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Long-Term Tartrazine Azo dye (E102) Exposure Altered the Concentration of Blood Corpuscles and their Indices in Male Albino Rats

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Abstract

To evaluate the chronic impact of tartrazine (E102) on red blood cells, haemoglobin, haematocrit, white cells and differential white cells, platelets, platelet indices, red blood cells, and red blood cell indices in albino rats. Eighty (80) male albino rats of average body weight of 0.15 kg were used as experimental rats in the study. The 80 experimental rats were divided into control and treatment groups. The treatment group was further divided into phase 1: made up of 17 rats, phase 2: made up of 25 rats, and phase 3: made up of 17 rats. While the control group; made up of 21 rats. All groups were put in separate ventilated cages and allowed to acclimatize to the new environment. Freshly Prepared tartrazine (E102) solution (of industrial grade) of a single daily dose of 7.5mg/kg per body weight of rat was orally administered to each rat in phase 1; for 30 days, phase 2; for 60 days, and phase 3; for 90 days. The control group was not administered tartrazine during the experiment. The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria within a period of 12 months (April 2022– March 2023). At the end of treatment duration, five (5) milliliters of whole blood specimen from each rat was collected by means of cardiac puncture into properly labeled K_3EDTA bottles. Each specimen was mixed properly with a haematology mixer and then analyzed immediately using Mindray 5300 haematology autoanalyzer. Statistical analysis was performed using GraphPad Prism version 8.02 (San Diego, California, USA). Platelet showed significantly higher and lower values in 30 days and 90 days treatments respectively. The 60 days treatment indicated significantly higher values in MPV, PDW, MCHC, RDW-CV, and RDW-SD while MCH showed significantly lower value. In addition, the 90 days treatment indicated aberrations of PLTs, RBCs, HCT, HB, MCV, MCH and MCHC. PLT, MCV, and MCH showed significant decrease while RBCs, HB, HCT, and MCHC showed significantly higher values. However, PCT (%) remained undisturbed in all administered phases when compared with the control group at $p<0.05$. The results obtained indicate that daily consumption of tartrazine (E102) at ADI doses of 7.5mg/kg bw for periods over 90 days induced altered the plasma concentration of blood cells and their indices triggering immunological and bone marrow responses in albino rats.

Keywords: Tartrazine Azo dye, ADI dose, Platelet and Platelet Indices, Red Blood Cells and Indices, White Blood Cells, and Differentials.

Introduction

Coloured foods are attractive and presentable. Sometimes, harvested food crops from the farm lose their natural pigments, when these food crops are cooked or converted to food products, they may appear pale and not attractive (Elekima *et al.,* 2017). To enhance foods quality and attraction; food manufacturers apply certain colorants to certain foods. A typical example is the application of azo dyes as colorants in some cooked foods and food products like ice-cream, which is aimed at attracting the consumers, and also, the addition of Sudan III and IV to palm oil by local palm oil producers to influence the

buyer's choice in some parts of West Africa (Sampson *et al*., 2020).

Azo dyes, also known as azoic dyes, are synthetic dyes (Benkhaya *et al*., 2020) distinguished by the presence of an azo bond (-N = N-) between two or more aromatic rings (Pandey *et al.,* 2007; Saratale *et al*., 2011). Azo dyes have several applications in the food, pharmaceutical, textile, cosmetic, and leather industries (Hunger *et al*., 2000; Nikfar & Jaberidoost, 2014). Azo pigments are the oldest and most commonly used food colorants (Diacu, 2016). They were founded in 1858 by Peter Griess (Diacu, 2016). Although natural dyes are the safer and more environmentally friendly option, they are more expensive, time consuming to apply, and difficult to obtain (Cooksey & Tyrian, 2013). In place of natural dyes, which already generate a vivid color in trace amounts, synthetic dyes like azo dyes have taken over as the main colouring agents in pharmaceutical, food, cosmetics, and textiles industries (Singh & Singh, 2017).

Some azo dyes, such as pigment orange 1, 2, and 5, (dinitroaniline orange, ortho-nitroaniline orange, and nitroaniline orange) are mutagenic and carcinogenic (Eva *et al*., 2008). According to Golka *et al.* (2004), azo dyes made from benzidine are known carcinogens and have historically been linked to bladder cancer. Workers who worked in the dye industry were found to have higher bladder cancer rates as early as 1895. Numerous studies showing the toxicity of azo dyes have been carried out since then. According to Chavan (2011), the dye itself or one of its metabolites may be carcinogen. Intake of azo dyes may also raise the incidence of hepatocarcinomas, nuclear abnormalities, and splenic sarcomas in humans (Chung *et al.,* 1992). They can also result in dermatitis, allergies, and DNA damage, which can lead to the growth of cancerous tumors (Khan & Malik, 2014; Carmen & Daniela, 2012). As a result, the manufacture of benzidine azo dyes was stopped in many western countries in the 1980s (Hunger *et al*. 2000). Among the more well-known azo dyes used in the food industries include tartrazine, brilliant black BN, sunset yellow FCF, ponceau 4R, azorubine, Allura red AC, and amaranth (Mota *et al*., 2021).

Amongst food dyes, tartrazine (E102) is the most frequently used food dye in food products in the study region and the most consumed. When taken in excess or even at the authorized amount, tartrazine has been shown to cause or initiate a number of clinical derangements (Daffalla *et al*., 2015; Elekima *et al*., 2017; Elekima & Ben-Chioma, 2018). Tartrazine, an azo dye, is a synthetic food colorant frequently used in many meals and food products to improve some food products' look (Amin *et al.,* 2010, Elekima & Ben-Chioma, 2018). Like other azo dyes, tartrazine enters the gastrointestinal tract after oral ingestion, there, the intestinal microflora or mammalian azoreductases cleave the azo bond (Chung *et al*., 1992; Chen, 2006). Following hydroxylation or acetylation, the aromatic amines, which are frequently the byproduct of azo dye reduction, become more toxic (Ngo & Tischler). Not surprisingly, the toxicity of azo dyes has been studied extensively.

The xenobiotic nature of these dyes necessitates an accurate assessment of their negative consequences. The importance of blood to humans cannot be overstated; its role in health and disease is crucial (Christian *et al.,* 2018). Several toxic studies place more emphasis on the organs and enzymes of the body on exposure to xenobiotics than the blood and its indices. The major developments that have occurred in all fields of medicine over the last decade have been accompanied by a greater understanding of the biochemical, physiological, and immunological processes involved in normal blood cell formation and function, as well as the disturbances that may occur in various diseases (Hoffbrand and Moss, 2011). The study is centered on the long-term impact of tartrazine (E102) consumption on blood cells indices. Blood is made up of erythrocytes, leukocytes, and thrombocytes that are suspended in fluid called plasma (Glenn & Amstrong, 2019). In the distribution process of pharmacokinetics, the blood plays significant role in the transport of xenobiotics to the target organ, tissue or cell and transports the metabolites or waste products of the xenobiotics to the skin, lung, and kidneys for elimination. The blood is the most frequently exposed and contact tissue to all xenobiotics in the body and most times these xenobiotics or

their metabolites stay in the blood for a long time. It is very candid to evaluate the morphology, production, physiology, immunology, and disturbances of the blood frequently on exposure to all xenobiotics.

In clinical practice, Full Blood Count (FBC) is usually assayed to assess one's health (Osei-Bimpong *et al.,* 2012; Lugos *et al*., 2019). Blood component and their indices considered in this study include; red blood cells (RBCs), red blood cell indices [mean corpuscular volume (MCV), mean corpuscular heamologlobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width- CV (RDW-CV), and red cell distribution width-SD (RCDW-SD)], Platelet (PLT), and platelet indices [mean platelet volume (MPV), platelet distribution width (PDW) and PCT %].

Materials and Methods

Materials

Materials used in this study include; Albino rats, 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%, Ohaus Scout-Pro Electronic weigh balance (Ohaus Corporation, New Jersey, USA), K_3EDTA bottles, polypropylene gavage tubes (Intech Laboratory Incorporated, Plymouth Meeting, USA), Haier thermocool refrigerator (China), haematology mixer, Mindray BS5300 haematology, autoanalyser Other materials used include hypodermic syringe, automatic pipettes, and chloroform.

Experimental Animals

All rats used in the study were male albino rats; they were purchased from the University of Port Harcourt, Rivers State, Nigeria. The rats weighed approximately 0.15kg on average.

Preparation of Tartrazine treatment solutions

The tartrazine treatment solution was prepared by dissolving 1.33 g of tartrazine powder in 1 litre of sterile distilled water in a sterile container. This implies that 1.0 ml of the freshly prepared tartrazine solution contains 0.00113 g, which is equivalent to 7.5mg/kg when administered to a 0.15 kg rat.

Experimental Design and Administration of Tartrazine dye solutions

Eighty (80) male albino rats of average body weight of 0.15 kg were used as experimental rats in the chronic (toxicity) study. The 80 experimental rats were divided into control and treatment groups. The treatment group was further divided into phase 1: made up of 17 rats, phase 2: made up of 25 rats, and phase 3: made up of 17 rats while the control group contained 21 rats. The different groups of rats were put in separate well-ventilated cages. 1.0 ml of the freshly prepared tartrazine solution was administered orally by using gavage tube to ensure complete delivery of the dye solution. The contents of the containers were properly mixed to ensure complete mixture before administration.

Dose Administration and Treatment

From the prepared 1 liter tartrazine solution, 1.0 ml of a single daily dose of 7.5mg/kg (7.5mg of tartrazine per kg body weight of rat) was orally administered to each rat in the treatment phases as follow: phase 1: a single daily dose of 7.5mg/kg tartrazine for 30 days, phase 2; a single daily dose of 7.5mg/kg tartrazine for 60 days, and phase: a single daily dose of 7.5mg/kg tartrazine for 90 days while the control group was not administered tartrazine.

Study Area

The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt. However, whole blood samples collected in K_3EDTA bottles were immediately transported (45-minute drive) to the Haematology Unit, University of Port Harcourt Teaching Hospital where all the haematological parameters were analyzed using Mindray BS 5300 haematology autoanalyzer.

Specimen Collection, Preparation, and Analysis

At the end of treatments, all rats were anaesthetized with chloroform and five (5) milliliters of whole blood sample from each rat was collected by means of cardiac puncture into K_3EDTA bottles. At the haematology laboboratory, the whole blood samples were assayed quickly using Mindray BS5300 haematology auto-analyzer.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.02 (San Diego, California, USA). Results were presented as Mean±Standard deviation (SD). Inferential statistics using Students' statistical t-test was employed to compare the values of the treated rats and control rats. In addition, the One-Way ANOVA (Post Hoc: Tukey's multiple comparative tests) was also used to evaluate the influence of treatment duration over 90 days. Statistical significance was set at $P<0.05$.

Results

Results of Platelet and platelet indices of Rats treated with Tartrazine over a Period of 30, 60, and 90 days

The platelets and platelet indices of phase 1, phase 2, and phase 3, were compared with the control group at $p<0.05$, there was significant difference in the levels of platelet in phase 1 (30 days treated rats) and phase 3 (90 days treated rats). The phase 2 (60 days treated rats), showed significant difference in the mean platelet volume (MPV) and platelet distribution width (PDW). However, there was no significant difference in plaletetcrit (PCT) (%) across all groups (Table 1).

Results of Red Cells and Red Cell Indices of Rats treated with Tartrazine over a Period of 30, 60, and 90 days

The red blood cells and its indices levels of phase 1, phase 2, and phase 3, were compared with the control group at $p<0.05$, phase 3 (90 days treated rats) showed significant increase in the level of red blood cells (RBC) and the mean corpuscular volume (MCV). Phase 2 (60 days treated rats) and phase 3 (90 days treated rats), showed significant increase in mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC). Phase 2 (60 days treated rats) showed significant increase in the red cell distribution width-CV (RDW-CV) and red cell distribution width-SD (RDW-SD) (Table 2).

Haematological Parameters of Duration on Tratrazine Treated Male Rats (7.5mg/kg) Over a Period of 30, 60 and 90 Days

The haematological parameters considered all indicated significant differences when compared to control group as well as within the different phases. RBC, HB, and HCT indicated significantly higher values in the 90 days treatment except in HB were significantly higher values were seen in 60 and 90 days treated rats. Platelets also indicated significantly higher and lower values in 30 days and 90 days treatments respectively. However, 60 days treatment indicated no significant difference. WBC indicated significantly higher values in 90 days treatment compared to control and other phases of treatments. In the differential count, Neutrophils and Lymphocytes indicated significantly lower and higher values respectively in 30-, 60-, and 90-days treatment groups compared to control. No significant differences were seen within the treatment phases. In addition, Monocyte indicated significantly lower values in the treated groups except in the 90 days treatment. More so, Eosinophil and Basophils also showed significantly lower values in the 30, 60, and 90 days treated groups. However, significantly lowest values in Eosinophils were observed in 30- and 60-days treatments (Table 3).

Table 1: Platelet and platelet indices of Rats treated with tartrazine over a period of 30, 60, and 90 days

Parameters	Control (n=21)	Phase 1 (n=17)	Phase 2 (n=25)	Phase 3 (n=17)	F value	P value	Remark
PLT (x10^9/L)	557.8±28.8^{a}	806.4±223.9^{b}	555.7±270.6^{a}	271.4±170.6^{c}	11.59	<0.0001	S
MPV (fL)	7.07±0.67^{a}	6.97±0.42^{a}	7.92±0.92^{b}	6.85±0.56^{a}	7.813	0.0002	S
PDW	15.48±0.24^{a}	15.32±0.18^{a}	16.87±1.32^{b}	15.92±1.17^{a}	5.933	0.0016	S
PCT (%)	0.40±0.20^{a}	0.56±0.14^{a}	0.46±0.22^{a}	0.50±0.19^{a}	1.102	0.3578	NS

PostHoc: *Values within the same row with different superscripts (a, b, c) or (a,b) differ significantly ($p<0.05$) when phases were compared.* ***Keys:*** *NS= Not Significant, S = Significant, n= No of Rats, MPV=Mean Platelet Volume, PDW= Platelet Distribution Width, (PDW) and Plateletcrit (PCT).*

Table 2: Red Cells and Red Cell Indices of Rats treated with tartrazine over a period of 30, 60, and 90 days.

Parameters	Control n=21	Phase 1 n=17	Phase 2 n=25	Phase 3 n=17	F value	P value	Remark
RBC(x10^12/L)	6.58±0.88[a]	6.40±0.52[a]	6.42±1.20[a]	8.01±0.63[b]	11.76	<0.0001	S
MCV (fL)	63.90±2.32[a]	64.01±2.53[a]	67.69±10.61[a]	57.21±2.46[b]	7.872	0.0003	S
MCH (pg)	18.97±0.90[a]	18.90±0.85[a]	17.68±0.61[b]	17.91±0.75[b]	7.781	0.0003	S
MCHC (g/dl)	29.70±0.64[a]	29.51±0.83[a]	31.19±1.05[b]	31.32±0.67[b]	14.49	<0.0001	S
RDW-CV	0.17±0.01[a]	0.17±0.02[a]	0.23±0.09[b]	0.16±0.02[a]	6.193	0.0014	S
RDW-SD (fL)	45.17±2.31[a]	46.87±5.24[a]	63.96±28.75[b]	41.11±9.72[a]	4.250	0.0110	S

PostHoc: *Values within the same row with different superscripts (a, b) differ significantly (p□0.05) when phases were compared.*
Keys: *NS= Not Significant, S = Significant, n= No of Rats. RBC =Red Blood Cells, MCV=Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC =Mean Corpuscular Haemoglobin Concentration, RDW-CV= Red Cell Distribution Width-CV, and RDW-SD =Red Cell Distribution Width-SD.*

Table 3: Haematological Parameters of Duration on Tratrazine Treated Male Rat (7.5mg/kg) Over a Period of 30, 60 and 90 Days.

Parameters	Control (n=21)	Phase 1 n=17	Phase 2 n=25	Phase 3 n=17	p-value	F-value	Remark
HCT (%)	37.67±8.16[a]	35.49±9.46[a]	39.69±2.42[a]	42.04±9.23[b]	0.0340	3.592	S
HB (g/dl)	11.43±2.12[a]	10.73±2.74[a]	12.25±0.82[b]	12.58±2.63[b]	0.0247	3.959	S
RBC(x10^12/L)	6.58±0.88[a]	6.40±0.52[a]	6.42±1.20[a]	8.01±0.63[b]	0.0484	3.197	S
PLT (x10^9/L)	557.8±28.8[a]	806.4±223.9[b]	555.7±270.6[a]	271.4±170.6[c]	0.0137	4.027	S
WBC(x10^9/L)	7.91±9.86[a]	8.10±4.73[a]	8.40±3.72[a]	10.28±4.06[b]	0.0446	2.453	S
N (%)	34.61±9.27[a]	26.88±11.55[b]	26.0±10.30[b]	27.28±6.70[b]	0.0213	3.161	S
L (%)	52.27±13.94[a]	64.81±10.23[b]	66.38±9.31[b]	62.89±7.61[b]	0.0027	3.344	S
M (%)	7.81±3.31[a]	5.66±4.24[b]	4.41±2.62[b]	6.25±2.58[a]	0.0436	2.870	S
E (%)	4.38±3.44[a]	1.55±0.90[b]	2.84±1.35[b]	3.19±1.44[c]	0.0008	8.079	S
B (%)	0.56±0.36[a]	0.51±0.29[a]	0.38±0.22[b]	0.24±0.12[b]	0.0340	3.592	S

PostHoc: *Values within the same row with different superscripts (a, b) or (a, b, c) differ significantly ($p^{<}0.05$) when phases were compared.* ***Keys:*** *NS= Not Significant, S = Significant, n= No of Rats. HCT=Haematocrit, HB=Haemoglobin, RBC=Red Blood Cells, PLT=Platelet, WBC=White Blood Cells, N=Neutrophil, L=Lymphocyte, M=Monocyte, E=Eosinophil, and B=Basophil.*

Discussion

In this work, the impact of tartrazine (E102) on blood cells and blood cells indices in male albino rats were evaluated. Haemopoietic stem cells in the bone marrow produce platelets and red blood cells. Red blood cells mature through several processes before enucleating and entering the circulatory system, many of which are regulated by the hormone erythropoietin (Klinken, 2003). According to Osei-Bimpong *et al*. (2012) and Akinbami *et al*. (2013), red blood cell counts fluctuate with age, gender, and geographic location and are crucial in the diagnosis of anaemia and polycythemia. Our study recorded significant increase in red blood cells in the experimental rats orally administered with daily

dosed of 7.5mg/kg tartrazine b.w for 90 days, which in contrasts to Aboel-Zahab et al. (1997), Sharma *et al.* (2009), and Daffallah *et al.* (2015), who reported in their separate studies that tartrazine induced a significant decrease in RBCs counts in rats treated with tartrazine. However, our present study coincided with Mehedi *et al.* (2013), and Sobokta *et al.* (1997) who reported increased red blood cells when tartrazine was fed to rats. Erythrocytosis refers to a rise especially in the quantity of red blood cells in the blood, whereas polycythemia refers to any increase in haematocrit and/or haemoglobin. Absolute polycythemia can result from physiologic adjustments to one's surroundings, drugs, and/or other medical problems (Mithoowani *et al*., 2020). It can also result from genetic changes in the bone marrow (referred to as "primary polycythemia"). The viscosity of blood increases with polycythemia due to increased hematocrit and red cell mass, which impairs blood flow and raises the risk of clotting (thrombosis) (Wallach, 2007). Balcells (1998) hypothesized that dehydration may have contributed to the observed alterations. Genetic tests and laboratory investigations into serum erythropoeitin levels may be useful in determining the origin of polycythemia (McMullin *et al.,* 2005).

The regular red cell indices produced by automated blood analyzers include the red cell distribution width (RDW), which offers a quantitative assessment of the heterogeneity of red cells in the peripheral blood. Anisocytosis is the medical term for an increased RDW (red blood cells of different sizes) (Evans & Jehle, 1991). Instead of being used as a stand-alone conclusive test, the RDW may be most useful as guidance in the differential diagnosis of anaemia (Carlos *et al*., 1987). In our study, we found RDW-CV and RDW-SD to be significantly increased in the 60 days oral tartrazine administration when compared with the control, 30 days and 90 days treatments.

The significantly lower values of MCV and MCH over the period of 90 days treated rats could also indicate smaller sizes of red blood cells and lower concentration of haemoglobin in the red blood cells. This could also imply that the intrinsic mechanism of RBCs of carrying and transporting oxygen to cells is affected negatively. The higher values of MCHC observed in 60- and 90-days treatment groups could be due to the significantly reduced size of the red blood cells as seen with the MCV values. MCHC defines the concentration of haemoglobin or space occupied by haemoglobin relative to the red cell size. Therefore, a smaller red blood cell size without a corresponding change would result in higher MCHC as observed in our findings. More so, the higher MCHC could be a cell membrane damaging attempt on the red blood cells by the oxidizing species giving rise to heterogeneity of red cells in the plasma as reflected by the RDW results. The observed significant increase in MCHC values of the treated rats in our study contradicts the report done by Ramez *et al.* (2019), who observed no significant difference of MCHC in tartrazine treated rats when compared with the control.

On the other hand, regarding platelet count, our investigations recorded significant increase in the platelet count in tartrazine oral administered rats in the 30 days administration, which is in accordance with our previous studies (Elekima & Christian, 2019; Ramez *et al.*, 2019) and the study done by Golli *et al.* (2016), who also found significant increase in platelet count. However, as the duration of exposure was increased to 60 and 90 days, the platelet values begin to fall and fell significantly in the 90 days treated groups. The initial increase as seen in 30 days treatment group could be due to platelet response in repairing micro-tears at cellular levels due to oxidative induced damages. According to Perkins (2009), platelets play a major role in preserving the haemostasis and structural integrity of blood vessels. Platelets are also used to evaluate a person's susceptibility to pathological and chemical alterations (Lugos *et al*., 2019; Perkins, 2009; Bloom & Brandt, 2010). However, their decline especially in the 90 days treated group could be due to overwhelming presence of oxidizing or reactive species inducing cellular toxicity and damages. There is a correlation between the quantity of megakaryocytes in bone marrow and the number of circulating platelets, and it is widely known that megakaryocytes are the source of circulating platelets (Long, 1998; Deutsch & Tomer, 2006).

Additionally, Sulani & Tefferi (2012) hypothesized that conditions like an infection, inflammation, iron deficiency, or stress may be reactive or secondary to the increase in platelet counts. However, in an overview consideration of platelet count from 30, 60, and 90 days, there was a significantly decreasing trend from 30 days treatment through 60 days to 90 days. The increase observed in 30 days treatment could be initial response, then normalized in 60 days treatment and then decreased in the 90 days treatment which agrees with the report of Himri *et al*. (2011). The decrease observed in the 90 days treatment could be due to continuous platelet depletion following persistent oxidative-induced cellular damages associated with tartrazine metabolism.

MPV and PDW were observed to be significantly higher values in the 60 days tartazine-treated groups compared control group and other treatment groups (30 and 90 days). Our findings agree with Himri *et al*. (2011), who reported significantly lowered platelet count but disagree with increased mean platelet volume (MPV) over 90 days exposure. The higher values of MPV and PDW observed in our study could be a response from the bone marrow (megakaryocytes) in producing new platelets (young platelets are usually larger in size than older ones) into the circulation due to the gradual platelet depletion from the circulation. More so, PDW has been indicated to be increased in a biological system with chronic illness, disturbances, or persistent presence of injurious agents such as reactive oxidative species. The PDW predicts platelet variability in size. The higher PDW values further suggest that there could be presence of micro-tears of the tissues, thus indicating platelet activation required for vascular repairs. In our study, plateletcrit (PCT (%) was observed to be not significant across the treatment periods when compared with the control and within phases of treatment periods. However, there is no report seen from other authors on the influence of tartrazine on PCT (%) in comparison to our findings.

Other haematological parameters observed in the study include haemoglobin, which showed significant increase in the 60 days and 90 days administrations which is in concordance with the study of Sobokta *et al.* (1997) but disagrees with the study of Sharma et al. (2009) who reported significant reduction of haemoglobin. Haematocrit (HCT %) was observed to be significantly raised in the 90 days administration compared to the control, 30 days and 60 days administrations. However, Mehedi *et al.* (2013) also reported an increased HCT % in their study, which our findings align with. However, out study took longer time (90 days) to observe raised HCT %. Our HCT % observation contradicts the significant reduction of HCT % in Sharma et al. (2009), study. The significantly lower and higher value of Neutrophil and Lymphocytes respectively in the treated groups could indicate cytotoxic effect of the dye on Neutrophil due to oxidative-induced damages and immunological response on the part of lymphocytes. Monocytes, Eosinophil, and Basophil also indicated similar significant reduction in the treated groups as observed in Neutrophil. The fall in these cell lines in the treated groups suggest cytotoxic impact of tatrazine on blood cells over a period 90 days consistent use.

The significant increase observed in WBC could reflect the significant increases observed n lymphocyte over-compensating the cell line losses observed seen in Neutrophil, Monocyte, Eosinophil, and Basophil. The increases observed in WBC and specifically in lymphocyte further suggest that azo dyes like tartrazine can trigger immunological response over a period following its metabolism in the biological system releasing reactive species culpable in inducing cellular damages.

Conclusion

The results obtained indicate that daily consumption of tartrazine (E102) at the recommended dose of 7.5mg/kg bw for a period over 90 days could induce alterations in the concentration blood corpuscles and their indices in male albino rats.

Recommendation

It is advised that high doses of tartrazine in foods or food products should be monitored and avoided. Because of the mild aberrations

observed in the chronic study, it is also advised that duration far above 90 days and higher dose should be considered in further studies.

Limitation of the Study

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.

Consent

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. 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.

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Competing Interests

Authors have declared that no competing interests exist.

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