https://dx.doi.org/10.4314/dujopas.v8i1b.12
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 8 No. 1b March 2022*

Sub-acute Toxicity Study on Tartrazine in Male Albino Rats

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Abstract
The study aimed to compare the sub-acute toxicity of tartrazine azo dyes that is used extensively as food colorant at low and high dose on biochemical parameters, lipid profiles and histological abnormalities. Twelve male albino rats were grouped into 3 groups of 4 rats each. Group 1 was fed normal diet and water, Group 2 was administered tartrazine 7.5mg/kg body weight and Group 3 was administered tartrazine 75mg/kg body weight. The albino rats were sacrificed after 7 weeks; tissue and blood samples were collected to assess the histopathological changes, lipid profiles and biochemical parameters of the liver and kidney. The findings revealed significant elevation ($P < 0.05$) in serum total cholesterol (TC) (4.88±0.31mg/dl to 8.18±0.45 mg/dl), triglyceride (TG) (0.92±0.05 mg/dl to 1.63±0.14 mg/dl), low density lipoprotein cholesterol (LDL) (3.59±0.26 mg/dl to 7.05±0.39 mg/dl), urea (42.35±2.43 mg/dl to 50.53±2.96 mg/dl) and creatinine (0.97±0.05 mg/dl to 1.46±0.17 mg/dl), alanine amino transferase (ALT) (12.87±2.64 U/L to 37.74±5.76 U/L), aspartame amino transferase (AST) (47.33±3.92 U/L to 134.88±6.51 U/L) and alkaline phosphatase (ALP) (76.80±8.58 U/L to 124.01±1.51 U/L) levels in groups administered low and high doses of tartrazine and significant decrease in high density lipoprotein (HDL) (0.81±0.05 mg/dl to 1.11±0.10 mg/dl), in comparison to the control. Furthermore, Periportal lymphocytic infiltrates and moderate mesangial cell proliferation were observed in kidney and liver respectively of rats administered tartrazine at both low and high dose. Hence, excessive use of tartrazine for long time may result in kidney and liver toxicity.

Keyword: Tartrazine, Histopathological, lipid profile, liver, and kidney function.

INTRODUCTION
With the increase in production of processed and convenience foods, the continuous use of food colorants to enhance the appearance of food products or replace the color lost due to processing is on the increase. Although natural food colorants such as turmeric, carotene, caramel and others have been used for long without any reported side effects, the increased production and use of synthetic colorants in recent years has piqued the interest of numerous researchers, nutritionists, and food safety authorities worldwide.

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Globally, roughly 800 tons of artificial colorants are generated yearly, with azo colorant accounting for 60-70% of the total (El-desoky *et al*., 2017). Synthetic azo dyes find wide range of applications because of the stability and low cost (El-wahab and Moram, 2013). Numerous studies on the health effect of synthetic azo dyes has been conducted focusing on the toxicity of azo dyes on rats and mice with an emphasis on immunological, serological and biochemical aspects (Elbanna *et al.,* 2017; Hashem *et al.,* 2010). The most widely used among the synthetic colorants are tartrazine, sunset yellow, and quinoline yellow.

Tartrazine is a synthetic soluble yellow azo color obtained from coal tar. It is universally used in food, cosmetics and pharmaceutical products (El-borm *et al.,* 2020). It is considered as substitute colorant for preparation of food in many nations due to its low-cost (Mehedi *et al.,* 2009).

Previous studies found that exposure to tartrazine, caused elevated eosinophil and lymphocyte counts as well as stomach lining inflammation in rats following administration over period of time (El-desoky *et al.,* 2017). Some other studies focused on effect of tartrazine on the brain of Albino rats (Hosieny *et al.,* 2020), immunohistochemical (Abdel-Aziz *et al.,* 2019) and hematological (Abd-Elhakim *et al.,* 2018) parameters. Biotransformation of tartrazine in the liver has been associated with the generation of reactive amines and free radicals and these have been implicated in the pathogenesis of toxicity following the consumption of the colorants (Araga *et al.,* 2005). There is little or no information available on the comparative sub-acute toxicity study at both high and low dose.
This study was conducted to determine the sub-acute toxic effect associated with tartrazine as well as compare the observed changes at both low and high dose in male Albino rats.

Materials and methods

Tartrazine
Tartrazine was purchased from solag Allied Chemicals Nigeria limited

Experimental Animals
Twelve (12) male albino rats weighing 65 – 80 grams were bought from the Biological Science Department of Bayero University Kano and used for the study. The animals were allowed to acclimatize for one week under controlled condition (12 hours light/dark cycle) and with *ad libitum* free access to water and basal diet (chukun brand). All the experimental procedures were done according to the Bayero university kano Institutional Animal Ethics Committee guidelines.

Experimental Design
The animals were randomly divided into 3 groups of 4 rats each.
Group A: Control received daily 1ml of distilled water throughout the experiment
Group B: Administered 7.5 mg tartrazine dissolved in 1ml of distilled water per kg body weight daily for 7weeks
Group C: Administered 75 mg tartrazine dissolved in 1ml of distilled water per kg body weight daily for 7 weeks.

Sample collection and preparation
After seven weeks, the animals were humanely killed following chloroform anesthesia and blood samples collected into plain tubes. Blood sera was obtained from blood cells by centrifugation at 4000 rpm for 15 minutes and used for the analysis of the lipid profile and biochemical parameters of liver and kidney. Liver and kidney tissues were also collected and

fixed in neutral buffered formalin (10%) and processed using routing histological techniques for histopathological studies..

Serum biochemical analysis

The Total cholesterol (TC) was estimated by enzymatic colorimetric method of Kenny, (1952). High Density Lipoprotein (HDL) estimation was done using Randox kit according to the method of Mcgowan *et al.,* (1982). TG estimation was done by enzymatic colorimetric method. Low Density Lipoprotein (LDL) was calculated using the formular; LDL = [Total Cholesterol – (HDL + triglycerides/5)]. The activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined according to the method of Reitman and Frankel, (1957). The Alkaline phosphatase (ALP) level was estimated using colorimetric method. Urea was estimated according to the method of Patton and Crouch, (1977) while creatinine estimation was done according to the method of Husdan and Rapoport, (1968).

Histological analysis

The formalin-fixed liver and kidney tissues were dehydrated in graded concentrations of ethanol and embedded in paraffin. Tissues were sectioned using a microtome at a thickness of 4 µm, stained with Haematoxylin and Eosin and viewed under light microscope for histopathological changes.

Statistical analysis

All data were analyzed using Statistical Package for Social Sciences (SPSS) version 20. Differences among the means of the experimental groups were analized using one-way ANOVA. The result of the study was reported as mean ±standard deviation (SD). The results were considered significant at *P*-values of less than 0.05 ($P < 0.05$).

Results

Lipid profile

The result on **Table 1** revealed significant ($P < 0.05$) rise in TG, TC, LDL levels and a significant decrease in HDL level at both 7.5 mg/kg and 75 mg/kg treatment groups in comparison with the control. Though the levels were significantly higher in the 75 mg/kg treatment group. ($p < 0.05$) as compared to the 7.5mg/kg treatment group.

Table 1: Serum level of TG, TC, HDL and LDL in rats administered tartrazine orally for seven weeks.

Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (control)	0.92±0.05^{a}	4.88±0.31^{a}	1.11±0.10^{a}	3.59±0.26^{a}
Group II Tat 7.5 mg/kg	1.16±0.17^{b}	6.86±0.31^{b}	0.99 ±0.01^{b}	5.64±0.34^{b}
Group III Tat 75 mg/kg	1.63±0.14^{c}	8.18±0.45^{c}	0.81±0.05^{c}	7.05±0.39^{c}

Values are Mean±SD of four rats. Means having different letter(s) within a column show significant difference at $P < 0.05$. Tartrazine (Tat).

Effect of Tartrazine on Liver Biochemical Indices

Table 2 reveals significant increase ($P < 0.05$) in levels of AST, ALT and ALP of rats administered tartrazine at both 7.5 mg/kg and 75 mg/kg doses as compared to the control. Also remarkable increase ($P < 0.05$) in groups fed with higher dose of tartrazine (75mg/kg) was observed in comparison with the low dose (7.5mg/kg)

Table 2: Serum level of AST, ALT and ALP in rats orally administered tartrazine for seven weeks

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I (Control)	47.33±3.92[a]	12.87±2.64[a]	76.80±8.58[a]
Group II (Tat 7.5 mg/kg)	73.67±4.09[b]	20.55±2.46[b]	92.98±5.11[b]
Group III (Tat 75 mg/kg)	134.88±6.51[c]	37.74±5.76[c]	124.01±1.51[c]

Values are Mean±SD of four rats. Means having different letter(s) within a column show significant difference at $P < 0.05$. Tartrazine (Tat)

Effect of tartrazine on Kidney indices

Table 3 shows the result of the effect of tartrazine on kidney function indices (creatinine and urea). Significant increase ($P < 0.05$) in creatinine level was observed in the group administered 75 mg/kg of tartrazine while no significant increase was observed in groups administered 7.5 mg/kg as compared to the control. Also, significant increase ($P < 0.05$) in urea level was observed at 7.5 mg/kg and 75 mg/kg as compared to the control. Furthermore, significant increase in urea levels was observed in 75 mg/kg treated group as compared to the 7.5 mg/kg treated group.

Table 3: Serum level of creatinine and urea in rats orally administered tartrazine for seven weeks

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Group I (Control)	0.97±0.05[a]	42.35±2.43[a]
Group II (Tat 7.5 mg/kg)	1.14±0.06[a]	44.20±1.17[b]
Group III (Tat 75 mg/kg)	1.46±0.17[b]	50.53±2.96[c]

Values are Mean±SD of four rats. Means having different letter(s) within a column show significant difference at $P < 0.05$. Tartrazine (Tat)

Histology of kidney

The result of histopathology of kidney is presented in **figure 1** and **2**. Photomicrographs showing normal glomerulus in control group (**Figure 1**). There was mild glomerular cellularity in kidney of rats administered with low dose of tartrazine (7.5 mg/kg) (**Figure 2A**) while high dose of tartrazine (75 mg/kg) showed moderate mesangial cell proliferation (**Figure 2B**).

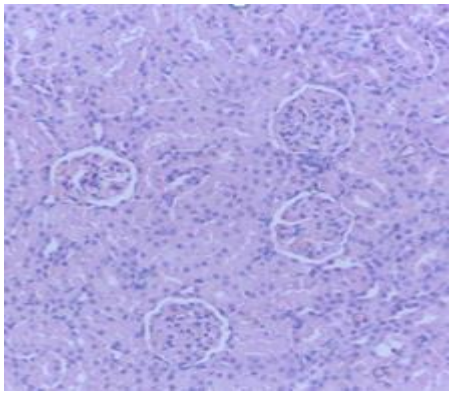

Figure 1: Photomicrographs of the kidney H&E X400 of rats showing normal glomerulus

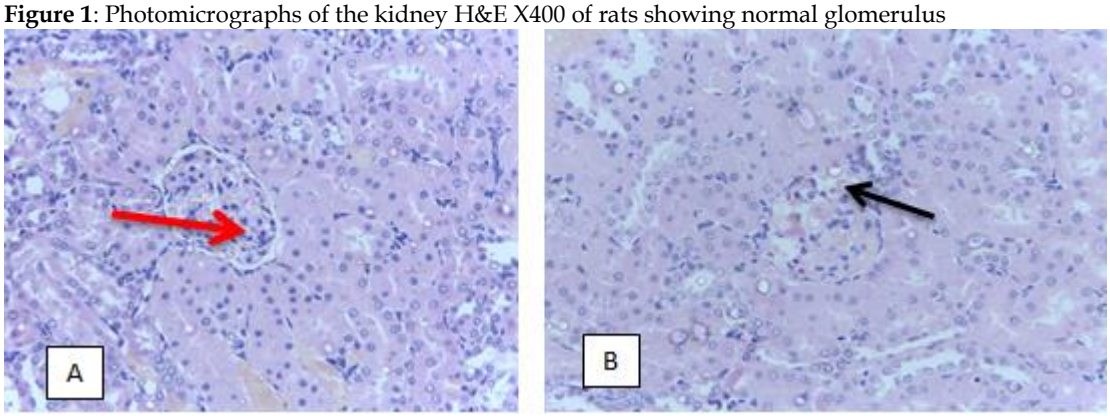

Figure 2: Histopathological changes in the kidney of rats administered low and high tartrazine dose (H&E X400) (A) 7.5 mg/kg tartrazine showing normal tubules with mild glomerular hypercellularity (B) 75 mg/kg tartrazine showing moderate mesangial cell proliferation

Histology Result of the Liver

The result of the histopathological study of the liver revealed normal hepatocytes in the control group (**Figure 3**). Periportal inflammatory aggregates mainly lymphocyte in rat's hepatocytes was observed at 7.5 mg/kg (**Figure 4A**) while 75 mg/kg treated group showed periportal lymphocytic infiltrates (**Figure 4B**).

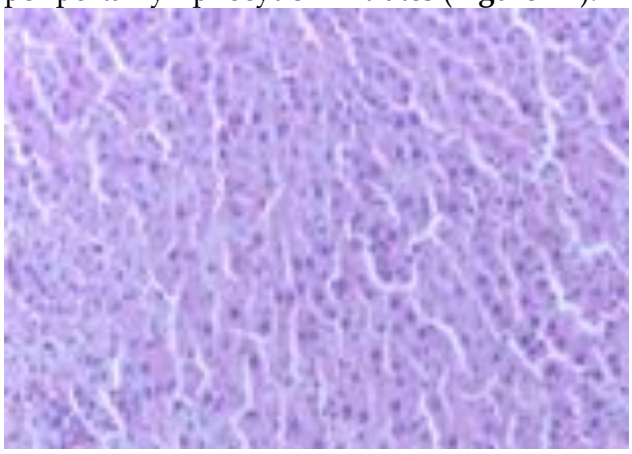

Figure 3: A Photomicrographs of the liver H&E X400 of rat showing normal liver

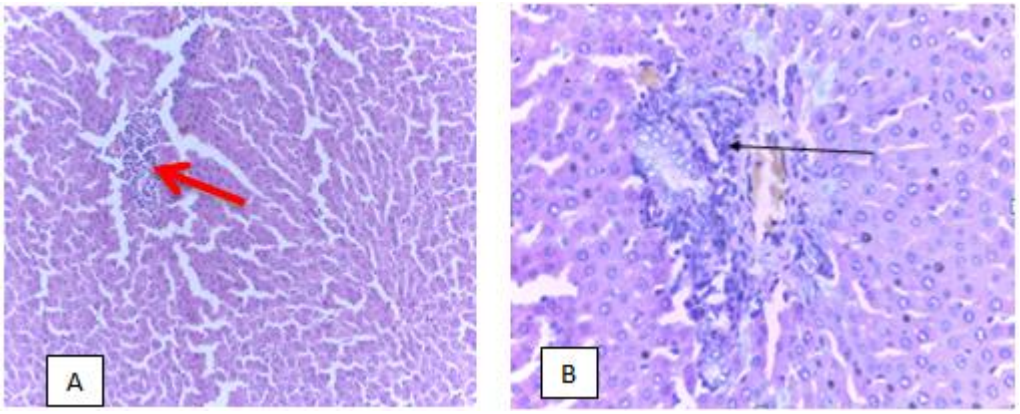

Figure 4: Histopathological changes in the liver of rats administered low and high dose of tartrazine (H&E X400) (A) 7.5 mg/kg tartrazine showing periportal inflammatory aggregates mainly lymphocytes (B) 75 mg/kg tartrazine showing periportal lymphocytic infiltrates.

DISCUSSION

Colorants are added to food to enhance its attractiveness and also replace lost colour due to processing. Tartrazine azo dye is added to a wide variety of products such as ice cream, soft drinks, biscuit, pharmaceutical products, cosmetics among others (Rovina *et al.,* 2017). The recommended daily intake of tartrazine for human is around 0-7.5mg/kg body weight (JFWEC, 1965). The present study assesses the subacute toxic effect of tartrazine azo dye on the liver and kidney of Albino rats. at a low dose which is the acceptable daily intake (7.5 mg/kg body weight) and a higher dose (75 mg/kg body weight).

The result of the present study showed an increase in the level of TG, TC, LDL and a decrease in HDL in both groups administered tartrazine in comparison with the control. The increase was more significant in the groups treated with 75 mg/kg of tartrazine. The remarkable increase in TC, TG, LDL and decrease in HDL levels observed is an indication that tartrazine may affect lipid metabolism which maybe as a result of the activities of reactive oxygen species and free radicals generated following as a result of metabolism of tartrazine which in turn causes changes in the levels of the hepatic enzymes such as lecithin cholesterol transferases and hepatic lipoprotein lipase which are needed for the catalysis of cholesterol and triglycerides respectively (Amin *et al.,* 2010; Nabila *et al.,* 2013). The result of this study corroborates with the work of Elekima et al., (2017) who reported increase in levels of lipid profile parameters in albino rats administered different doses of tartrazine, while differing from the findings of Amin *et al.,* (2010) who reported decreased levels of TG and TC.

Our result also revealed significant increase in the serum levels of ALT, AST and ALP in the tartrazine treated rats in comparison with the untreated. The results are consistent with the work of El-Golli *et al.,* (2016) who reported elevated serum AST, ALT & ALP levels in rats that underwent subchronic oral tartrazine treatment at a dose of 300mg/kg for 30 days. Also, Alsolami, (2017) reported increase in the serum level of ALT, AST and ALP in young male rats administered allura red color. Findins of this studey also corroborates with the findings of Amin *et al.,* (2010) who reported that oral administration of tartrazine dye for 30 days at a dose of 500 mg/kg in albino rats causes a remarkable increase in serum aminotransferases and ALP levels. Liver plays an important role in the metabolism of xenobiotics, and hence elevated ALT and AST activities in the serum may be due to liver tissue damage.
The significant increase ($P < 0.05$) in levels of creatinine and urea in tartrazine treated groups in comparison with the control is consistent with the work of Abdelaziz and Ashour, (2010)

who reported significant elevation in rats administered fast green azo dye for 35 days. Similarly, El-wahab and Moram, (2013) reported remarkable increase in serum creatinine and urea in rats administered tartrazine at a dose of 75mg/kg body weight. Furthermore, Nabila *et al.,* (2013) reported elevated serum creatinine and urea level in swiss mice administered 2.5% tartrazine for 13 weeks. Increase in creatinine and urea levels is closely related to damage in kidney and affects kidney function since blood urea and creatinine increases in all forms of kidney disease.

Histopathology assessment of the liver revealed periportal inflammatory aggregates mainly lymphocytes in liver cells administered low dose (7.5 mg/kg) of tartrazine while high dose of tartrazine (75 mg/kg) showed more periportal lymphocytic infliltrates in their liver. Furthermore, the histology of the kidney reveal mild glomerular hypercellularity at 7.5 mg/kg while group administered 75 mg/kg showed moderate mesangial cell proliferation. The result is in line with the findings of Alaa *et al.,* (2016), who reported mild mononuclear leucocytes inflammatory cells infiltration in hepatocytes and focal mononuclear cell infiltration with congestion of renal blood vessels in rats administered 200 mg/kg tartrazine. The lymphocytic infiltration in the hepatocytes might be as a result of the reactive oxygen species generated by the metabolism of tartrazine. Renal glomerular damage may be linked to toxic chemical filtration by the kidney which induces immune responses (Himri *et al.,* 2011).

CONCLUSION

Administration of tartrazine for 7weeks at 75 mg/kg and 7.5 mg/kg showed adverse effect on lipid profile as well as; liver and kidney biochemical parameters resulting in histopathological changes in the liver and kidney tissues. Hence, the use of tartrazine in food should be reduced or replaced with natural colorants.

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