



Assessment of the changes in some diagnostic parameters in male albino rats fed on an Azo Dye

Mohammed S. Al-Shinnawy and Nabawy A. Elkattan

Department of Biology, Faculty of Science, University of Tabuk, KSA.

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ABSTRACT

Food additives are used for various purposes, including preservations, colouring and sweetening. Some food additives, however, has been prohibited from use because of their toxicity. Azo dyes are one of these food additives which widely used as colorants in foods. The present study was conducted to evaluate the possible influence of an azo dye (tartrazine) on some hematological and biochemical parameters of male albino rat *Rattus norvegicus*. Sixty adult male rats weighing 100-110g were divided into 3 groups; the first one served as a control, the second received 10mg/kg, b.w. of tartrazine and the third group was treated with 25mg/kg, b.w. of tartrazine. Rats were treated orally for 30 days followed a recovery for another 30 days. The data obtained reveal a marked decrease in the percentage of body weight gain, red blood cells (R.B.Cs) counts, hemoglobin (Hb) content, mean corpuscular hemoglobin concentration (MCHC), serum total lipids and serum total cholesterol of rats treated with the high dose of tartrazine. On the other hand, a noticeable increase in hematocrit (Hct) value, mean corpuscular volume (MCV), activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose level, serum total protein and globulin were found in rats treated with the high dose of tartrazine. In general, there was appreciable improvement after the recovery period.

1. INTRODUCTION

The wide range of food additives, used to preserve, dye or enhance foods (Hirschbruch & Torres, 1998; Toledo, 1999) are a consequence of industrialization and the development of food processing technology. The great bulk of artificial colourings used in food are synthetic dyes. For decades synthetic food dyes have been suspected of being toxic or carcinogenic and many have been banned whenever possible, choose foods without dyes. They are mostly used in foods of questionable nutrition worth anyway. Many researchers (Reyes *et al.*, 1996; Tanaka, 2005; Zrally *et al.*, 2006; Gao *et al.*, 2011) studied the metabolic and toxicological disorders induced by the administration of specific food colourant additives to rats and other mammals.

Many azo compounds are genotoxic in short-term tests and carcinogenic in laboratory animals (Combes & Haveland - Smith, 1982; Sasaki *et al.*, 2002).

Among the food dyes which are widely used is tartrazine. It is a synthetic lemon yellow azo dye, water soluble powder used worldwide as food additives to colour several foods, drugs and cosmetics. Moreover, this food colorant is used in cooking in many countries as a substitute for saffron (Mehedi *et al.*, 2009).

Tartrazine has been implicated as the food additive which is most often responsible for allergic reactions in specific human populations (Stenius & Lemola, 1976; Neuman *et al.*, 1978; Devlin & David, 1992).

The study of the carcinogenetic and mutagenetic effects of tartrazine was established by some authors which gives variable results (Jones *et al.*, 1964; Sasaki *et al.*, 2002). When the Tartrazine reaches the intestine, it can undergo metabolic reduction by intestinal microflora (Levine, 1991).

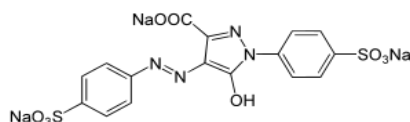
Tsuda *et al.* (2001) reported that some azo dyes as amaranth and allura red induce colon DNA damage at a very low dose in mice. Yet further investigations are required from other points of view in order to confidently predict the potential danger of these food dyes to mankind. The present study was aimed to determine the effect of tartrazine on some hematological and biochemical parameters in male albino rats. Also, the study is an attempt to determine if high doses of tartrazine can affect liver and kidney functions?

2. MATERIAL AND METHODS

2.1 Synthetic dye used:

2.1.1 Tartrazine:

- Synthetic azo dye.
- Lemon yellow azo dye.
- Very soluble in water.
- It is also known as E number E102, C.I. 19140, FD&C Yellow 5, Acid Yellow 23, Food Yellow 4, and Trisodium 1-(4-sulfonatophenyl)-4-(4 sulfonatophenylazo)-5-pyrazolone-3-carboxylate).



2.2 Animals:

Sixty mature male albino rats (*Rattus norvegicus*) ranging in weight from 100-110 g was obtained from *Schistosoma* Biological Supply Program (SBSP), Theodor Bilharz Research Institute. The rats were kept under normal laboratory conditions, fed on standard diet and water *ad libitum*. All rats were starved for 12 hrs before treatment, but allowed free excess to water. They were allocated at random into two groups; the first group (20 rats) was considered as controls. The second group (40 rats) was in turns-assigned into equal subgroups (i.e. 20 rats each). Rats in the first subgroup were orally

administered a dose of 10 mg/kg body weight/day of rats in the second subgroup were treated orally with a dose of 25 mg/kg body weight/day of tartrazine. After 30 days of treatment 10 rats were chosen randomly from each group and sacrificed. The rest of rats were left without any treatment for further 30 days as a recovery period after which the last batch of rats was sacrificed to assay the impact of recovery.

All of the control and treated rats were individually weighed in order to detect any changes that may take place in their body weights. The percentage of body weight gain was calculated as follows:

$$\frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial body weight}} \times 100$$

2.3 Hematological studies:

A part of blood samples were collected on heparinized capillary tubes for hematocrit value, which was determined according to the method of Rodak (1995). Another part of blood was collected on EDTA for the hematological experimentation. Red and white blood cells counts were performed using improved hemocytometer according to Dacie and Lewis (1991). Hemoglobin concentration was estimated according to Dacie and Lewis (1991). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Dacie and Lewis (1993).

2.4 Biochemical analysis:

Samples of blood were withdrawn and left to clot in a clear dry centrifuge tubes for each rat, then centrifuged at 3500 r.p.m. for 15 minutes. A portion of the clear supernatant serum was used immediately for glucose determination according to the enzymatic colorimetric method described by Trinder (1969).

The remaining serum was frozen at -20°C for subsequent analysis. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated according to the method described by BergMeyer and Bernt (1974).

Table 1: Effect of treatment with tartrazine on the percentage of body weight change (%) of male albino rats.

Groups	After 30 days (treatment)	After 30 days (recovery period)
Control	42.66 ± 1.03	84.50 ± 2.62
Tartrazine (10mg/kg)	39.12 ± *0.97	75.85 ± 2.09
Tartrazine (25mg/kg)	31.32 ± **2.13	73.12 ± *1.98

All values were expressed as mean ± standard error.

* (Significant). ** (Highly Significant).

Results presented in Table 2 show the alterations of the various hematological parameters in the blood of the studied rats induced by tartrazine. Rats supplemented

Serum alkaline phosphatase (ALP) was determined by the method of Belfield and Goldberg (1971). Serum contents of total cholesterol and total lipids were assayed according to Allain *et al.* (1974) and Knight *et al.* (1972), respectively. Serum total protein and albumin levels were estimated according to the methods described by Doumas (1975) and Doumas *et al.* (1971), respectively. Serum globulin was calculated according to Latner (1975). Serum content of urea and creatinine were estimated according to the methods described by Patton & Crouch (1977) and Bartels & Bohmer (1972), respectively.

2.5 Data analysis:

The obtained results were statistically analyzed by using the student "t"-test according to the method of Snedecor and Cochran (1980).

3. RESULTS

The data represented in Table (1) displayed the effect of treatment with tartrazine on the percentage of body weight gain of male albino rats. The findings revealed a significant decrease ($p < 0.05$) after 30 days treatment with the low dose (10mg/kg) of tartrazine and recorded a highly significant decrease ($p < 0.01$) with the high dose. These results remain significantly decrease ($p < 0.05$) after the recovery period. with 25mg/kg of tartrazine exhibited highly significant decrease ($p < 0.01$) in red cell count (R.B.Cs) and the mean corpuscular hemoglobin concentration (MCHC). Furthermore, the same dose recorded a significant decrease ($p < 0.05$) in hemoglobin (Hb) content, while it showed a highly significant ($p < 0.01$) increase in the mean of corpuscular volume (MCV) and of the hematocrit (Hct) value. Same results of R.B.Cs, Hb, MCV and MCHC were recorded in the recovery groups. Whereas, leucocytes (W.B.Cs) counts and the mean corpuscular hemoglobin (MCH) of albino rats were not affected by tartrazine throughout the experiment.

Table 2: Blood picture values of male albino rats treated with tartrazine.

Groups	R.B.Cs ($\times 10^6/\text{mm}^3$)	Hb (g/dl)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (%)	W.B.Cs ($\times 10^3/\text{mm}^3$)
After 30 days (treatment)							
Control	8.2 \pm 0.04	14.38 \pm 0.31	38.38 \pm 1.32	46.28 \pm 0.18	17.38 \pm 0.18	37.50 \pm 0.83	4.98 \pm 0.98
Tartrazine (10mg/kg)	8.3 \pm NS0.06	14.59 \pm NS0.22	38.48 \pm NS1.36	45.84 \pm NS0.72	17.42 \pm NS0.14	37.97 \pm NS0.81	5.04 \pm NS1.20
Tartrazine (25mg/kg)	7.3 \pm **0.12	13.02 \pm *0.45	41.88 \pm *1.04	56.63 \pm **0.59	17.66 \pm NS0.29	31.12 \pm **0.76	5.82 \pm NS1.71
After 30 days (recovery period)							
Control	8.4 \pm 0.09	14.32 \pm 0.42	37.58 \pm 1.61	44.24 \pm 0.86	16.90 \pm 0.26	38.13 \pm 1.04	4.37 \pm 1.03
Tartrazine (10mg/kg)	8.2 \pm NS0.08	14.40 \pm NS0.39	38.08 \pm NS1.57	45.91 \pm NS0.83	17.41 \pm NS0.24	37.84 \pm NS1.00	5.22 \pm NS1.74
Tartrazine (25mg/kg)	8.1 \pm *0.1	13.26 \pm *0.17	41.2 \pm NS1.22	51.51 \pm **0.67	16.63 \pm NS0.14	32.23 \pm **0.71	5.14 \pm NS1.60

All values were expressed as mean \pm standard error.

NS (Insignificant).

*(Significant).

** (Highly Significant).

The data presented in Table 3 displayed the effect of treatment with tartrazine on enzymatic activities which reflect the liver function of male albino rats. A highly significant increase ($p < 0.01$) in AST and ALT was detected in groups treated with the high dose of tartrazine; these results

were continued after a recovery period. This elevation was also recorded in groups treated with the low dose which revealed a significant increase ($p < 0.05$). Obvious amelioration appeared in AST and ALT activities in groups treated with the low dose after the recovery period.

Table 3: Effect of treatment with tartrazine on some serum enzymes activities indicative of liver function of male albino rats.

Groups	AST(U/ml)	ALT(U/ml)	ALP(U/l)
After 30 days (treatment)			
Control	163.94 \pm 0.17	64.67 \pm 1.02	95.64 \pm 4.25
Tartrazine (10mg/kg)	167.10 \pm *1.13	69.33 \pm *1.54	100.86 \pm NS6.85
Tartrazine (25mg/kg)	178.10 \pm **1.04	75.33 \pm **1.68	115.12 \pm *3.15
After 30 days (recovery period)			
Control	162.69 \pm 0.19	64.08 \pm 1.13	95.74 \pm 4.81
Tartrazine (10mg/kg)	164.01 \pm NS0.93	66.92 \pm NS1.39	101.65 \pm NS3.09
Tartrazine (25mg/kg)	166.41 \pm **0.71	72.83 \pm **1.94	112.96 \pm *3.12

All values were expressed as mean \pm standard error.

NS (Insignificant).

*(Significant).

** (Highly Significant).

A significant increase ($p < 0.05$) in ALP activity was also detected in groups treated with the high dose of tartrazine in both treated and recovery groups.

Table 4 shows the changes in serum total lipids, total cholesterol and glucose levels. The data revealed a significant decline in measuring total serum lipids and

total serum cholesterol ($p < 0.05$). On the contrary, the amount of glucose serum level was significantly increased. Both results were obvious at the high concentration. Moreover, the glucose levels seemed to return around its normal values after the recovery period.

Table 4: Effect of treatment with tartrazine on total serum cholesterol, total serum lipids and glucose levels of male albino rat.

Groups	Serum total lipids (mg/dL)	Serum total cholesterol (mg/dL)	Serum glucose level (mg/dL)
After 30 days (treatment)			
Control	232.71 ± 4.82	83.33 ± 2.91	78.56 ± 1.44
Tartrazine (10mg/kg)	227.14 ± ^{NS} 3.91	79.15 ± ^{NS} 3.13	80.32 ± ^{NS} 1.60
Tartrazine (25mg/kg)	216.92 ± *3.07	74.02 ± *2.64	84.96 ± *2.70
After 30 days (recovery period)			
Control	236.15 ± 4.33	87.13 ± 2.24	76.71 ± 1.49
Tartrazine (10mg/kg)	230.66 ± ^{NS} 4.01	81.82 ± ^{NS} 2.16	77.15 ± ^{NS} 1.81
Tartrazine (25mg/kg)	223.86 ± *4.17	78.95 ± *2.37	80.76 ± ^{NS} 2.11

All values were expressed as mean ± standard error.

NS (Insignificant).

* (Significant).

Table 5 demonstrates the effect of tartrazine on total serum proteins, albumin, globulin levels and A/g ratio. The two doses of tartrazine caused a significant increase (p<0.05) in total serum proteins only as compared with the corresponding control

group. However, treated rats with the high dose caused a significant increase (p<0.05) in serum globulin. No significance was recorded in all these parameters after the recovery period.

Table 5: Effect of treatment with tartrazine on serum total proteins, albumin, globulin and A/g ratio of male albino rats.

Groups	Serum total protein (g/dL)	Serum albumin (g/dL)	Serum globulin (g/dL)	A/g ratio
After 30 days (treatment)				
Control	7.15 ± 0.15	3.77 ± 0.15	3.37 ± 0.12	1.15 ± 0.31
Tartrazine (10mg/kg)	7.89 ± *0.30	4.14 ± ^{NS} 0.20	3.73 ± ^{NS} 0.21	1.12 ± ^{NS} 1.82
Tartrazine (25mg/kg)	8.25 ± *0.24	3.89 ± ^{NS} 0.14	4.38 ± *0.37	0.81 ± ^{NS} 0.93
After 30 days (recovery period)				
Control	7.22 ± 0.10	3.73 ± 0.09	3.47 ± 0.32	1.02 ± 0.42
Tartrazine (10mg/kg)	7.86 ± ^{NS} 0.33	3.63 ± ^{NS} 0.10	4.21 ± ^{NS} 0.60	0.88 ± ^{NS} 0.46
Tartrazine (25mg/kg)	8.36 ± ^{NS} 0.21	3.91 ± ^{NS} 0.13	4.44 ± ^{NS} 0.49	0.91 ± ^{NS} 0.53

All values were expressed as mean ± standard error.

NS (Insignificant).

* (Significant).

Serum urea and creatinine levels were determined to study the effect of tartrazine on kidney function test. Data presented in Table 6 show that neither serum urea nor

creatinine levels were significantly changed in any treated groups throughout the total experimental period, as compared with the control group.

Table 6: Effect of tartrazine on urea and creatinine concentrations of male albino rats.

Groups	Serum urea (mg/L)	Serum creatinine (mg/L)
After 30 days (treatment)		
Control	32.93 ± 0.98	15.37 ± 1.61
Tartrazine (10mg/kg)	33.02 ± ^{NS} 1.13	16.09 ± ^{NS} 1.95
Tartrazine (25mg/kg)	36.21 ± ^{NS} 2.06	18.37 ± ^{NS} 2.05
After 30 days (recovery period)		
Control	35.34 ± 1.08	15.52 ± 1.06
Tartrazine (10mg/kg)	38.15 ± ^{NS} 1.66	16.90 ± ^{NS} 1.63
Tartrazine (25mg/kg)	36.69 ± ^{NS} 1.38	17.07 ± ^{NS} 2.11

All values were expressed as mean ± standard error.

NS (Insignificant).

4. DISCUSSION

The present study is concerned with the effect of tartrazine (azo dye) on some hematological and biochemical parameters of male albino rats. Azo dyes have been tested by the oral route in mice, rats and dogs and by the subcutaneous route in rats. Two oral administrations of Azo dye studies in rats indicated a carcinogenic effect (IARC, 1975).

The present results indicated that the percentage of body weight gain of male albino rats treated with low and high dose of tartrazine recorded a marked reduction in body weight throughout the experimental periods as compared with the control. These result continued after the recovery period. The weight loss encountered in this study may be a consequence to the effect of tartrazine, which appears to have a hypocholesterolemic and hypolipidic effects as revealed by a decrease in total serum content and total serum lipids especially with the dose treated groups. These results were in agreement with other researchers (Shaker *et al.*, 1989; Takeda *et al.*, 1992; Aboel-Zahab *et al.*, 1997).

Hematological parameters were valuable tools for assessing the injury that caused by certain substances. The RBC counts were the most useful as raw data for calculation of the erythrocyte indices MCV and MCH. Decreased RBC is usually seen in anemia of any cause. Results of the present investigation revealed that erythrocytes (RBCs) count, hemoglobin (Hb) and MCHC in rats treated with the high dose were decreased. These results were continued after the recovery period. These changes induced by tartrazine may be due to the prevention of red blood cell synthesis via inhibition of erythropoiesis in the bone marrow. In agreement with Chakravarty *et al.* (2005) work, who showed a decrease in hemoglobin content and total erythrocyte count at all dose levels of the food dye used.

On the contrary to the above findings, Ford *et al.* (1987) stated that carmoisine (given in high doses for 6 months) did not

cause any changes in the hematological investigations of rats. The marked discrepancies observed between the various research studies might be attributed to dose variations as well as the duration of food colourant intake. The total leucocytic count and MCH in the present study remained unchanged in all experimental groups. This finding was in agreement with Borzelleca and Hallagan (1988).

Serum aminotransferases activities are known as toxicity markers in the study of hepatotoxicity caused by chemicals (Govindwar and Dalvi, 1990). An increase in the activities of these enzymes was termed as the early recognition of toxic hepatitis. Results of the present investigation revealed a marked elevation in AST and ALT activities throughout the entire experimental period in groups treated with both doses of tartrazine. This elevation in AST and ALT activities continued after a recovery period in the group treated with the high dose only. The elevation of the aminotransferases activities in blood has been considered as an indicator of tissue damage. Similar results were reported by Abdel-Rahim *et al.* (1989) who found significant increase in both serum AST and ALT of rats fed on brown food dye for three months, he attributed these changes in liver function to hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood.

The present study recorded an elevation in alkaline phosphatase (ALP) activity in rats treated with high dose of tartrazine. The elevation in serum ALP may be an evidence of obstructive damage in the liver tissue due to tartrazine administration. This observation is in agreement with that reported by Chakravarty *et al.* (2005). Regarding to the serum total lipids, it was found that administration of tartrazine with a dose 75 mg/kg led to a significant decrease throughout the experimental period. This effect may be consequently due to the nature of the studied food additive which works as cholesterol – lowering agents (Ahmed *et al.*,

1987). The present results showed that rats which were administered the high dose of tartrazine exhibited a significant elevation in serum glucose during the first period of the experiment. The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis by the liver with a temporarily loss of endocrine functions of pancreas leading to hyperglycemia.

Serum total protein in this study exhibited a significant increase in rats received the high dose of tartrazine. Our finding is in agreement with the findings of El-Saadany (1991). The accumulation of serum protein can be attributed to the stimulation of protein biosynthesis to produce the specific enzymes required for all processes. The globulin fraction in the serum of rats studied, recorded an increase induced by high dose of tartrazine. The specific elevation in globulin fraction pointed towards an increase in immunoglobulin synthesis, the defense mechanism which aims to protect the body from the toxic effects of this synthetic food colourant.

The present study revealed that the blood urea and serum creatinine in rats supplemented with low and high doses of tartrazine.

In conclusion, it was clear that the administration of the high dose of tartrazine to rats caused many disturbances in the hematological and biochemical parameters. Finally, more extensive assessments of azo dye additives in general and tartrazine in particular is warranted.

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