Original Research Article

BIOCHEMICAL AND HISTOLOGICAL CHANGES ASSOCIATED WITH AZO FOOD DYE (TARTRAZINE) IN MALE ALBINO RATS

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ABSTRACT

Aim: to study the effect of chronic exposure of tartarzine at ADI doses on some biochemical parameters of male albino rats.

Study Design: The design involved chronic study. In the study, the experiment was divided into phase 1, 2, and 3 which lasted for 30, 60 and 90 days respectively. In each phase, 80 rats were used and were divided into treatment and control groups. The treated groups were given 7.5mg/kg of tartrazine orally on daily basis over the stipulated periods while the control groups were not treated with tartrazine.

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 (December 2017 – December 2018).

Methodology: At the end of the chronic study, 5mls of whole blood specimens was collected by means of cardiac puncture into Lithium Heparin bottles and fluoride oxalate bottles (for glucose only). The collected specimens were spun, plasma collected and analyzed for glucose, Lipase, AST, ALT, ALP, total protein, albumin and globulin. Renal, hepatic, and pancreatic tissues collected were fixed in 10% formol saline and later examined histologically using H&E stain. Statistical analysis was performed using GraphPad Prism version 5.03 (San Diego, California, USA).

Results: In the chronic treatment, glucose indicated significant increases after 30, 60, and 90 days of chronic treatment at ADI doses. Urea, AST, and ALT showed significantly higher values after 60 of treatment while creatinine, ALP, total protein, albumin and globulin indicated significantly higher values after 90 days of treatment. However, lipase did not show any significant difference after 30, 60, and 90 days of treatment. Histologically, hepatic distortions such as fatty degeneration, vacuolation, pcynosis, and compression of central vein were seen in the liver section. In the renal section, hyaline cast in proximal tubules, hypercellularity of messengial cells, and inflammation of the glomerulus were observed in the treated rats while the histology of the pancreas indicated mild vacuolation of the islet region. However, the pancreatic ducts and acinar cells were not distorted.

Conclusion: The administration of tartrazine over a period of 30 days at ADI dose did not indicate hepatocellullar and renal derangements as well histological distortions in liver, pancreas and kidneys. However, after 60 and 90 days, mild hepatocellular, pancreatic, and renal derangements were seen.

Keywords: Tartrazine, Lipase, Pancreas, Liver function, Renal function, protein and globulins

1. INTRODUCTION

Colours are vital components of foods and food products which gives the first impression on the mind of the consumer [1][2]. Food dyes are substances when added to foods or food products, they change, maintain or improve on the colour of the foods or food products by

covalently binding to the food particles [3]. They are vital in food industries in order to make food look more attractive and appetizing, providing identity and for artistic or decoration purposes as seen in cakes [4]. Because of the unstable state of natural food dyes, synthetic dyes are preferred in food processing and storage [2]. Tartrazine, erythrosine, fast green, carmoisine, etc are synthetic dyes and are mainly organic compound (azo dyes) in origin [5]. The applications of dyes are also seen in textile, leather, paper, rubber, cosmetics and pharmaceutical industries [6].

Tratrazine (E102) is widely used in food, pharmaceutical, and cosmetic industries to produce yellow colours [7]. They are present in edibles such as soft drinks, energy drinks, cereals, ice creams, some coloured rice, biscuits, chocolates, yoghurts and so on [2][7]. Consumption of tartrazine in food products have been reported to have induce asphyxia, insomnia, depression, anxiety, migraines, itching, weakness and blurred vision [8][9]. Tartrazine is also known as FD & C yellow no 5 with IUPAC name of trisodium 5-hydroxy-1-(4-sulfonato phenyl)-4-(4-sulfonato phenylazo)-H-pyrazol-3-carboxylate.

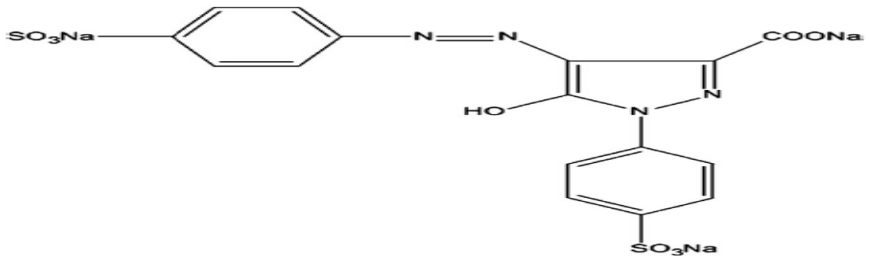

Figure 1: Structure of Tartrazine Dye

The Acceptable Daily Intake (ADI) of tartrazine is 0 - 7.5mg/kg [5][7]. The Food and Agricultural Organization (FAO) and World Health Organization (WHO), because of the toxicity of synthetic food dyes, have been put in place laws and regulations for the approval and regulation of the use of synthetic food dyes [8][10]. Reviews of literature reveals that tartrazine as a synthetic food dyes originate from coal tar which is toxic and carcinogenic [9]. The toxicity of tartrazine has been linked to the reductive biotransformation of the azo bond during their metabolism in the intestine and liver producing reactive amines, aryl amines and free radicals [11]. However, the extent to which a particular product is hazardous is assessed by the dose, duration of exposure, age, sex, body weight and race as well as interaction with other product [12].

The exposure of food dyes cuts across almost everyone due to their diverse application in various industries and their toxic effect even when consumed at the recommended acceptable daily intake (ADI) is still scientifically unclear or controversial. For example, though the ADI for New Coccin or Ponceua 4R (CAS NO: 2611-82-7) is 0–4.0mg/kg but its usage in the USA is not approved because few scientific studies have shown that it causes DNA damage of gastrointestinal mucosal cells in rodents [9][13]. However, such dyes (e.g. Ponceau 4R) are still used in developing countries like Nigeria in the production of sausage roll food products. Therefore, the purpose of this research is to evaluate the chronic effect of tartrazine dye on the liver, pancreas, and kidneys as well as its effects on the biochemical components of these organs of albino rats.

The liver detoxifies substance ingested into the body and the detoxification process may produce reactive intermediate metabolites that can attack macromolecules leading to direct toxicity and hypersensitivity [14][15][16]. Hepatocellular damages and alteration of the liver architecture occur when this mechanism of conjugating metabolites by glutathione is saturated or where the rate of toxic metabolites produced exceeds the bioavailability of

glutathione [14]. Liver enzymes like alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are sensitive indicators of hepatocellular damages. ALT and AST play vital roles in metabolism of amino acid particularly, in their synthesis and degradation in a reversible reaction called transamination [7]. AST catalysis the transfer of amino group from glutamate to oxoglutarate to from oxaloacetate and aspartate while ALT catalyses the transfer of amino acid from glutamate to oxoglutarate to form pyruvate and alanine [17]. ALT is more hepatocellular specific than AST and an increase in ALT is seen in acute hepatocellular damage than AST [17]. AST tends to increase in chronic hepatocellular damage compared to ALT due to their presence in cytoplasmic and mitochondrial component of the cells [7][17]. In a study carried out by Al-Shinnawy & Elkathan [18], Al-Shinnawy & Elkathan [18], stated that tartrazine administered in rats caused a significant increase in hepatic AST and ALT enzymes in the plasma when rats were fed with 10mg/kg of tartrazine for 30 days. In addition, ALP is a hydrolase enzyme that catalyses the release of inorganic phosphates from phosphate-ester substrates [17]. It is present in all body tissues mostly in bones, liver, placenta, erythrocytes and renal tubules [7]. Higher values of ALP are seen in infants due to increased bone activities and in third trimester of pregnancy but in adults, ALP mostly originates from the cells of the liver [7]. Increase in ALP is seen in cholestatic hepatic (obstructive) disorder, metastatic malignancy and chronic viral hepatitis [17]. In a study carried out by Amin et al. [7], it was reported that ALP levels were increased in rats when treated with 15mg/kg of tartrazine for 30 days.

The integrity of the kidney is very essential in maintaining of body homeostasis, removal of metabolic wastes,regulation of intracellular and extracellular fluid, synthesis and release of renin and erythropoietin hormones, electrolyte balance, as well as acid-base balance [19]. The kidney receives blood supply from the renal artery and when toxicant is the delivered to the kidney through the blood most times the functional integrity of the kidney is impaired [20]. In assessing the renal functional integrity, biochemical parameters such as urea and creatinine are used [19]. Creatinine is nitrogen containing by-product formed by the actions of creatine-kinase on creatine which is synthesized in the liver from arginine, glycine and methionine [21]. Diet and state of hydration or dehydration does not influence creatinine much compared to urea. An elevated level of plasma creatinine is usually associated with renal dysfunction [21]. Tartrazine at a dose above ADI have been reported to induce renal dysfunction in rats even though there are still contradictory scientific review reports. Studies by Amin et al. [7]; Mehedi et al. [22], demonstrated that tartrazine when fed to albino rats induced increased level of serum creatinine. More so, the measurement of plasma urea level in conjunction with plasma creatinine is essential clinically in defining the state of the kidneys [19]. Measurement of plasma urea alone is not very reliable in defining the glomerular filtration rate (GFR) due to certain factors such as high protein diet, increase protein breakdown (e.g. burns), muscle wasting (e.g. starvation), haemorrhage, state of hydration or dehydration and some chronic hepatic disorders [19]. Increased urea level is seen in primary and secondary renal failure as well as renal obstruction (post-renal disorder) and malignancies [19]. Studies by Amin et al. [7]; Mehedi et al. [22], demonstrated that tartrazine when fed to albino rats induced increased level of urea.

Glucose is the simplest form of carbohydrate that acts as a major source of energy to cells and tissues through the Kreb's cycle [23]. Maintenance of plasma glucose concentration within a relatively narrow interval is essential to avoid metabolic disorders such as hyperglycaemia or hypoglycaemia [23]. Insulin is the most vital hormone maintaining glucose level in the plasma. Therefore, pancreatic injury or insult directly or indirectly affects insulin production and release from the islet of Langerhans which in turn affects the maintenance of plasma glucose concentration. Several etiologic agents such as drugs, chemical, viruses, trauma, etc can induce pancreatic insufficiency that may affect its endocrine functions [23]. As reported by Sharma et al. [24], tartrazine when fed orally to albino rats induced

hypoglycaemia. However, Amin et al. [7], reported a significant increase in glucose concentration when tartrazine is administered at low and high doses in male albino rats for 30 days.

2. MATERIAL AND METHODS

2.1 Materials

Materials used in this research include Polypropylene gavage tubes (Intech Laboratory Incorporated, Plymouth Meeting, USA), Haier thermocool refrigerator (China), MPW bucket centrifuge Model 351 (MPW Medical Instruments, Warsaw, Poland), Ohaus Scout-Pro Electronic weigh balance (Ohaus Corporation, New Jersey, USA),Albino rats, Vis spectrophotometer (Axiom Medical Limited, United Kingdom), 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% guaranteed by the manufacturer. Glucose, Urea, Creatinine, Total Protein, Albumin, Lipase, ALT, ALP and AST kits were purchased from Atlas Medicals (Cowley Road, Cambridge, United Kingdom) except ALP reagent that was purchased from Teco Diagnostics. Other materials used include automatic pipettes and glass test-tubes.

2.2 Experimental Animals

Male and female albino rats used for the study weighed 150gm approximately. The reason for selecting male rats for the study was based on the fact that we wish to avoid the influence of pregnancy in the study. All the rats used for the experiment were obtained by breeding. However, the parent rats used for the breeding were purchased from the University of Port Harcourt, River State, Nigeria. The rats were fed with rat pre-mix rat feed and water *ad libitum*. The animals were placed in a well-ventilated rat cages with water cans and feed containers in place.

2.3 Preparation of Tartrazine Food Dye

In the chronic study, 1.13 grams of tartrazine was weighed and dissolved in 1.0 litre of distilled water. This means that, 1.0ml of the tartrazine solution contains 0.00113grams, which is equivalent to 7.5mg/kg when given to 0.15kg rat.

2.4 Experimental Design and Administration of Food Dyes

The method of treatment involved oral techniques. In the oral method, the food dyes were administered using orogastric tube to ensure complete delivery of the dye.

2.4.2 Chronic Treatment and Toxicity Study

In the study, the experiment was divided into three phases depending on the duration of exposure of the rats to tartrazine dyes. The phase 1, 2 and 3 of the chronic toxicity studies lasted for a duration of 30, 60 and 90 days respectively. Forty (40) experimental rats were used in each phase of the study (with a total of 116 male rats of which 4 died in the course of the experiment). In each phase of the experiment, the rats were divided into two groups designated T_T (tartrazine treated group), and C (control, untreated group). Rats in each of these groups were further distributed randomly into ten cages with four rats per cage, designated $T_{T1,}$ $T_{T2...}T_{10}$. In the treatment pattern, 7.5mg/kg of tartrazine was administered orally. The control group, were not treated with tartrazine. At the end of the chronic study, the animals were anaesthetized with chloroform and pancreas, kidney and liver organs were

harvested for histologic examination while blood samples collected by means of cardiac puncture for biochemical investigations.

2.5 Study Area

The study was carried out and samples analyzed in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt while the histological examinations of the selected organs was carried out in the anatomical laboratory, College of Medical Science, University of Port Harcourt.

2.6 Specimen Collection, Preparation and Analysis

At the end of the study, the animals were anaesthetized with chloroform and 5mls of blood samples was collected by means of cardiac puncture into lithium heparin bottle for all biochemical parameters except glucose sample that was collected into fluoride oxalate bottle. The blood specimens were spun at 4500 rpm for 10 minutes to obtain plasma which was transferred into other sets of labeled plain bottles and stored at -4^0C. The laboratory analysis of ALP was determined using spectrophotometer as described by Kind and King [25]. Plasma ALT and AST were also measured with spectrophotometer as described by Reitman & Frankel [26]. Plasma Urea was estimated using Berthelot's enzymatic method as described Patton & Crouch [27]. Creatinine was determined as described by kinetic colorimetric-Kinetic method as described Vaishya *et al*. [28]. Plasma glucose was determined by oxidase enzymatic method as described Trinder, [29]. Plasma total protein was determined using biuret reaction as described Henry, [30]. Lipase concentration was also determined kinetic colorimetric method as described by Panteghini et al., [31]. Albumin was estimated using the bromo-cresol green dye binding method Speicher et al. [32]. Globulin concentration was calculated by subtracting albumin concentration from total protein concentration as described by Busher, [33].

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. In addition, the One-Way ANOVA (Post Hoc: Tukey's multiple comparative tests) was also used to analyze the influence of treatment duration. Statistical significance was set at P=.05.

3. RESULTS AND DISCUSSION

3.1 Biochemical Parameters of Male Rats Chronically Treated with Tartrazine

When male control and male treated rats were considered over a treatment period of 30 days, the comparison showed significant increase only in Glucose concentration at P=.05 (table 1a). Also, after 60 days of tartrazine treatment, Glucose Urea, AST, ALT and ALP showed significantly higher value in treated male rats compared with male control rats at P=.05 (table 1b). More so, when 90 days treatment was considered, Glucose, Urea, CRT, AST, ALT, Total protein, ALB, globulin and ALP also indicated significantly higher values in treated male rats compared with control male rats (table 1c).

Table 1a: Biochemical Parameters of Male Rats Chronically Treated with Tartrazine Over a period of 30 Days

Parameters	Control Rats n=18	Treated Rats n=17	P value	T value	Remark
GLU (mmol/l)	2.18±0.90	3.09±1.54	0.0383	2.158	S
UREA (mmol/l)	4.09±0.23	4.21±0.421	0.3547	0.939	NS
CRT (µmol/l)	145.6±45.76	162.9±55.24	0.3196	1.011	NS
AST (U/L)	50.33±32.51	51.41±24. 07	0.9123	0.111	NS
ALT (U/L)	25.41±13.43	21.21±10.19	0.3064	1.039	NS
ALP (U/L)	27.36±14.92	26.83±15.09	0.9162	0.106	NS
Lipase (U/L)	121.3±76.63	175.7±85.78	0.0777	1.831	NS
T. Protein (g/dl)	4.68±1.29	5.11±1.43	0.3898	0.873	NS
Albumin (g/dl)	2.22±0.46	2.44±0.48	0.2125	1.276	NS
Globulin (g/dl)	2.46±1.55	2.68±1.37	0.6979	0.406	NS

n= no of Rats, NS= Not Significant, S= Significant

Table 1b: Biochemical Parameters of Male Rats Chronically Treated with Tartrazine Over a Period of 60 Days

Parameters	Control Rats n=20	Treated Rats n=25	P value	T value	Remark
GLU (mmol/l)	2.62±0.81	3.53±1.02	0.0019	3.309	S
UREA (mmol/l)	4.0±0.41	4.56±0.40	<0.0001	4.544	S
CRT(µmol/l)	174.6±152.1	180.5±45.93	0.8537	0.186	NS
AST (U/L)	33.22±19.47	51.28±18.76	0.0029	3.156	S
ALT (U/L)	17.26±5.78	21.81±3.86	0.0029	3.158	S
ALP (U/L)	22.48±7.71	28.25±7.95	0.0184	2.451	NS
Lipase (U/L)	121.3±76.63	157.9±53.19	0.1396	1.521	NS
T. Protein (g/dl)	4.68±1.29	5.24±1.05	0.2036	1.302	NS
Albumin (g/dl)	2.22±0.46	2.57±0.69	0.1109	1.646	NS
Globulin (g/dl)	2.46±1.55	2.66±1.09	0.6787	0.419	NS

n= no of Rats, NS= Not Significant, S= Significant

Table 1c: Biochemical Parameters of Male Rats Chronically Treated with Tartrazine Over a Period of 90 Days

Parameters	Control Rats n=19	Treated Rats n=17	P value	T value	Remark
GLU (mmol/l)	2.94±1.39	6.81±3.13	<0.0001	4.886	S
UREA (mmol/l)	4.0±0.51	5.61±1.18	<0.0001	5.417	S
CRT (µmol/l)	137.2±100.9	205.9±81.25	0.0323	2.232	S
AST (U/L	32.0±15.03	66.75±22.13	<0.0001	5.564	S
ALT (U/L)	16.58±6.25	35.96±12.52	<0.0001	5.971	S
ALP (U/L)	21.04±8.48	42.75±17.88	<0.0001	4.736	S
Lipase (U/L)	121.3±76.63	142.5±55.08	0.3917	0.870	NS
T. Protein (g/dl)	4.68±1.29	6.63±1.09	0.0001	4.483	S
Albumin (g/dl)	2.22±0.46	2.75±0.88	0.0458	2.091	S
Globulin (g/dl)	2.46±1.55	3.88±1.43	0.0143	2.612	S

n= no of Rats, S= Significant

3.2 Weights of Organs Extracted from Male Rats Chronically Treated with Tartrazine Over a Period of 30 Days

When the comparison of male treated rats and control rats were considered, no significant differences were observed in the weight of organs over the period of 30, 60 and 90 days at $p<0.05$ (table 2a, 2b and 2c).

Table 2a: Weights of Organs Extracted from Male Rats Chronically Treated with Tartrazine Over a Period of 30 Days

Parameters	Control Rats n= 18	Treated Rats n=17	P value	T value	Remark
Kidneys (gm)	1.25±0.31	1.17±0.34	0.4666	0.7366	NS
Liver (gm)	5.99±1.36	5.45±1.26	0.2254	1.2350	NS
Pancreas (gm)	0.49±0.11	0.47±0.12	0.7286	0.3506	NS

n= no of Rats, NS= Not Significant

Table 2b: Weights of Organs Extracted from Male Rats Chronically Treated with Tartrazine Over a Period of 60 Days

Parameters	Control Rats n=20	Treated rats n=25	P value	T value	Remark
Kidney (gm)	1.19±0.22	1.05±0.26	0.0615	1.920	NS
Liver (gm)	5.13±1.00	4.75±1.08	0.2272	1.225	NS
Pancreas (gm)	0.49±0.11	0.42±0.14	0.1588	1.448	NS

n= no of Rats, NS= Not Significant.

Table 2c: Weight of Organs Extracted from Male Rats Chronically Treated with Tartrazine Over a Period of 90 Days

Parameters	Control Rats n=19	Treated Rats n=17	pvalue	tvalue	Remark
Kidney (gm)	1.21±0.28	1.07±0.30	0.1585	1.442	NS
Liver (gm)	5.41±0.83	5.05±0.93	0.23	1.232	NS
Pancreas (gm)	0.49±0.11	0.43±0.15	0.2431	1.192	NS

n= no of Rats, NS= Not Significant

3.3 Biochemical Parameters of Duration on Chronically Treated Rats with Tartrazine Over a Period of 30, 60 and 90 Days

Table 3a showed biochemical parameters for 30 (phase 1), 60 (phase 2) and 90 days (phase 3) tartrazine treated male rats. The ANOVA results indicated significantly higher values in GLU, UREA, ALT and ALP in tartrazine treated male rats from phase 1 to phase 3 at $p<0.05$. When the various phases were compared using multiple turkey comparison test, the significantly higher values were seen between phase 1 and 3 as well as phase 2 and 3.

Table 3a: ANOVA of Biochemical Parameters on Duration of Tartrazine Treated Rats Over a Period of 30, 60 and 90 Days

Parameters	Phase 1 n=17	Phase 2 n=25	Phase 3 n=17	P value	F value	Remark
GLU(mmol/l)	3.09±1.54[a]	3.53±1.02[a,c]	6.81±3.13[b,d]	<0.0001	18.79	S
UREA(mmol/l)	4.21±0.421[a]	4.56±0.40[a,c]	5.61±1.18[b,d]	<0.0001	17.43	S
CRT(µmol/l)	162.9±55.24[a]	180.5±45.93[a,b]	205.9±81.25[a,b]	0.1234	2.173	NS
AST (U/L)	51.41±24.07[a]	51.28±18.76[a,b]	66.75±22.13[a,b]	0.0504	3.153	NS
ALT (U/L)	21.21±10.19[a]	21.81±3.86[a,c]	35.96±12.52[b,d]	<0.0001	15.53	S
ALP (U/L)	26.83±15.09[a]	28.25±7.95[a,c]	42.75±17.88[b,d]	0.0012	7.558	S
Lipase (U/L)	175.7±85.78[a]	157.9±53.19[a,b]	142.5±55.88[a,b]	0.1271	0.938	NS
T. Protein (g/dl)	5.11±1.43[a]	5.24±1.05[a,c]	6.63±1.09[b,d]	0.0018	7.400	S
Albumin (g/dl)	2.44±0.48[a]	2.57±0.69[ac]	2.75±0.88[bc]	0.4738	0.760	NS
Globulin (g/dl)	2.68±1.37[a]	2.66±1.09[a,c]	3.88±1.43[b,d]	0.0196	4.336	S

Values in the same row with different superscript letter (a, b) differ significantly ($p<0.05$) when comparing phase 1 with other phases. Values in the same row with different superscript letter (c, d) differ significantly ($p<0.05$) when comparing phase 2 with other phases. NS= Not Significant, S = Significant, n= No of Rats.

3.4 Weights of Organs Extracted from Rats Chronically Treated with Tartrazine Over a Period of 30, 60 and 90 Days

Table 4.52a showed weights of organs extracted from male rats treated with tartrazine for 30, 60 and 90 days. The ANOVA results obtained indicated no significant differences in the weight of the kidney, liver and testis from phase 1 to 3 at $p<0.05$.

Table 4.52a: ANOVA on Weights of Organs Extracted from Male Rats Treated with Tartrazine Over a Period of 30, 60 and 90 Days

Parameters	Phase 1 n=17	Phase 2 n=25	Phase 3 n=17	P value	F value	Remark
Kidney (gm)	1.17±0.34[a]	1.05±0.26[a,b]	1.07±0.30[a,b]	0.4159	0.1393	NS
Liver (gm)	5.45±1.26[a]	4.75±1.08[a,b]	5.05±0.93[a,b]	0.1393	2.042	NS
Pancreas (gm)	0.47±0.12[a]	0.42±0.14[ab]	0.43±0.15[ab]	05425	0.6205	NS

Values in the same row with same superscript letter (a) do not differ significantly ($p<0.05$) when comparing phase 1 with other phases. Values in the same row with same superscript letter (c) do not differ significantly ($p<0.05$) when comparing phase 2 with phase 3. NS= Not Significant, n= No of Rats.

3.8 Histological Examination of Liver, Kidneys, and Pancreas

The histologic examination of the liver, kidney and pancreas over the periods of 30, 60 and 90 days are shown in figure 2-4.

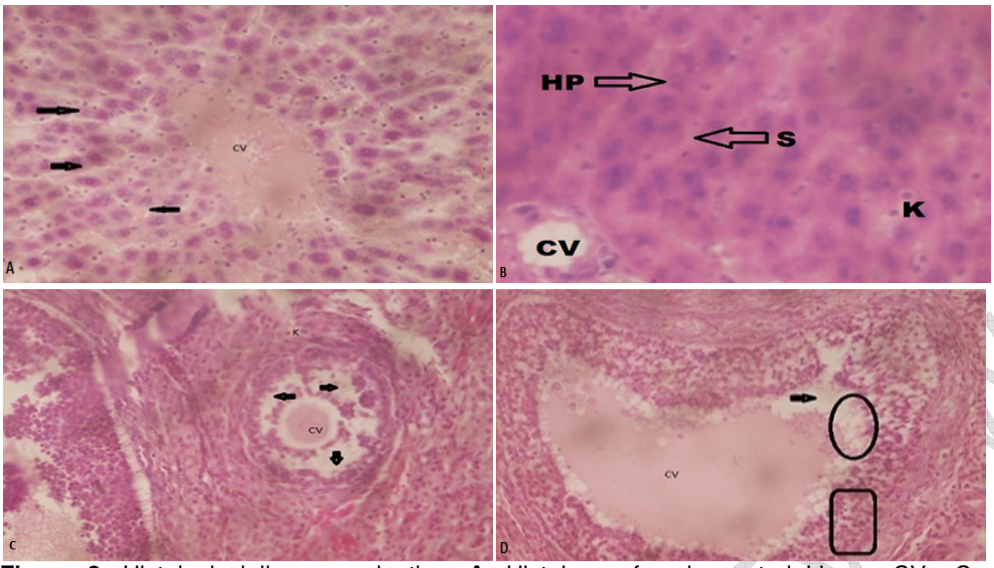

Figure 2: Histological liver examination. **A.** Histology of male control Liver. CV= Central Vein appearing normal, hepatocyte appears in thick plate radiating (arrow) between thick plates are sinusoids. **B.** 30 days. CV= Compressed central vein, K= Kupffer cells. S= Sunusoids and HP = Hepatic Plate. Hepatocytes appears inflamed but with nuclear content. **C.** 60 Days, CV = Central vein surrounded by radiating hepatic cells. Hepatic cells are destroyed leaving vacuoles (arrows), K= Kupffer cells. **D.** 90 Days. CV=Central Vein with Granule, Rectangular Portion Shows Cell Cluster. The circled portion shows hepatocytes with no nuclear content. The cells with fatty cyst (arrow), pockets of kupffer cells infiltration. inference: nuclear degeneration. H&E stain. X400.

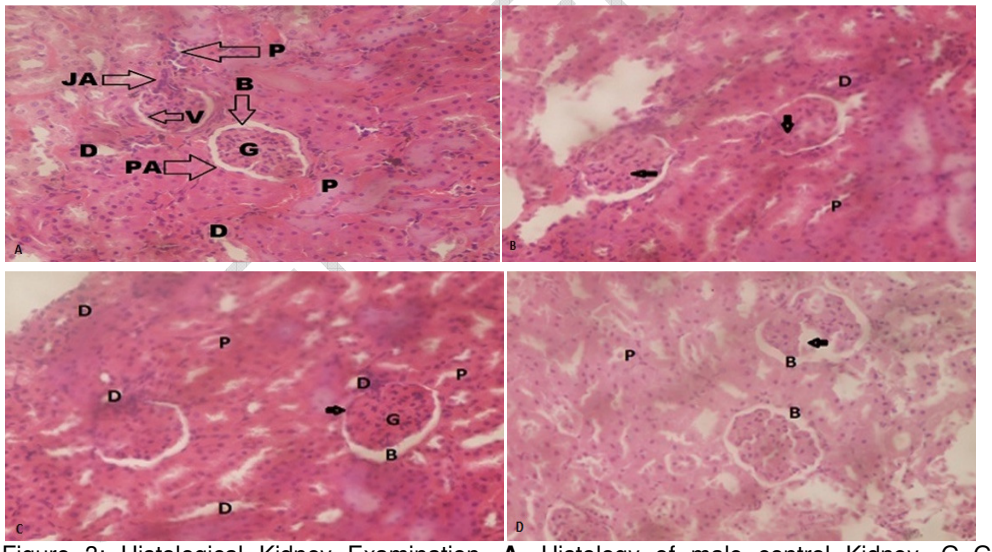

Figure 3: Histological Kidney Examination. **A.** Histology of male control Kidney. G=Glomerulus (normal), visceral layer (v), b=bowman's capsular space (normal), parietal layer (PA) with juxtaglomerular apparatus (JA), P&D=proximal and distal convoluted tubule (normal) inference: kidney slide appears normal. **B**. 30 Days. Normal glomerulus, bowman's capsule and space. Hypercellularity (arrows) of the mesengial area. proximal and distal convoluted tubules appear normal. Inference: normal histology of kidney with hypercellularity of mesengial cells. **C**. 60 Days. G =Glomerulus (Normal), B= Bowman's space (normal) with compressed area (arrow). P=Proximal convoluted tubule with hyaline cast within the lumen. D =Distal convoluted tubule (normal). Inference: Normal Histology of Kidney with Hyaline Cast in Proximal Tubule. **D**. 90 Days. Distorted glomerular arrangement (arrow) which is vacuolated, B = Bowman's capsule appears normal, P = Podocyte. Inference: Possible glomerulonephritis. H&E stain. X400.

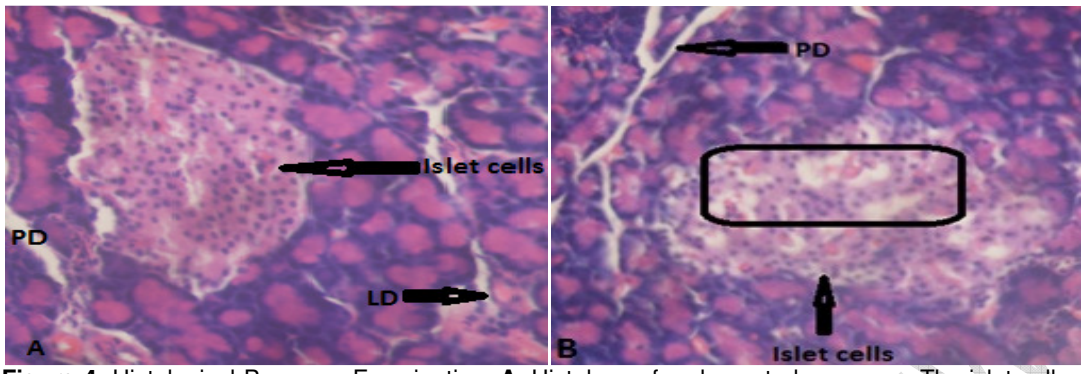

Figure 4: Histological Pancreas Examination. **A.** Histology of male control pancreas. The islet cells of the pancreas appear normal and distinct. Pancreatic duct (PD)and interlobular duct (LD) appears normal without obstruction. **B**. 90 Days. Islet cells of the pancreas scanty with presence of vacuolation especially in the rectangular shaped structure. However, the Pancreatic duct (PD) and interlobular duct (LD) still appears normal without obstruction. H&E stain. X400.

4. DISCUSSION

When the effect of tartrazine was considered on glucose and lipase after 30, 60, and 90 days of treatment, glucose indicated significantly higher values in tartrazine treated male rats when compared with control rats. The increase in glucose seen in our study is in line with the finding of Rehman et al. [34]; Lahmass et al. [35]; but contrary to the report of Al-Shinnawy & Elkattan, [18]; Mehedi et al. [22]. Rehman et al. [34] recorded a significant increase in glucose concentration in tatrazine treated rats compared to control rats at a dose of 10mg/kg for 30 days. More so, Lahmass et al. [35], also reported a significant increase in glucose concentration when adult male rats were treated with tartrazine at a dose of 10mg/kg for 60 days. However, Al-Shinnawy & Elkattan [18], reported no significant difference in glucose concentration of tartrazine treated rats compared with control rats at a dose of 10mg/kg for 30 days as well. Mehedi et al., 2013, documented no significant change in glucose when rats were fed with very low dose (0.1%) of tartrazine for 13weeks. Furthermore, when lipase was considered, the non-significant difference observed in lipase concentration seen in our work after 30, 60, and 90 days of treatment contradicts the reports of Rehman et al. [34]. Rehman et al. [34], documented a significant increase in lipase when male rats were treated with tartrazine at a dose of 10mg/kg for 30 days. The significant increases seen in glucose in the 30, 60, and 90 days of treatment, in our opinion could be as a result of negative pharmacological interaction between these azo dyes and islets of langerhans of the pancreas that affected the optimum production of insulin as seen in the histologic examination of the after 90 days of treatment (figure 4, B) and the non-significant increase in pancreatic lipase. Increase in lipase has been reported to be associated with inflammation of the pancreas. Therefore, the non-significant increase in lipase observed in our work could also be a pointer to pancreatic disturbance. Meanwhile, the scanty and mildly distortion with vacuolation of the islets region seen in the histologic examination of the pancreas (Figure 4, B) suggest loss of islets cells and physiological function which in turn might have also affected the regulation of glucose in the plasma. Although the islet region of the pancreas appeared mildly vacuolated, but the pancreatic duct and interlobular duct still appears normal without any obvious derangement. The distortion and scanty nature of the Islets of langerhans could probably by as a result of direct oxidative insult on the pancreas by reactive oxygen spices of azo dye metabolism.

When liver enzymes and proteins were observed, significantly higher values were seen in AST and ALT of tartrazine treated rats after 60 days compared to control rats. More so, after

90 days, significantly higher values were also seen AST, ALT, ALP, total protein, albumin, and globulin in tartrazine treated male rats compared to control rats. The increase in ALT seen in our work is supportive of the reports of Lahmass et al. [35]; El-Rabey et al. [36]. Lahmass et al. [35], reported significant increase in ALT concentration when adult male rats were treated with tartrazine at a dose of 10mg/kg for 60 days. However, they also reported no significant difference in AST concentration after 60 days at a dose 10mg/kg. Furthermore, El-Rabey & colleagues [36], also recorded significantly increased liver enzymes in rats treated with low doses of tartrazine that was attenuated with honey. In addition, Al-Shinnawy & Elkattan [18], also reported significant increase in liver enzymes (AST and ALT) at a dose of 10mg/kg for 30 days. However, contrary to our findings, Himri et al., 2011reported no significant change in AST and ALT in tartrazine treated male rats when fed for 90 days at a dose of 7.5mg/kg. More so, Mehedi et al. [22], also documented no significant change in AST and ALT when rats were fed with very low dose (0.1%) of tartrazine for 13weeks. When protein components were considered, the significant increase seen in total protein of tartrazine treated rats contradicts the findings of Mehedi et al., 2013 but support the reports of Himri et al. [37]; Al-Shinnawy & Elkattan [18]. Mehedi et al. [22], reported a significant reduction in total protein concentration when male rats were treated with tartrazine at a low dose of 0.1%. However, in line with our work, Himri et al. [37], recorded a significant increase in total protein concentration when male rats were treated with tartrazine for 90 days at a dose of 7.5mg/kg. Albumin in our study indicated significantly higher level in treated rats which is also in line the findings of Mehedi et al. [22]. They also reported significantly higher values of albumin in tartrazine treated rats when tartrazine was administered to rats at a dose of 0.1% for 13 weeks. The significant increase in globulin concentration seen in our work after 90 days of treatment contradicts the reports of Mehidi et al. [22]. Mehidi et al. [22], documented no change in globulin proteins when male rats were treated with tartrazine at a dose of 0.1% for 13 weeks. However, Amin et al. [7], documented significant higher level of globulin in male rats treated with tartrazine at a high dose of 500mg/kg. The significant increase in AST, ALT, and ALP enzymes observed in our work suggest hepatocellular damage leading to the increase presence of these enzymes in the plasma. In particular, elevated ALT activities in the plasma reflect hepatic derangement because of its specific for hepatic insult or injury compared to AST since ALT is contained in the cytoplasm and organelle such as the mitochondria of hepatocyte. The histological examination revealed the presence of inflamed hepatocytes, vacuolation, compression of the central vein (figure 2 B), vacuolations, loss of hepatic plates and presence of pigmented kupffer cells within the sinusoids (figure 2, C) distorted lobular boundary, clusters of inflamed hepatocytes, loss of nuclear content of the hepatocytes (pynosis), hydropic degeneration of the central vein, fatty materials at the periphery of the central vein and pockets of kupffer cells (Figure 2, D), loss of hepatic plates and pigmented kupffer cells at the sinusoids (figure 2, C). Our histologic findings also concur with the finding of Himri et al. [37]; Mehidi et al. [22]. Himri et al. [37], reported the presence of fatty degeneration and kupffer cells in the renal tissue when tartrazine was given to male rats at a dose of 7.5mg/kg and 10mg/kg for 90 days. Mehidi et al. [22], also documented mild hydropic degeneration (dilation) of the central vein and condensed nuclear materials in the hepatocytes when tartrazine at a dose of 0.1%, 0.45% and 1% were given to rats for 13 weeks. The presence of kupffer cells and vacuolation might probably indicate immunological response and hepatocellular damages. More so, the presence of fatty materials could also indicate fatty degeneration as a result of increased lipid peroxidation products as well as poor endogenous hepatic anti-oxidative functions. The histopathological results obtained correlates with our biochemical findings were significant increases in liver enzymes: AST and ALT and ALP were observed in both tartrazine treated rats. In addition, the significant increase in total protein, albumin and globulin seen in our study could also be connected with the hepatic derangements, immunological, and inflammatory response owing to the presence of kupffer cells observed. Our opinion is in agreement with the report of Amin et al. [7], who mentioned that liver

damage release greater than normal levels of plasma proteins such as albumin into the blood.

Furthermore, significant increase in urea was observed after 60 days of tarrazine treatment. However, after 90 days of treatment, significantly higher values in urea and CRT in tartrazine treated male rats compared to control rats. The increase in creatinine supports the reports of Lahmass et al. [35]. Lahmass et al. [35], reported a significant increase in Creatinine when tartrazine azo dye was given to rats at a dose of 10mg/kg. The increase seen in urea and creatinine after 90 days is also in line with the reports of Amin et al. [7]. Amin et al. [7], documented a significant increase in urea and creatinine when male rats were fed with tatrazine at a dose of 15mg/kg. However, contrary to our findings, Himri et al. [37], reported no significant change in urea in tartrazine treated male rats compared to control rats when fed for 90 days at a dose of 7.5mg/kg. More so, Mehedi et al. [22], documented no significant change in urea and creatinine when rats were fed with very low dose (0.1%) of tartrazine for 13weeks. The increase in CRT and Urea suggest renal derangement associated with the azo dye administered. In our opinion, the compressed capsular space observed could be as a result of hydropic dilation of the glomerulus (figure 3, C) while the clustered mesengials area with hypercellurity (figure 3, C) distorted glomerulus, and vacuolation seen within the glomerulus (figure 3, D) suggest glomerular inflammation or an indication of inflammatory responses of nephritic damages. More so, the hyaline cast observed (figure 3, C) probably indicates early tubular degeneration of the nephrons that might affect tubular re-absorption of substances such as urea, sodium, potassium etc from the lumen into the interstitial tissues. Also, our histologic findings are supportive of the work of Himri et al. [37]; Mehedi et al. [22]. Himri et al. [37], reported distorted glomerulus and tubular degeneration when male rats were treated with 7.5mg/kg of tartrazine. Similarly, Mehedi et al. [22], reported glomerular damages and compressed lumen of tubular cells when rats were treated with 1% of tartrazine for 13 weeks. Therefore, it is possible that the histopathological alterations observed could account for the increase in creatinine and urea seen in our biochemical assay in tartrazine treated rats. The presence of reactive oxygen species tends to reduce cell viability by disrupting cell membrane integrity thereby inducing cell membrane leakage.

Meanwhile, when the absolute weight of the kidneys, liver and pancreas were considered, it was observed that no significant difference was seen in the treated rats compared to the control rats after 30, 60, and 90 days. Our recent finding support the reports of Mehedi et al. [22]; Himri et al. [37]; Lahmass et al. [35]. Mehedi et al. [22], reported no significant change in the weight of kidney and liver when male rats were fed with tartrazine at a low dose of 0.1% for 90 days. Also, Himri et al. [37], also documented no significant change in the weight of kidney and liver when male rats were fed with tartrazine for 90 days at a dose of 7.5mg/kg. More so, Lahmass et al. [35], further reported no significant when male rats were treated with tartrazine a a dose of 10mg/kg for 60 days. The non-significant decrease observed in the weight of these organs could be due to insufficient loss of cellular mass. Although, the histologic findings indicated loss of parenchymal cells of the kidneys, liver, and pancreas (vacuolated areas) in the treated male rats but were not significant enough when compared with the control.

Finally, when the influence of the different periods (30, 60 and 90 days) on biochemical parameters were compared using ANOVA, significantly higher values in glucose, urea, ALT and ALP, total protein, and globulin in tartrazine treated male rats were seen. The significantly higher values seen in glucose, urea, ALT, and ALT over 30, 60, and 90 days in our opinion could be as a result of the progressive derangements. The progressive derangement could be associated the cells inability to adapt to oxidative stress induced by the dyes over time. The higher values seen in urea concentration without a corresponding

increase in CRT might also suggest dehydration and increased protein degradation as earlier reported in our work. Our opinion also agrees with reports of Mehedi et al. [22] who mentioned that tartrazine induced dehydration. Also, the significant increase observed in total protein could also be associated with progressive distorted of the hepatic tissue which in turn induced immunological or inflammatory responses. In addition, the increase in globulin points towards enhanced immunoglobin production by the body defense mechanism which is targeted towards the toxic effect of the azo dye. The presence of kupffer cells as seen in this study and the increased presence of lymphocytes in the peripheral blood system after 90 days of tartrazine treatment at a dose of 7.5mg/kg as reported by Elekima & Serakara [38], further support our opinion on immunological response that resulted in increased globulin fraction and total protein. More so, when weights of organs extracted from rats were considered over the period of 30, 60, and 90 days using ANOVA, it was observed that the kidney, pancreas, and liver indicated non-significant lower values in the tartrazine treated male rats as the duration of treatment increased to 90 days. The non-significant reduction seen in the weight of these organs may suggest loss of parenchymal cells and might probably be more evidential if the duration surpasses 90 days.

5. CONCLUSION

In this study, when ADI doses were administered over a given period, 30 days did not indicate hepatocellullar and renal derangements as well histological distortions in liver, pancreas and kidneys. However, in the 60 and 90 days of chronic studies, there were mild hepatocellular and renal derangements as well as histologic distortions in liver, pancreas and kidneys. When the influence of duration of exposure was considered, it was observed that derangements and toxicity of tartrazine azo dyes were more pronounced in the 90 days exposure.

6. RECOMMENDATION

Because of the mild alterations seen in the chronic study, it is also advised that duration far above 90 days should be considered in further studies.

7. LIMITATION OF THE STUDY

The duration of the chronic aspect of this study was not more than 90 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 be the same in humans. Therefore, our findings are subject to further research and verification especially in humans.

COMPETING INTERESTS

We declare that there is/are no competing interests exist.

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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