 3. Results of atherogenic indices in rats treated with tartrazine over a period of 60 days with their respective control

Parameters	Control Rats (n=12)	Treated Rats (n=16)	P-value	t-value	Remark
nHDL (mmol/L)	1.02±0.55	1.59±0.31	0.025	2.342	S
AIP	0.03±0.01	0.11±0.10	0.165	1.421	NS
CRI-1	2.09±0.79	2.48±1.73	0.156	1.590	NS
CRI-2	0.70±0.63	1.02±0.95	0.221	1.088	NS
AC	1.09±0.40	1.64±1.23	0.457	0.794	NS

S= Significant, NS=Not significant at P<.05, nHDL=Non-High Density Lipoprotein, AIP, AtherogenicIndex of Plasma, CRI-1= Castelli Risk Index 1, CRI-2= Castelli Risk Index 2, AC= Atherogenic Coefficient. n= No of Rats: Results were expressed as Mean ± SD

Table 4. Comparison of atherogenic indices in rats treated with tartrazine over a period of 30 and 60 days

Parameters	Treated Rats (30 days)	Treated Rats (60 days)	P-value	t-value	Remark
nHDL (mmol/L)	1.36±0.34	1.59±0.31	0.213	1.128	NS
AIP	-0.02±0.01	0.11±0.10	0.206	1.287	NS
CRI-1	2.06±1.41	2.48±1.73	0.101	1.565	NS
CRI-2	0.73±0.63	1.02±0.95	0.268	0.975	NS
AC	1.04±1.41	1.64±1.23	0.210	1.516	NS

NS=Not significant at P<.05, nHDL=Non-High Density Lipoprotein, AIP=Atherogenic Index of Plasma, CRI-1= Castelli Risk Index 1, CRI-2= Castelli Risk Index 2, AC= Atherogenic Coefficient. Results were expressed as Mean ± SD

Table 5. Results of cardiac markers in rats treated with tartrazine over a period of 30 days

Parameters	Control Rats (n=15)	Treated Rats (n=20)	Pvalue	Tvalue	Remark
CK-MM (ng/ml)	92.17±28.62	116.2±49.37	0.103	1.679	NS
CK-MB (ng/ml)	5.03±1.62	4.63±2.36	0.580	0.559	NS
TOTAL CK (U/L)	8.40±4.52	8.47±4.87	0.966	0.043	NS
cTn-I (pg/ml)	324.3±137.4	339.0±138.1	0.757	0.315	NS
cTn-T (pg/ml)	5.34±4.50	6.795±4.99	0.400	0.852	NS

NS=Not significant at P<.05, CK=CreatineKinase, CK-MM=CreatineKinase MM, CK-MB= CreatineKinase MB, cTn-I= Cardiac Troponin I, cTn-T=Cardiac Troponin T, n= No of Rats: Results were expressed as Mean±SD

Table 6. Results of cardiac markers in rats treated with tartrazineover a period of 60 days

Parameters	Control Rats (n=12)	Treated Rats (n=16)	Pvalue	Tvalue	Remark
CK-MM (ng/ml)	95.41±29.95	107.4±36.64	0.309	1.033	NS
CK-MB (ng/ml)	5.27±2.95	6.00±3.12	0.490	0.698	NS
TOTAL CK (U/L)	9.086±4.74	12.60±5.05	0.045	2.089	S
cTn-I (pg/ml)	340.2±151.0	389.7±125.3	0.297	1.060	NS
cTn-T (pg/ml)	5.49±4.57	8.89±3.66	0.020	2.438	S

S=Significant at P<.05, NS=Not significant at p<0.05, CK=Creatine Kinase, CK-MM=CreatineKinase MM, CK-MB= CreatineKinase MB, cTn-I= Cardiac Troponin I, cTn-T=Cardiac Troponin T, n= No of Rats: Results were expressed as Mean±SD

3.4 Histological Examination of the Cardiac Tissues

The histological findings of the cardiac tissues examined are shown in Fig. 2.

4. DISCUSSION

The aim of this work is to evaluate the effects of chronic oral administration of tartrazine at the ADI level on the cardiac parameters of albino rats using the atherogenic indices and the cardiac biomarkers Troponin I & T, Total CK, CK-MM, and CK-MB.

When atherogenic indices were considered, there was significantly higher value of nHDL-C in tartrazine treated rats against the control rats over a period of 30 and 60 days of treatment. However, non-significant increases were seen in AC, AIP, CRI-1 and CRI-2. The significantly elevated level of nHDL-C observed in this work is in line with the reports of Elekima & Ben-Chioma [32]. They reported that the oral administration of 1%, 1.5%, 2% and 2.5% of tartrazine induced elevated nHDL-C and other atherogenic indices over a period of 30 days. In a similar work, Amin et al. [7], reported significantly higher levels of TC, TG, and LDL-C which are atherogenic lipids while a significantly reduced level of HDL-C was observed when rats were treated with tartrazine over a period of 30 days. It could be further deduced that, increased atherogenic lipids and reduced HDL-C points in the direction of increased nHDL-C. The increase in atherogenic markers as seen in our work predicts cardiovascular risks induced by tartrazine. nHDL-C tells the overall fraction of the atherogenic lipoproteins that make up the total cholesterol thereby serving as a sensitive predictor of lipid atherogenicity. The increase in nHDL could be as a result of low activities of lipoproteins lipases such as lecithin cholesterol transferases which could be attributed to the degenerative impact of ROS generated in course of tartrazine metabolism. Tartrazine metabolites such as sulfanilic acid have also been seen to induce the production of ROS leading to the generation of oxidized-LDL which is also a risk factor of atherosclerosis. Lipoproteins lipases are responsible for the hydrolysis of circulating atherogenic lipoproteins like Chylomicron, VLDL, and LDL. Therefore, their poor activities are usually associated with increased TG as well as decreased HDL-C levels in the plasma promoting the formation of atherosclerotic plagues viz-a-viz CVD risks.

Table 7. Results of cardiac markers in rats treated with tartrazine over a period of 30 and 60 days

Parameters	Treated Rats 30 Days	Treated Rats 60 Days	Pvalue	Tvalue	Remark
CK-MM (ng/ml)	116.2±49.37	107.4±36.64	0.528	0.638	NS
CK-MB (ng/ml)	4.63±2.355	6.00±3.12	0.125	1.567	NS
TOTAL CK (U/L)	8.47±4.873	12.60±5.05	0.012	2.631	S
cTn-I (pg/ml)	339.0±138.1	389.7±125.3	0.231	1.217	NS
cTn-T (pg/ml)	6.80±4.99	8.89±3.66	0.139	1.508	NS

S=Significant at P<0.05, NS=Not significant at p<0.05, CK=CreatineKinase, CK-MM=CreatineKinase MM, CK-MB= CreatineKinase MB, cTn-I= Cardiac Troponin I, cTn-T=Cardiac Troponin T, n= No of Rats: Results were expressed as Mean±SD

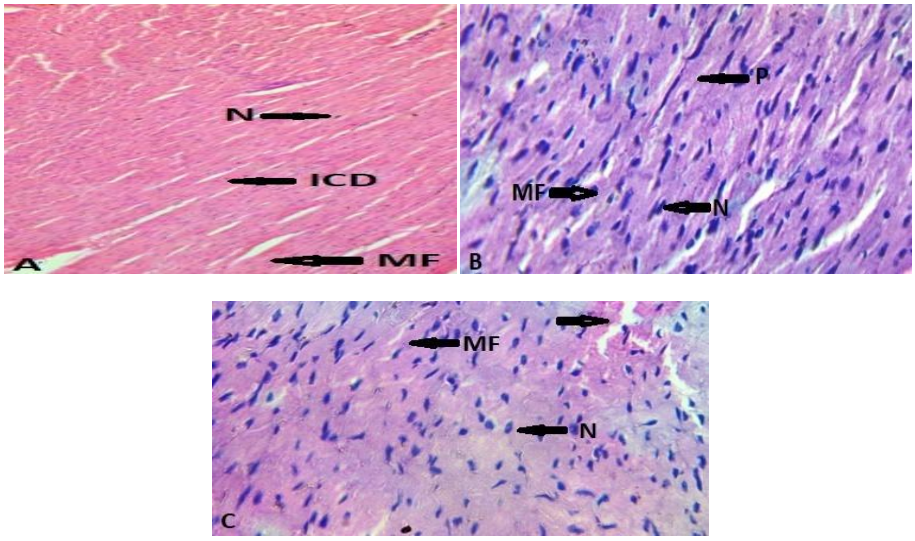

Fig. 2. A. Control 30 days.Cardiac tissue (myocardium) indicates the presence of normal cardiomyocyte with nucleus (N). The myofibrile (MF) are distinct and intercalated disc (IC). H&E stain. 200X. B. 30 days treatment: cardiac tissue (myocardium) showing normal cardiomyocytes and very distinct (N). The myofiber (MF) are distinct linked by the intercalated disc. Parenchymal materials (P) were seen to be intact. H&E X400. C. 60 days treatment. Cardiac tissue (myocardium) indicates hypertrophied nucleus (N) of cardiomyocytes. Fibrous connective tissues (MF) are distinct. Parenchymal materials were seen to be intact. H&E. X400

In addition, when markers of cardiac injury were considered, significantly higher values were observed in Total CK and cTnT in rats treated with tartrazine against controls over the period of 60 days. Again, significantly higher value in total CK was seen in 60 days treated rats against 30 days treated rats. Other cardiac markers such as CK-MB, cTn-I, and complementary marker like CK-MM were non-significantly elevated after 30 and 60 days of tartrazine treatment at a dose of 7.5 mg/kg bodyweight. The significant increase in total CK as seen in our results concur with the finding of Oyewole & Johnson [33]. They reported elevated CK and LDH1 level in rats treated with tartrazine at a dose of 250 mg/kg for 21 days. In a related study, Amin [34], also reported an increase in CK and LDH when carmoisine azo dye at doses of 5, 10 and 20 mg/kg were fed to rats orally for 30 days. However, our finding concerning CK-MB is contrary to the reports of Ahmad & Hussain [35]. Ahmad & Hussain [35], reported significant increase in CK-MB when rats were treated orally with 2, 4 6, and 8mg/kg of tartrazine for 60 days. The significant increase seen in total CK and cTn-T in conjunction with the non-significant increases seen CK-MB, CK-MM and cTn-I over the period of 30 and 60 days indicates cardiac derangements especially of myocardial origin. Though, total CK is a non-specific indicator of cardiac muscular dysfunction, but the increase in cTn-T is specific and points towards cardiac

injury or inflammation therefore validating the increase in total CK alongside the non-significant increases seen in CK-MB and CK-MM. cTn-T and cTn-I have been reported to have increased specificity and sensitivity as indicators of myocardial disorders relatively to CK-MB. The observed elevated cardiac markers are suggestive of cardiac injury induced by ROS generated by tartrazine.

In addition, the histological examination of the cardiac tissue after 30 days of treatment with tartrazine indicated normal cardiomyocytes and very distinct nuclei. The myofibers were distinct with intact parenchymal materials (Fig. 2B). However, after 60 days treatment, hypertrophied nuclei of the cardiomyocytes were observed with intact connective tissues and the parenchymal materials. In a related work, Oyewole & Johnson [33], also reported deformities in the sizes and shapes in the nuclei of cardiomyocytes of rats treated with tartrazine at 250 mg/kg for 21 days. The derangement reported by Oyewole & Johnson [33] is obviously more severe than what we recorded in our work because they further documented disarray of myofibers and connective tissue deposits. The hypertrophied nuclei are suggestive of gradual degeneration of the cardiac cells may be due to oxidative damages.

The biochemical and histological changes observed in this study could be as a result of oxidative damage to the cardiac tissues induced by tartrazine as tartrazine metabolism generates free radicals and increases oxidative stress. This is supported by the work of Uyota et al. [35], in which there was a significant increase in MDA as well as a non-significant decrease in SOD in rats treated with tartrazine over the period of 30 and 60 days. Amin et al. [7] and Sexena [36], in their respective works also reported significant increment and reduction in MDA and SOD respectively in rats orally treated with tartrazine. SODs are anti-oxidative enzymes involved in the removal or scavenging of free radicals and reactive oxygen species generated during metabolism. On the other hand, MDA is a produced as a result of peroxidation of lipids commonly associated with oxidative derangements. The maintenance of anti-oxidant activities in biological system is very critical. Loss of anti-oxidant activities or over-production of oxidative agents can induce several pathophysiological alterations. Therefore, the non-significantly reduced value of SOD is suggestive of increased utilisation of SOD in course of eradicating or neutralizing ROS generated during tartrazine metabolism. More so, the significantly elevated MDA levels are also suggestive of oxidative stress on lipids of cellular membranes.

5. CONCLUSION

Orally administered tartrazine over a 60 days period induced cardiac injury as shown by the significant increase in the cTn-T and total CK as well as hypertrophied nuclei of cardiomyocytes. This goes to say chronic administration of tartrazine even at the recommended daily dose could pose the risk of cardiovascular disease over a period of time. This is also supported by an increase in nHDL cholesterol.

6. RECOMMENDATION

It is gathered from this study that the daily consumption of tartrazine even at the accepted daily dose over a period of 60 days may constitute a cardiovascular risk. Therefore, it is advised that tartrazine food dye should be used with caution.

7. LIMITATION OF THE STUDY

The duration of the study was not more than 60 days. Moreover, our present findings were in rats and therefore these effects cannot be directly interpreted in humans. Therefore, our findings are subject to further research and verification.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

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. Experimental procedures concerning the use of animals were 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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