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Toxicity Induced after Subchronic Administration of the Synthetic Food Dye Tartrazine in Adult Rats, Role of Oxidative Stress

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Abstract

The present study was conducted to evaluate the toxic potential of tartrazine, a food color, in different tissues in adult rat: blood, liver, kidneys, and spleen. Tartrazine was administered orally at a dose of 300 mg/kg of body weight to adult male Wistar rats during a period of 30 days. Tartrazine treatment led to an increase in platelets count, a reduction in peripheral lymphocytes and in spleen T CD8-lymphocytes. Furthermore, tartrazine increased the activities of hepatocellular enzymes and promoted changes in kidney biomarkers. In order to explore the possible mechanism involved, oxidative-stress assessment was performed. Results identified critical oxidative alterations in all tested organs, as shown by the promotion of lipid peroxidation and the modification of endogenous antioxidant-defense enzymes. Thus, tartrazine is able to induce in adult rats' hematotoxicity, immunotoxicity, and liver and kidney injuries by changing the whole balance between oxidants and antioxidants.

Keywords: E102; Foodstuff; Toxicity; Oxidative stress; Adult rats.

Abbreviations

ADI: acceptable daily intake; ALT: alanine transaminase; A/G: albumin/globulin ratio; ALP: alkaline phosphatase; AST: aspartate transaminase; BW: body weight; CDNB: 1-chloro, 2,4-dinitrobenzene; EDTA: Ethylenediaminetetraacetic acid; GSH: Glutathione; GST: Glutathione S-transferase; HDL: high-density lipoprotein; HGB: hemoglobin concentration; HT: hematocrit; JECFA: Joint FAO/WHO Expert Committee on Food Additives; LDH: lactate dehydrogenase; LDL: low-density lipoprotein; MDA: malondialdehyde; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; NOAEL: no observed adverse effect levels; PLT: platelets; RBC: red blood cell count; ROS: reactive oxygen species; SEM: standard error mean; TBA: thiobarbituric acid; TC: total cholesterol; TCA: trichloroacetic acid; TG: triglyceride; vLDL: very low density lipoprotein; WBC: white blood cell count.

1. INTRODUCTION

Food additives are now in almost all of our food or liquids. They make it more attractive and appetizing, improving their appearance and taste. Within the food industry, many kinds of natural and synthetic dyes are used. Compared with natural dyes, synthetic ones have numerous advantages: chemical stability to oxidation and to the change of pH and temperature, intensity and uniformity of the color obtained, a long life and low production costs. Although there are strict guidelines for chemicals to be approved as food additives, the safety of food colorants has not been proven. Therefore, acceptable daily intake (ADI) has been used to minimize any potential deleterious effect of synthetic dyes.

Most popular synthetic dyes are azo dyes: quinoline yellow (E104), sunset yellow (E110), and also tartrazine, known as E102, FD & C Yellow No. 5, and C.I. 19140. Tartrazine is widely used in drugs, cosmetics, and food products. This dye is produced by assimilation with saffron, prized for its flavor and aroma as a natural food coloring. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1996 established an ADI of 0-7.5 mg/kg of body weight (BW)/day.

In humans, tartrazine induces food-intolerance reactions and hyperactivity, especially in children [1]. As regard to its potential toxicological risk in rodents, Davis *et al*. have designed a chronic toxicity study (2 years) in rats given tartrazine (0.5-5%) in the diet [2]. They showed that tartrazine didn't affect the incidence of tumors and the common incidental diseases. Another 2 years toxicity study conducted by Maekawa *et al*. reported that tartrazine in drinking water (1-2%) didn't show any carcinogenic effects on rats [3]. Borzelleca and Hallagan have also reported that the no observed adverse effect levels (NOAEL) of tartrazine were 2,641 and 3,348 mg/kg of BW/day for males and females, respectively [4]. More recently, tartrazine was shown to inflame the rat stomach lining, increasing the number of lymphocytes and eosinophils [5]. Amin *et al*., who were interested in young male rats, showed adverse effects in biochemical markers of liver and kidney with 15 or 500 mg of tartrazine/kg of BW in drinking water [6].

Interestingly, recent studies have reported tartrazine as a potential agent for inducing oxidative stress by the production of reactive oxygen species (ROS) and disturbance of pro-oxidant and antioxidant balance, respectively, in rat liver [6, 7] and brain [8]. Tartrazine is transformed into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal microflora [5], and the formed aromatic amines can generate ROS by interaction of these amino groups with nitrite or nitrate-containing foods or in the stomach. The ROS such as superoxide anion, hydroxyl radical, and H_2O_2 could be produced in the metabolism of nitrosamines and increase oxidative stress [9] leading to organ injuries.

In order to evaluate the general toxic potential of tartrazine, adult male Wistar rats were exposed to a high subchronic tartrazine dose, and damages in vital organs were challenged using hematological and immunological balances, liver and kidney biomarkers, and lipid profile. Furthermore, in order to bring up insights into the mechanism possibly involved in tartrazine toxic effects, some biomarkers of the oxidative stress were tested.

2. MATERIALS AND METHODS

2.1. Chemicals

Tartrazine used in this study is a product composed of 60% tartrazine and 40% starch (commercialized product in France).

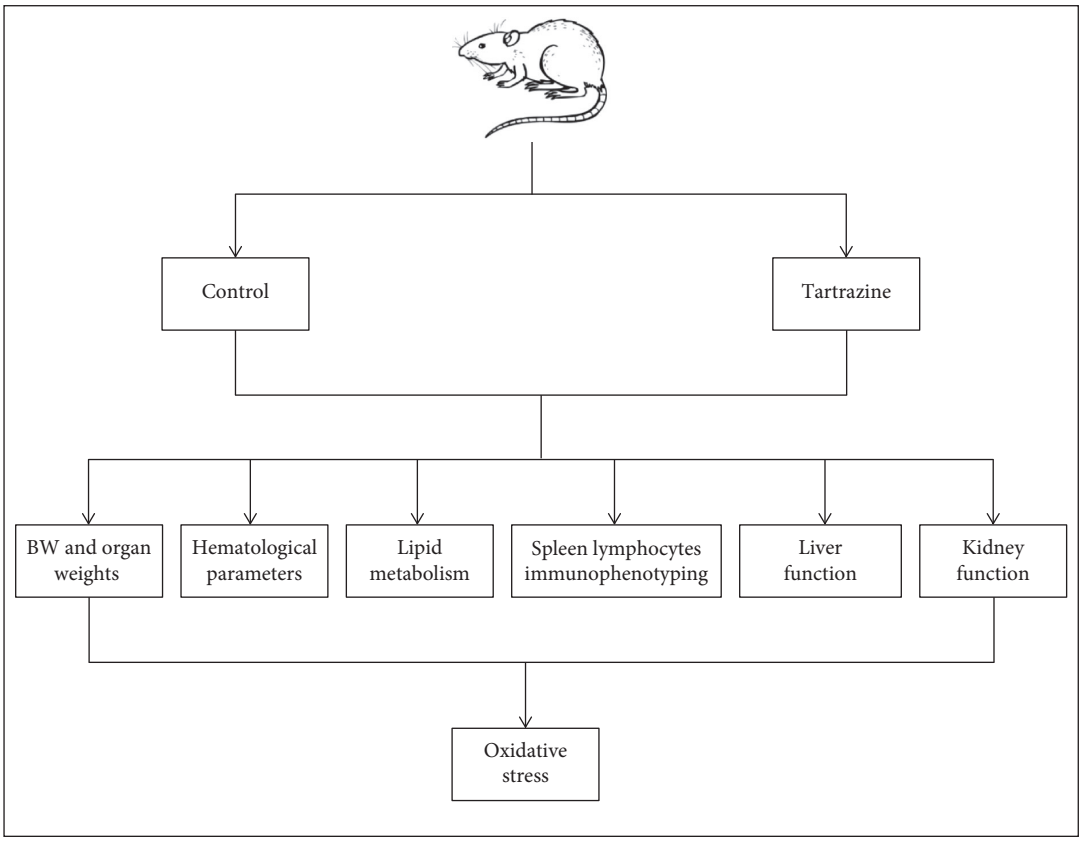
2.2. Animals

Male Wistar rats weighting 130 ± 40 g were purchased from (Société des Industries Pharmaceutiques de Tunisie) SIPHAT (Tunis, Tunisia). Before beginning the experiment, all animals were acclimated for 1 week under well-controlled conditions of temperature (22 ± 2°C), relative humidity (70 ± 4%), and a 12/12 h light-dark cycle with 07:30-19:30 being light phase. Animals were housed 2 in a polypropylene cage. They were fed with standard pellet diet Société Industrielle de Concentré de Sfax (SISCO, Sfax, Tunisia) and given free access to water *ad libitum* all along the experiment. Procedures involving the animals and their care were conformed to the Guidelines for Ethical Control and Supervision in the Care and Use of Animals.

2.3. Experimental Design

A total of 20 rats were randomized into 2 groups of 10 animals each. The first group, corresponding to the control group was given distilled water during a period of 30 days, and the second one (tartrazine-treated group) received 300 mg of tartrazine/kg of BW/day diluted in distilled water (less than 15% of the rat lethal dose 50), by oral route during the same period. Rats were sacrificed by decapitation 24 h after the last treatment. Experimental procedure is depicted in Figure 1.

Figure 1: Experimental procedure. 20 rats were divided as follows: 10 rats control, 10 rats treated with tartrazine (300 mg/kg). We determined body weight and organ weights, hematological and lipid parameters, spleen lymphocytes immunophenotyping, liver, kidney, and oxidative stress markers.



2.4. Body Weight and Organ Weights
BWs were determined at the beginning and on the last day of experiment and were used to calculate the BW gain (BW gain = Final BW minus initial BW). After decapitation, spleen, liver, and kidneys were carefully dissected out, made free from adherents, weighed immediately on an electronic balance to the nearest mg and stored at −80°C.

2.5. Blood Sampling
After decapitation, arteriovenous blood was quickly collected and centrifuged at 1,000 g for 10 min at 4°C. Plasma aliquots were stored at −80°C until use.

2.6. Hematological Parameters
Blood samples were quickly collected on Ethylenediaminetetraacetic acid (EDTA) tubes and immediately analyzed using an automatic hematological assay analyzer (BC-2800 VET mindray auto hematology analyzer) in order to determine red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cell count (WBC), lymphocytes, monocytes and granulocytes.

2.7. Lipid Profile
Plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglyceride (TG) concentrations were measured using commercially available diagnostic kits supplied by Randox Laboratories (UK).

2.8. Spleen Lymphocytes Immunophenotyping by Flow Cytometry
Spleen was transferred to a petri dish containing 50 ml of RPMI-1640 and was chopped up using two frosted slides. Tissue dispersion was centrifuged at 1,200 g at 4°C for 10 min, and the pellet was resuspended in 3 ml of RBC lysing containing 0.83% NH_4Cl in 100 mM Tris buffer (pH 7.4) and kept at room temperature for 3 min. Cells were washed three times with RPMI-1640 and finally suspended in 1 ml of complete medium. Spleen cells phenotyping was done as previously described [10]. Splenocytes cell suspension in RPMI-1640 (1×10^6 cells/ml) was washed with PBS and incubated with anti-CD3, anti-CD4, and anti-CD8 antibodies according to manufacturer's instruction. Cell subtypes were determined using BD FACS Vantage™ flow cytometer.

2.9. Liver and Kidney Biochemical Markers
Activities of cellular enzymes: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were assayed in plasma using Randox Laboratories (UK) diagnostic kits. Enzyme activities were expressed in international units per liter (U/l). Creatinine, uric acid, and urea concentrations were assayed in plasma. Total protein content and albumin level in serum were determined spectrophotometrically according to the method of Biuret using Bovine Serum Albumine as standard. As serum proteins comprise albumin and globulins, globulins concentration was calculated as follows: total proteins concentration – albumin concentration. Thus, albumin/globulin ratio (A/G) was estimated.

2.10. Oxidative Stress Measurement
2.10.1. Lipid Peroxidation
Lipid peroxidation was evidenced by measuring the formation of malondialdehyde (MDA) using the method of Buege and Aust [11]. Briefly, 0.1 ml of tissue supernatant and 1.9 ml of 0.1 M sodium phosphate buffer (pH 7.4) were incubated at 37°C for 1 h. After the incubation, the mixture was precipitated with 10% TCA (trichloroacetic acid) and centrifuged (2,300 g for 15 min at room temperature) to collect supernatant. Then 1 ml of 1% TBA (thiobarbituric acid) was added to the supernatant and placed in boiling water for 15 min. After cooling to room temperature, absorbance was taken at 532 nm and was converted to MDA and expressed in nmol per mg protein using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.10.2. Determination of Total Thiol Groups Level
50 μl of tissue supernatant was mixed with 1 ml of the Tris base (0.25 M)-EDTA (20 mM) buffer, pH 8.2, and absorbance was measured at 412 nm. To this, was then added 20 μl of 10 mM DTNB. After 15 min at ambient temperature, absorbance was measured again with a DTNB blank [12]. Results were expressed in mM.

2.10.3. Catalase Activity Assay
Catalase activity was assayed by the method previously descried [13]. Briefly, the assay mixture consisted of 0.05 M phosphate buffer (pH 7.0), 0.019 M H_2O_2, and 0.03 ml PMS in a total volume of 0.3 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of μmol of H_2O_2 consumed per minute per mg of protein.

2.10.4. Glutathione S-transferase Assay
Glutathione S-transferase (GST) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig *et al*. [14]. S-2,4-dinitrophenyl glutathione (CDNB)

was used as a substrate. The principle of the method is based on the measurement of the conjugation of CDNB with reduced glutathione. The formation of adduct of CDNB was monitored by measuring the net increase in absorbance at 340 nm against the blank. GST activity was expressed in terms of nmol of CDNB-GSH conjugate formed per minute per mg of protein.

2.11. Statistical Analysis

Data were expressed as mean ± standard error mean (SEM). Statistical test t-student was carried out to find significant difference between means recorded for control and treated animals. We used the "STATISTICA" software to assess whether differences were significant or not. $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Clinical Observations, Body Weight, and Organ Weights

The administered dose of tartrazine didn't show any mortality or sign of toxicity. No variation of absolute or relative weights of liver, kidneys, or spleen was recorded after tartrazine treatment (Table 1). However, despite this apparent nontoxicity, tartrazine-treated rats decreased significantly in BW gain (−28%) when compared to the control group.

3.2. Hematological Parameters and Immunological Toxicity

3.2.1. Effect on Blood Cells

No statistically significant changes were found in RBC, HGB, HT, MCV, MCH, and MCHC values (Table 2). However, a significant increase was observed in PLT count in treated rats (from 369.86 ± 69.79 × $10^3/\mu l$ in control rats to 617.86 ± 40.51 × $10^3/\mu l$). Moreover, compared to control, lymphocytes number showed a significant decrease in tartrazine-treated group (−22%). There was no significant change regarding WBC, monocytes, and granulocytes values (Table 2).

Table 1: Effect of tartrazine on body weight gain, liver, kidneys, and spleen weight in rats.

	Control group	Treated group
Initial weight (g)	114.1 ± 4.6	126.1 ± 5.3
Final weight (g)	270.4 ± 4.8	239.4 ± 6.2
Body weight gain (g)	156.3 ± 5.2	113.3 ± 4.2***
Absolute liver weight (g)	10.95 ± 0.43	10.58 ± 0.34
Relative liver weight (%)	4.07 ± 0.11	4.37 ± 0.11
Absolute kidneys weight (g)	0.98 ± 0.04	0.95 ± 0.03
Relative kidneys weight (%)	0.37 ± 0.01	0.39 ± 0.01
Absolute spleen weight (g)	0.86 ± 0.08	0.92 ± 0.06
Relative spleen weight (%)	0.32 ± 0.04	0.38 ± 0.03

Results were expressed as the mean ± SEM ($n = 10$). Values significantly different from control group were indicated as ***$p < 0.001$.

Table 2: Effect of tartrazine on hematological parameters in blood rats.

	Control group	Treated group
RBC ($10^6/\mu l$)	7.86 ± 0.22	8.01 ± 0.20
HGB (g/l)	124.14 ± 2.91	125.71 ± 2.21
HT (%)	44.01 ± 1.22	44.69 ± 0.78
MCV (fl)	56.10 ± 0.67	54.67 ± 0.47
MCH (pg)	15.77 ± 0.19	15.67 ± 0.14
MCHC (g/l)	281.86 ± 1.79	287.14 ± 1.61
PLT ($10^3/\mu l$)	369.86 ± 69.79	617.86 ± 40.51*
WBC (cells/μl)	6,700 ± 770	5,543 ± 482
Lymphocytes (cells/μl)	4,400 ± 432	3,414 ± 273*
Monocytes (cells/μl)	286 ± 34	229 ± 29
Granulocytes (cells/μl)	2,014 ± 347	1,900 ± 206

Results were expressed as the mean ± SEM per microliter of blood. ($n = 10$). Values significantly different from control group were indicated as *$p < 0.05$.

3.2.2. Effect on Spleen Cellularity

In order to evaluate the tartrazine impact on spleen cellularity, flow cytometry was performed on splenocytes preparations using anti-CD3, anti-CD4, and anti-CD8 antibodies. Data are presented in Figure 2. A significant decrease in the percent of CD8+ cells was observed in tartrazine-treated rats (22.12 ± 0.59%) when compared to control rats (26.42 ± 1.46%), whereas there was no significant change in the proportions of CD3+ and CD4+ cells.

3.3. Effect on Hepatic Function

To evaluate tartrazine hepatotoxicity, liver enzymes such as AST, ALT, LDH, and ALP were used as biomarkers. As shown in Table 3, there was a significant increase in transaminases (+50% for AST and +28% for ALT), ALP (+16%), and LDH (+33%) activities in the plasma of tartrazine-treated rats. Moreover, total protein, albumin and globulin levels were significantly reduced after tartrazine treatment (respectively −8, −9, and −35%). The calculated A/G ratio was increased in tartrazine-treated group compared with control (+97%).

3.4. Effect on Kidney Function

As shown in Table 3, a significant increase in creatinine (+19%), uric acid (+26%), and kidney proteins level (+15%) was observed following tartrazine exposure.

3.5. Effect on Lipid Status

Effects of tartrazine on lipid parameters are shown in Table 4. Tartrazine increased significantly plasma TG content (+82%) and decreased TC level (−30%). There was no change in the rate of HDL (Table 4). LDL rate was significantly decreased (−79%).

Figure 2: Effect of tartrazine on the proportions of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes in the spleen (CTR: control group, TAR: tartrazine-treated group). Results were expressed as the mean ± SEM (n = 10).*$p < 0.05$.

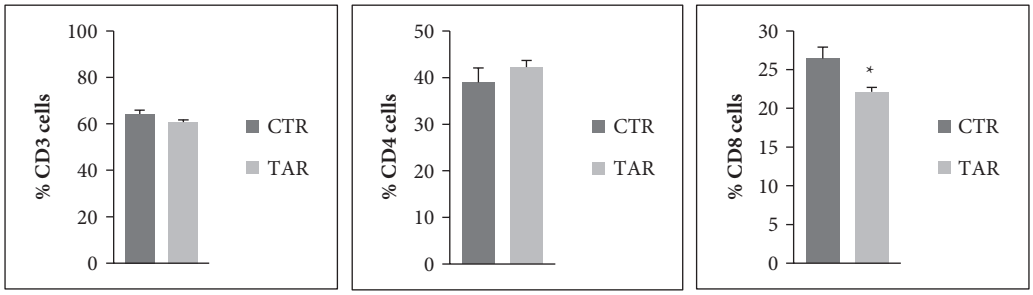


Table 3: Effect of tartrazine on hepatic and renal injury markers in rats.

	Control group	Treated group
AST (U/l)	80.64 ± 9.75	120.53 ± 11.59*
ALT (U/l)	29.01 ± 2.18	37.02 ± 3.24*
ALP (U/l)	323.98 ± 16.87	376.23 ± 26.88
LDH (U/l)	1,098.09 ± 70.27	1,457.10 ± 139.06*
Liver proteins (g/100 g)	11.75 ± 0.53	11.65 ± 0.19
Total proteins in serum (g/l)	53.06 ± 1.22	48.75 ± 1.73*
Albumin (g/l)	34.89 ± 0.32	31.70 ± 0.89*
Globulin	19.89 ± 1.34	13.00 ± 1.61*
Albumin/globulin ratio	1.69 ± 0.11	3.33 ± 0.45**
Creatinine (mg/l)	10.87 ± 0.28	12.93 ± 0.30**
Urea (g/l)	0.45 ± 0.01	0.42 ± 0.01
Uric Acid (mg/l)	15.16 ± 0.60	19.11 ± 0.88*
Kidneys proteins (g/100 g)	9.30 ± 0.39	10.71 ± 0.61*

Results were expressed as the mean ± SEM (n = 10). Values significantly different from control group were indicated as *$p < 0.05$ and **$p < 0.01$.

Table 4: Effect of tartrazine on lipid status in rats.

	Control group	Treated group
TG (g/l)	0.97 ± 0.05	1.86 ± 0.11***
TC (g/l)	0.54 ± 0.05	0.38 ± 0.02*
HDL (g/l)	0.069 ± 0.006	0.078 ± 0.003
LDL (g/l)	0.42 ± 0.04	0.09 ± 0.02***
vLDL (g/l)	0.19 ± 0.01	0.37 ± 0.02***

Results were expressed as the mean ± SEM ($n = 10$). Values significantly different from control group were indicated as*$p < 0.05$ and ***$p < 0.001$.

Table 5: Effect of tartrazine on oxidative stress status in rats.

		Control group	Treated group
Spleen	MDA (nmol/mg protein)	0.019 ± 0.003	0.035 ± 0.003*
	Total Thiol level (mM)	0.292 ± 0.082	0.140 ± 0.060
	Catalase (μmol/min/mg protein)	0.126 ± 0.013	0.193 ± 0.012**
	GST (nmol/min/mg protein)	0.488 ± 0.026	0.700 ± 0.054*
Liver	MDA (nmol/mg protein)	0.193 ± 0.023	0.320 ± 0.014**
	Total Thiol level (mM)	1.012 ± 0.016	1.115 ± 0.022*
	Catalase (μmol/min/mg protein)	0.062 ± 0.024	0.043 ± 0.006
	GST (nmol/min/mg protein)	12.666 ± 1.210	8.384 ± 0.579**
Kidney	MDA (nmol/mg protein)	0.049 ± 0.005	0.184 ± 0.024***
	Total Thiol level (mM)	0.678 ± 0.016	0.904 ± 0.016***
	Catalase (μmol/min/mg protein)	0.122 ± 0.025	0.176 ± 0.015*
	GST (nmol/min/mg protein)	1.670 ± 0.055	1.998 ± 0.080**

Results were expressed as the mean ± SEM ($n = 10$). Values significantly different from control group were indicated as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

3.6. Effect on Oxidative Stress Status

In spleen, tartrazine led to a significant increase of MDA and antioxidant enzymes (catalase and GST) activities. Thiol groups level was decreased. In liver, subchronic administration of tartrazine led to a significant increase in MDA and total thiol groups levels. Antioxidant enzymes activities were decreased. In kidney, MDA, total thiol groups levels and antioxidant enzymes activities were increased (Table 5).

4. DISCUSSION

To determine the safety of chemicals for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a "safe" dose in humans [15]. In this study, we strived to identify the effects of a food dye, still in use in many countries, on rat physiological parameters, and further to explore the possible mechanisms involved. We considered a dose of tartrazine less than 15% of the lethal dose 50 estimated by Sasaki *et al.* [16]. In fact, there isn't any well-established LD50 in rats.

Tartrazine-treated rats showed a significant decrease in BW gain. These data are in agreement with Amin *et al.* who reported a reduction of the gain of young male Wistar rats exposed to 15 or 500 mg of tartrazine/kg of BW for 30 days [6]. A similar finding was also reported by El-Wahab and Moram, with adult male albino rats treated with tartrazine during 42 days [7].

BW loss is considered to be a good reliable sensitive toxicity indicator. Thus, the BW loss in the present study may represent the first marker of dye adverse effect. Moreover, El-Wahab and Moram proposed that synthetic food colorants might bind to the bacterial cell surface in the rat's intestine, leading to a decrease in the number of the active bacterial cells and an inhibition of the food absorption capacity at the intestinal surface, leading to a BW decrease [7].

Regarding the hematological findings, there were few differences between the control group and the treated group. However, rats exposed to tartrazine showed a significant increase in the number of platelets and a significant decrease in the number of lymphocytes.

Only one study was interested in the hematological changes induced by a lower dose (10 mg/kg of BW/day) of tartrazine to adult Wistar rats. They didn't observe any variation in the number of platelets or lymphocytes [17]. The rise of platelet counts can be reactive and secondary to a pathological condition such as infection, inflammation, iron deficiency, stress ... [18]. So, the increase observed in our study could be explained by the potentially deleterious effect of this dye stuff. Moreover, an increase of the platelet counts could lead to a risk of thrombosis and cardiovascular disease.

Tartrazine-exposed rats showed decreased peripheral lymphocytes, suggesting a potential reduction of hematopoiesis, in particular, at the lymphoid lineage or an increase of cell death. They also exhibited a decreased proportion of CD8+ T lymphocytes in the spleen. T lymphocyte subpopulations play important roles in T lymphocyte immune function and the proportions of CD3+, CD4,+ and CD8+ cells are thus important to investigate immunotoxicity. To our knowledge, no other study was interested in immunophenotyping of T lymphocytes in the spleen after treatment with tartrazine. Tartrazine is able to bind to double-stranded DNA, causing its degradation in vitro in a dose of 0.02-8 mM [19]. Thus, in light of our previous findings, we hypothesize that tartrazine could have deleterious effects on lymphocyte proliferation, inducing cell death, acting on lymphocytes DNA. However, more investigations need to be performed in order to understand tartrazine immunosuppression.

To evaluate tartrazine hepatotoxicity, enzymes (AST, ALT, ALP, and LDH) were used as hepatic marker enzymes. In this study, subchronic exposure to tartrazine caused an increased activity of ALT, AST, ALP, and LDH. These data are in agreement with Amin *et al*. who showed an increase in serum ALT, AST, and ALP activities using 500 mg of tartrazine/kg of BW/day in young rats, during a 30 day period [6]. Same results were found by El-Wahab and Moram [7]. The disturbance in hepatic enzymes activities reflects the damage caused by tartrazine in hepatocytes, which subsequently causes the release of intracellular enzymes in the blood. It is known that ALT is located in the cytoplasm, and AST is located mainly in organelles such as mitochondria [20]. Therefore, increased plasma levels of AST and ALT suggested damage of both hepatic cellular and mitochondrial membranes. Similarly, the increase in plasmatic LDH could be attributed to cell injury induced by tartrazine.

On the other hand, liver synthesizes proteins, among which is albumin. Serum albumin was significantly reduced after tartrazine treatment, as well as serum total protein and globulin. Although this result is in contrast with the studies of Amin *et al*. and El-Wahab and Moram that reported a significant increase in serum albumin concentration [6, 7], it suggests that tartrazine exposure leads to deleterious effects on the synthetic function of treated rats' livers. Moreover, A/G ratio is a predictable marker of the toxicological potential of various substances. Tartrazine induced an increase in A/G ratio, suggesting liver impairment.

The significant decrease in the cholesterol level after tartrazine treatment is in agreement with results of Amin *et al*. showing depletion rates of cholesterol in rats administered 15 and 500 mg of tartrazine/kg of BW/day [6]. However, Himri *et al*. reported a significant increase in cholesterol rate after exposure to 10 mg of tartrazine/kg of BW/day [17]. Cholesterol is a lipid family of sterols produced from Acetyl-CoA. The TC content depends on the balance between cholesterol formed in the body and the rate absorbed from the diet. Since the liver is the main site of production of cholesterol (20-25% of daily production), the deviation of its normal in the blood is considered to be another marker of liver damage [21].

The increase in TG levels observed in our study is consistent with Amin *et al*. and Himri *et al*. [6, 17]. Several studies have reported that the increase in TG levels might be caused by an alteration of the activity of hepatic lipase, responsible for the catabolism of TGs [22]. Thus, in the case of liver impairment, the lipase activity will be decreased resulting in hypertriglyceridemia.

On the other hand, our results showed a significant decrease in LDL cholesterol of treated rats, which disagrees with Amin *et al*. [6]. LDL is produced by the liver from the very low density lipoprotein (vLDL). So, the significant decrease observed in our study could be attributed to the deterioration of hepatocytes, which will induce accelerated degradation of LDL cholesterol.

The kidney is one of the target organs attacked by tartrazine exposure. Our study showed that tartrazine induced a significant increase in plasma creatinine and uric acid. These results are partially consistent with that of Amin *et al*., Himri *et al*., El-Wahab and Moram [6, 7, 17] who found elevated creatinine in treated rats. Increased creatinine level is observed when renal function is disturbed. Although we haven't performed kidney histological studies, our results may suggest a toxic nephropathy due to changes in the structure of the kidneys. In fact, histological damages were revealed in the kidneys of guinea pigs receiving tartrazine in water at 1, 2, and 3% for 3 months [23].

Altogether, our results showed that a subchronic exposure to the food dye tartrazine has impaired effects in adult rat vital organs, causing changes not only in blood parameters and immune response but also in hepatic and renal functions.

Our results revealed an important imbalance between pro- and antioxidants markers in tartrazine-treated rats. Foremost, an increase of MDA, the most common peroxidation products of lipids group, was observed in all tested organs. This peroxidation could lead to a decrease of membrane fluidity and disruption of membrane integrity and function, inducing serious pathological changes [24].

In liver and kidney, tartrazine induced an increase in total thiols group, identifying a possible struggle of nephrotic and hepatic cells against ROS produced by tartrazine treatment.

Finally, the homeostasis of antioxidant defenses was altered, as shown by modifications in catalase and GST activities. Antioxidant activities pass through two phases during induced toxicity. Firstly, they increase gradually to a maximum, the initial adaptation of the cell in response to free radicals. When concentrations of free radicals exceed the antioxidant capacity to neutralize, the antioxidant activities fall.

Catalase accelerates the degradation of hydrogen peroxide in water molecules and oxygen to prevent the formation of hydroxyl radicals, acting as the first line of defensive antioxidant enzyme [25]. In our study, tartrazine increased catalase and

GST activities in spleen and kidneys, whereas, there were decreased in liver. So, we identified different responses of catalase and GST, depending on target organ, showing a tissue-specific disturbance of oxidative-stress status.

In liver, maximum MDA observed was associated with alteration in GST activity. GST plays an important role, especially in the detoxification of xenobiotics, as it catalyzed the reaction of such compounds with Glutathione (GSH), neutralizing their electrophilic sites and rendering their products more water soluble [6]. So, a subsequent reduction in hepatic GST activity is suggestive of impaired hepatic detoxifying capacity.

Tartrazine is known to generate aromatic amines [5], which produce ROS, increasing oxidative stress [9]. As a result of the ROS formation, the antioxidant defense mechanism of the cells including catalase and GST began to be consumed to prevent the cell death by these toxic radicals, so their levels in the tissue homogenate were changed. On the other hand MDA level was increased as a product of lipid peroxidation through the ROS action on lipids of cellular membrane. Produced ROS would induce the activation of a large number of enzymes like proteases, lipases, and DNases that are actively involved in the destruction of the cell. Proteases such as calpain degrade spectrin, part of the cytoskeleton, carrying the collapse of the cell and leading to cell death and tissue damage.

5. CONCLUSION

Subchronic exposition to tartrazine (only 30 days) leads to impaired vital organs in adult rats, causing impairments in blood parameters, immune response, hepatic and renal functions. This food dye must be used with care.

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Author Contributions
Narges El Golli: concept, acquisition and analysis of data, redaction.
Ines Bini-Dhouib: acquisition of data, redaction.
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