academicJournals

Vol. 12(28), pp. 4519-4529, 10 July, 2013
DOI: 10.5897/AJB2013.12125
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

A thirteen week *ad libitum* administration toxicity study of tartrazine in Swiss mice

Nabila Mehedi[1]*, Nawel Mokrane[1], Omar Alami[1], Soraya Ainad-Tabet[1], Chahinaize Zaoui[2], Omar Kheroua[1] and Djamel Saidi[1]

[1]Laboratory of Physiology of Nutrition and Food Safety, Department of Biology, University of Oran, El Menaouer 31000 Oran, Algeria.
[2]Laboratory of Developmental Biology, Department of Biology, University of Oran, El Menaouer 31000 Oran, Algeria.

Accepted 5 July, 2013

Tartrazine is a colorant widely used in food products, drugs and cosmetics. The current study evaluates the effect of sub-chronic ingestion of tartrazine in drinking water at doses of 0, 0.1, 0.45, 1 and 2.5% for 13 weeks in mice. Our results show that female body weight gain and food consumption decreased in all treated groups, while fluid consumption increased. The red blood cell count, hemoglobin and hematocrit were increased in male 2.5% treated groups and the white blood cell count decreased in all treated groups. In both sexes of the 2.5% doses groups, total proteins, albumin, creatinine, urea, uric acid, total bilirubin, alkaline phosphatase and transaminases were higher. Histological examinations showed brain, liver and kidney damages in animals treated with 1 and 2.5% doses. We concluded that at doses of 1 and 2.5% in drinking water, tartrazine induces weight depression and adverse effects on brain, liver and kidney.

Key words: Tartrazine, subchronic toxicity, hematology, biochemical parameters, histology.

INTRODUCTION

Color is a vital constituent of food which imparts distinct appearance to the food product. However, artificial coloring becomes a technological necessity as foods tend to lose their natural shade during processing and storage. tartrazine [FD&C no. 5, Colour Index (CI) food yellow 4] is a monazo dye, especially found in drinks and juices (Rao and Baht, 2003; Husain et al., 2006), cosmetics as well as drug products. Also, this food colorant is many used in cooking in developing countries as a substitute for saffron (Mehedi et al., 2009). The acceptable daily intake (ADI) of human is 0 to 7.5 mg/kg bw (JECFA, 1996).

In toxicological studies of tartrazine, some authors studied the carcinogenic and mutagenetic effects of tartrazine. No carcinogenic effect in chronic toxicity study (two years) of rats and mice have been reported at levels of 1.0 to 5.0% of tartrazine in drinking water or diet (Maekawa et al., 1987; Borzelleca and Hallagan, 1988a; Borzelleca and Hallagan, 1988b). Genotoxicology studies showed that tartrazine has possible clastrogenic activity (Ishidate et al., 1981; Ishidate et al., 1984; Giri et al., 1990; Durnev et al., 1995), may induce chromosomal aberrations in mammalian cells (Patterson and Butler,

*Corresponding author. E-mail: nabilamehedi@gmail.com. Tel: 213 550231610. Fax: 213 41513025.

Abbreviations: ADI, Acceptable daily intake; **ALP,** alkaline phosphatase; **ALT,** alanine aminotransferase; **AST,** aspartate aminotransferase; **γ-GT,** γ-glutamyl transferase; **Ht,** hematocrit; **Hb,** hemoglobin; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **MCV,** mean corpuscular volume; **RBC,** red blood cell count; **TB,** total bilirubin; **TC,** total cholesterol; **TG,** triglyceride; **TP,** total proteins; **WBC,** white blood cell count.

1982) and DNA damage in the colon of mice by comet assay (Sasaki et al., 2002). tartrazine has no mutagenic and no genotoxic in *Salmonella typhimurium* in the Ames test (Das and Mukherjee, 2004). Acute oral exposure to tartrazine did not induce genotoxic effect in the gut micronucleus assay in mice (Poul et al., 2009) but Mpountoukas et al. (2010) show that tartrazine has a toxic potential to human lymphocytes *in vitro* and it seems that they bind directly to DNA. tartrazine induced adverse effects in learning and memory functions in animals (Gao et al., 2011) and behavioral changes have been reported in children (Rowe and Rowe, 1994; Ward, 1997; Schab and Trinh, 2004; Bateman et al., 2004; McCann et al., 2007). Human studies indicated that tartrazine can induce wide range of allergic reactions in sensitive or atopic individuals (Devlin and David, 1992; Nettis et al., 2003; Inomata et al., 2006; Ould Elhkim et al., 2007; Hannuksela and Haahtela, 2009).

Data in the literature reported that intakes of tartrazine for children in developing countries like United Arab Emirates and India exceeded their ADIs (Husain et al., 2006; Rao et al., 2004; Dixit et al., 2010; Dixit et al., 2011) because children are the major consumers of colored food. The prescribed limit of tartrazine and other synthetic colorants in food product samples was also exceeded (Dxit et al., 2011). Thus, exposure of excessive colorants to those vulnerable in the population may pose a health risk. Most of the researches in the field of toxicological effects of tartrazine on hematological and biochemical parameters as well as histopathology was conducted on male rats or the sex of animals was not specified (Amin et al., 2009; Himri et al., 2011). Swiss mice are widely used in toxicology research because they respond favorably. Therefore, the aim of this work was to study the effect of subchronic excessive ingestion of tartrazine in Swiss mice.

MATERIALS AND METHODS

Chemicals

Tartrazine (C.I. 19140, CAS No 1934-21-0, Mw 534,37, synonyms: E 102,Food yellow 4, FD&C yellow No.5) is an azo dye with the chemical formula 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-((4-sulfophenyl)azo)-1H-pyrazole-3-carboxylic acid, and trisodium salt was obtained from Courtex International, France. Purity of 86.7% was guaranteed by the manufacturer. All chemicals used were purchased from Merck, Germany.

Animals and treatments

The studies were conducted on male and female Swiss albino mice, four weeks old. They were obtained from Pasteur Institute, Algiers, Algeria. Animals were kept under conditions of ambient room temperature and relative humidity. Tartrazine was diluted in water. Mice were divided into five groups of 12 animals each. The first group was given drinking water as a control; the second received drinking water that contained 0.1% tartrazine (low dose level), in the third, the drinking water contained 0.45% tartrazine (presents the no observed adverse effect levels (NOAEL)) (Tanaka, 2006; Tanaka et al., 2008), for the fourth, the drinking water contained 1% (high dose level) and for the fifth, the drinking water contained 2.5% (high dose level) tartrazine each for 13 weeks (Maekawa et al., 1987; Borzelleca and Hallagan, 1988a; Borzelleca and Hallagan, 1988b). Standard food pellets diet and water were given *ad libitum* for the duration of the experiment. All the animals were used following the instructions of the guidelines for animal use of Oran University.

Gross observation and measurement of body weight and food consumption

The animals were observed daily for general conditions. They were weighed on the first and the last days of the period, and on the day of necropsy. Food and liquid consumption were measured daily and mean daily intake of tartrazine (mg/kg/day) during the administration period was calculated.

Hematological examination

After the 13 weeks administration, all the animals were fasted for 18 h. Blood was taken from the retroorbital vena, using EDTA-2K as an anticoagulant, and then these animals were killed by cervical dislocation. The following items were measured manually with a hematocytometer: red blood cell count (RBC) and white blood cell count (WBC). Hemoglobin was determined by colorimetric method (cyanomethemoglobin). Hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Blood biochemistry

Parameters for serum biochemistry were analyzed using sera frozen after centrifugation of whole blood at 3080 × *g* for 15 min (Sigma 4 K10 bioblock scientific, Germany). The concentrations of total protein (TP), albumin, total cholesterol (TC), glucose, urea, uric acid, creatinine, triglyceride (TG), total bilirubin and the activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and δ-glutamyl transferase (δ-GTP) were measured with kits (Elitech, France). The assays were conducted according to the manufacturer's instructions. The absorbance was measured using a spectrophotometer JA.S.CO V-530 (UV/vis), Japan.

Pathological examination

Following the blood sampling, the animals were killed by cervical dislocation, autopsied and the following organs and tissues were weighed: brain, thymus, heart, lungs, liver, spleen, kidneys, testes, epididymides, salivary glands, adrenal glands, bladder, seminal vesicles, uterus and ovaries. In addition, the relative weights of these organs and tissues compared to the body weight were calculated. Brain, liver and kidney were fixed in buffered 10% formalin solution.

Statistical methods

The results were presented as means with standard errors. Statistical test one way ANOVA was applied to find significant difference between values of various parameters recorded for control and treated animals and $p < 0.05$ was considered statistically significant.

Table 1. Effect of tartrazine ingestion on body weight gain.

Group	Initial bw (g)	Final bw (g)	Gain bw (g)
Male			
Control	15.5 ± 1.3	37.9 ± 1.3	21.5 ± 1.5[a]
0.1%	15.8 ± 0.9	40.1 ± 0.7	20.1 ± 1.0[a]
0.45%	21.2 ± 0.5	41.3 ± 1.0	19.5 ± 0.8[a]
1%	19.5 ± 0.8	42.9 ± 2.0	26.2 ± 1.3[b]
2.5%	21.3 ± 0.9	38.3 ± 0.6	16.5 ± 0.9[c]
Female			
Control	16.2 ± 0.4	33.6 ± 0.7	21.3 ± 0.6[a]
0.1%	18.9 ± 0.7	34.7 ± 1.3	17.9 ± 1.2[b]
0.45%	21.3 ± 0.7	35.4 ± 1.0	14.1 ± 1.2[c]
1%	17.6 ± 1.2	32.1 ± 1.2	16.4 ± 0.7[b]
2.5%	21.0 ± 0.7	33.9 ± 1.0	13.3 ± 0.6[c]

Values are means ± SE for 12 animals. Means which have different letters are significantly different ($p < 0.05$).

Table 2. Daily intake of fluid containing tartrazine and food for 13 weeks.

Group	Fluid consumption (ml)	Food consumption (g)	Daily intake of tartrazine[a] [mg/kg/day]
Male			
Control	5.3 ± 0.3[a]	15.3 ± 1.7[a]	0.0
0.1%	6.1 ± 0.2[b]	11.8 ± 0.8[b]	173.9
0.45%	5.1 ± 0.2[b]	12.0 ± 0.5[a]	695.4
1%	6.3 ± 0.3[b]	11.0 ± 0.4[b]	1767.8
2.5%	7.9 ± 0.5[c]	11.9 ± 0.6[b]	5541.4
Female			
Control	3.5 ± 0.3[a]	13.6 ± 0.8[a]	0.0
0.1%	5.0 ± 0.3[b]	9.4 ± 0.4[b]	168.2
0.45%	4.8 ± 0.1[c]	10.0 ± 0.5[b]	594.0
1%	4.7 ± 0.2[c]	8.2 ± 0.4[c]	1759.2
2.5%	5.7 ± 0.1[b]	6.8 ± 0.5[c]	4710.7

Values are means ± SE for 12 animals. [a]Calculated from the daily fluid consumption per mouse. Means which have different letters are significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

For the 13 weeks subchronic toxicity study of tartrazine in Swiss albino mice, no death was observed in all the animals treated. The female body weight gain decreased significantly in 0.45, 1 and 2.5% dose groups and in male 2.5% dose group ($p<0.05$), while the male body weight gain increased significantly only in 1% as shown in Table 1; it seems that it is related to tartrazine ingestion. Similarly, Amin et al. (2010) showed a loss body weight gain when administrated tartrazine at low (15 mg/kg bw) and high (500 mg/kg bw) dose orally in males rats for 30 days. Body weight loss is considered to be a good reliable sensitive toxicity indicator (Ezeuko et al., 2007).

Daily food and liquid consumption through the administration period are shown in Table 2. Food consumption values were significantly decreased at all experimental groups compared to the controls ($p<0.05$). However, liquid consumption was increased at all experimental groups ($p<0.05$) except in male 0.45% dose group. The average tartrazine intake calculated from liquid consumption, in mg/kg/day, is shown in Table 2. The reduced food intake was probably due to the high volume of tartrazine fluid drank. Different studies showed an increased consumption of tartrazine in the diet by rats or mice (Borzelleca and Hallagan, 1988a; Borzelleca and Hallagan, 1988b; Collins et al., 1990; Collins et al., 1992).

It seems that tartrazine stimulates the substance

Table 3. Hematology of male and female mice treated with tartrazine for 13 weeks.

Group	RBC (10^6/mm^3)	Hb (g/dl)	Ht (%)	MCV (fl)	MCH (pg/dl)	WBC (x100/mm^3)
Male						
Control	3.8 ± 0.0^a	12.7 ± 0.2^a	36 ± 0.5^a	95.2 ± 0.0^a	33 ± 0.0^a	284.7 ± 16.9^a
0.1%	3.7 ± 1.2^a	12.5 ± 0.4^a	36 ± 1.0^a	95.2 ± 0.0^a	33 ± 0.0^a	133.3 ± 14.8^c
0.45%	4.5 ± 0.0^b	11.9 ± 0.1^b	42 ± 2.0^b	113.9 ± 3.2^c	33 ± 1.2^a	127.2 ± 15.2^c
1%	3.8 ± 0.1^a	12.5 ± 0.7^a	36 ± 1.0^a	95.6 ± 2.2^a	33 ± 1.5^a	76.2 ± 17.7^c
2.5%	4.4 ± 0.2^b	14.6 ± 0.7^b	42 ± 2.0^b	95.2 ± 0.0^a	33 ± 0.0^a	48.6 ± 18.5^c
Female						
Control	3.9 ± 0.3^a	12.9 ± 0.3^a	37 ± 2.0^a	95.2 ± 0.0^a	33 ± 0.0^a	93.2 ± 19.4^a
0.1%	3.6 ± 0.3^a	12.1 ± 0.2^a	35 ± 2.0^a	95.2 ± 0.0^a	33 ± 0.0^a	116.8 ± 26.0^a
0.45%	4.3 ± 0.0^a	11.6 ± 0.1^a	47 ± 2.0^c	110.1 ± 8.2^a	31 ± 2.8^a	94.5 ± 27.3^a
1%	3.7 ± 0.9^a	12.3 ± 0.5^a	35 ± 1.0^a	95.8 ± 2.9^a	34 ± 1.8^a	75.7 ± 10.4^a
2.5%	4.2 ± 0.5^a	13.9 ± 0.3^a	40 ± 2.0^a	95.2 ± 0.0^a	33 ± 0.0^a	22.3 ± 51.1^c

Data for MCHC, mean corpuscular hemoglobin concentration was excluded from this table; no significant changes were found in any group. Values are means ± SE for six animals. Means which have different letters are significantly different ($p < 0.05$).

T**able 4a.** Blood chemistry of male and female mice treated with tartrazine for 13 weeks.

Group	TP (g/dl)	Alb (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Glu (mg/dl)	TC (mg/dl)
Male							
Control	7.6 ± 0.3^a	3.6 ± 0.1^a	1.1 ± 0.1^a	28.3 ± 4.2^a	7.3 ± 0.6^a	170.9 ± 4.0^a	164.0 ± 0.1^a
0.1%	6.9 ± 0.3^c	4.8 ± 0.0^c	1.2 ± 1.0^a	43.1 ± 4.9^a	5.9 ± 0.6^a	174.4 ± 26.7^a	133.1 ± 0.2^a
0.45%	5.9 ± 0.7^c	5.8 ± 0.6^c	0.9 ± 0.1^b	45.5 ± 3.6^a	7.1 ± 0.4^a	224.9 ± 27.5^a	201.6 ± 16.0^b
1%	6.8 ± 0.3^c	5.7 ± 0.2^c	1.9 ± 0.1^b	39.7 ± 4.5^a	9.1 ± 1.2^a	179.4 ± 9.7^a	155.9 ± 0.3^a
2.5%	8.1 ± 0.7^c	6.2 ± 0.2^c	1.9 ± 0.2^b	70.7 ± 1.1^c	18.7 ± 3.5^c	110.0 ± 15.9^c	206.9 ± 0.5^b
Female							
Control	7.9 ± 0.3^a	3.4 ± 0.2^a	1.3 ± 0.1^a	32.4 ± 4.2^a	7.9 ± 0.7^a	163.1 ± 10.7^a	161.7 ± 0.2^a
0.1%	6.2 ± 0.3^c	5.2 ± 0.2^c	1.3 ± 0.2^a	47.4 ± 8.2^a	3.9 ± 0.5^c	164.3 ± 5.9^a	142.8 ± 0.2^a
0.45%	4.5 ± 0.2^c	4.9 ± 0.4^c	1.4 ± 0.2^a	48.9 ± 3.7^a	8.4 ± 1.1^a	236.6 ± 18.8^c	176.8 ± 0.2^a
1%	6.6 ± 0.2^c	5.7 ± 0.2^c	1.3 ± 0.2^a	51.2 ± 8.9^c	8.4 ± 0.5^a	216.4 ± 22.1^a	170.2 ± 0.4^a
2.5%	8.5 ± 0.3^c	6.4 ± 0.2^c	1.9 ± 0.1^b	72.6 ± 7.2^c	15.3 ± 1.5^c	133.9 ± 10.2^a	155.1 ± 0.2^a

Values are means ± SE for 6 animals. Means which have different letters are significantly different ($p < 0.05$).

consumption in which it is incorporated.

The data from hematological and serum biochemical examinations are summarized in Tables 3, 4a and 4b, respectively. In the present study, RBCs, Hb and Ht increased in the males of the 2.5% tartrazine treated group ($p<0.05$). These changes resulted from a heme concentration by dehydration (Balcells, 1998). Also, RBCs and Ht increased in the males 0.45% tartrazine treated group ($p<0.05$). In female, only the Ht was increased in 0.45% tartrazine treated group ($p<0.05$). These findings are similar to those of Sobotka et al. (1977) who indicated that *in utero* ingestion of 0.1 and 2% of tartrazine in rats increased RBCs and Hb during the lactation period. However, Aboel-Zahab et al. (1997) found a decrease in RBCs and Hb of rats whose diets were supplemented with chocolate colors A and B (sunset yellow, tartrazine, carmoisine and brilliant blue) in varying concentrations. The WBCs decreased in all male treated groups and in female 2.5% tartrazine group ($p<0.05$). A decrease in WBCs is a leukopenia which occurred when the toxic affects the bone marrow (Robert and Budinsky, 2000). This result is in contrary with that of Hashem et al. (2010); they declared that Amaranth and Sunset Yellow in daily doses of 47.0 and 315 mg/kg bw, respectively for 4 weeks did not affect the total leucocytes count in female rats. Also, Himri et al. (2011) showed that tartrazine at doses of 5.0, 7.5 and 10 mg/kg bw administered in rats for 90 days did not affect the total leucocytes count (the sex is not mentioned). These differences may be due to the colorants used or differences

of doses and animal sex.

In this study, subchronic tartrazine ingestion in albino mice for 13 weeks increased the level of serum total protein in male and female of 2.5% dose groups (p<0.05) and decreased the level in male and female of 0.1, 0.45 and 1% dose groups (p<0.05). These results are in accordance with those of Amin et al. (2010) who found a significant increase in serum total protein when administrated tartrazine at low and high dose in males rats for 30 days, and also is in accordance with Aboel-Zahab et al. (1997) who found the increase level on serum total protein with rats whose diets were supplemented with Chocolate colors A and B. Furthermore, our study demonstrate that all doses of tartrazine caused a significant increase in serum albumin (p<0.01), which is in accordance with the result of Amin et al. (2010). The increase in serum total protein and serum albumin is the result of a heme concentration caused by dehydration (Biou, 2007).

The daily intake for 13 weeks of tartrazine in both male and female 2.5% doses groups exhibited a significant increase in serum creatinine (p<0.05), urea (p<0.01) and uric acid concentration (p<0.01). In same time, a significant increase in serum creatinine (p<0.05) was observed in male 1% treated group and urea was increased in female 1% treated group (p<0.01). Furthermore, the present findings are in accordance with data reported by Ashour and Abdelaziz (2009) who observed a significant elevation in serum creatinine and urea level of rats dosed with organic azo dye (Fast Green) orally for 35 days. Also, Amin et al. (2010) showed a significant elevation in serum creatinine and urea level of rats that ingested either low or high doses of tartrazine. An increase level of urea or creatinine in the plasma indicates renal dysfunction (Timbrell, 2009).

The current study demonstrates that the cholesterol level was increased in male 0.45 and 2.5% dose group (p<0.05) and the triglycerides level was increased in female and male 1% as well as in 2.5% doses groups (p<0.01). These results correlate well with that reported by Aboel-Zahab et al. (1997) who observed a significant increase in serum total lipids, cholesterol and triglycerides in rats whose diets were supplemented with chocolate colors A and B (sunset yellow, tartrazine, carmoisine and brilliant blue) in varying concentrations. While our results are contrary with those of Amin et al. (2010) who observed a reduction in serum cholesterol level and an increase in triglycerides level in male rats that ingested either low or high doses of tartrazine. This discrepancy in results might to be attributable to the different ingested doses.

Our data shows that tartrazine at 0.45% dose in female mice caused an increase in glucose concentration but at 2.5% in male animals a significant decrease (p<0.01). This hypoglycemia might be explained by chan-ges in blood sugar concentration caused by foreign compounds, and this may involve a variety of mechanisms. Hypoglycemia as glycogen stores are depleted and gluconeogenesis is inhibited (Timbrell, 2009). In contrary, Amin et al. (2010) showed a significant increase in glucose concentration when administrated tartrazine at low (15 mg/kg bw) and high (500 mg/kg bw) dose orally in males rats for 30 days.

Our findings reveal a significant increase in serum ALT, AST, and alkaline phosphatase activities in male and female 2.5% tartrazine treated groups (p<0.01). These results are in concordance with the data of Amin et al. (2010) which revealed that rats that consumed high dose of tartrazine (500 mg/kg bw) for 30 days exhibited a significant increase in serum ALT, AST and alkaline phosphatase activities. The present findings are in accordance with those of Mekkawy et al. (1998) who indicated that two doses of synthetic dyes (low or high doses); tartrazine and carmoisine (Ponceau, Carmoisine, Erythrosine, sunset yellow, tartrazine, fast green, indi-gotine, brilliant blue and brilliant black) showed a signi-ficant increase in serum AST, ALT, and alkaline phosphates activities. Also, these results are in accor-dance with those of Aboel-Zahab et al. (1997) who found that liver enzymes ALT, AST, and Alkaline phosphatase were elevated in rats whose diets were supplemented with Chocolate colors A and B (Sunset Yellow, Tartra-zine, Carmoisine and Brilliant Blue in varying concen-trations). In addition, TBil was increased in female 2.5% treated group (p<0.01) and γGTP was increased in all female treated groups (p<0.05). Serum bilirubin concen-trations may be elevated from acute hepatocellular injury, cholestatic injury, or biliary obstruction (Roberts et al., 2000; Brissot et al., 2007). Therefore, ALT, AST and γGTP increased activities reflect primarily hepatocellular damage (Roberts et al., 2000; Brissot et al., 2007).

Data for absolute and relative organ weights are summarized in Tables 5a, 5b, 5c and 5d. The absolute and relative weights of kidney were increased in male 0.45 and 2.5% treated groups (p<0.05). The absolute weight of lung was decreased in male 0.45 and 2.5% treated group. However, the relative weight was increased only in 2.5% (p<0.05). Also, the absolute weight of liver and testes was increased in 0.45% treated group (p<0.05) but in female, the absolute weight of liver (p<0.01) and kidney (p<0.05) decreased in 1% dose group. In addition, the absolute weight of brain decreased in 1 and 2.5% dose groups (p<0.05). Also, salivary glands absolute and relative weight increased in 0.1% treated group (p<0.05). Maekawa et al. (1987) indicated that subchronic tartrazine ingestion in rats decreased the absolute and relative liver in 2% dose group. On the other hand, Osman et al. (1995) showed that oral ingestion of synthetic dyes such Fast Green at 12.5 mg/kg/day and Sunset Yellow at 5 mg/kg/day for 30 days in mice increased the liver and kidney weight. The change of the absolute and relative organs weight is a sign of toxicity.

A sub cortical edema in the brain was observed in 5/6 of mice treated with 1 and 2.5% tartrazine doses and in

Table 4b. Blood chemistry of male and female mice treated with tartrazine for 13 weeks.

Group	TG (mg/dl)	TBil (mg/dl)	ALP (UI/l)	ALT (UI/l)	AST (UI/l)	γGT (UI/l)
Male						
0.0%	101.8 ± 1.6[a]	1.2 ± 0.1[a]	6.3 ± 0.1[a]	35.1 ± 1.4[a]	31.6 ± 2.0[a]	5.9 ± 2.2[a]
0.1%	102.4 ± 1.6[a]	1.2 ± 0.1[a]	7.4 ± 0.7[a]	28.7 ± 1.4[a]	31.7 ± 2.0[a]	6.6 ± 0.4[a]
0.45%	79.1 ± 7.7[a]	1.2 ± 0.2[a]	12.4 ± 0.4[b]	30.6 ± 4.6[a]	35.0 ± 0.0[a]	6.7 ± 0.4[a]
1%	109.3 ± 0.0[c]	1.3 ± 0.3[a]	8.9 ± 0.4[a]	29.1 ± 2.7[a]	34.1 ± 2.1[a]	3.5 ± 0.3[a]
2.5%	103.8 ± 0.1[a]	1.5 ± 0.1[a]	23.4 ±0.3[c]	47.5 ± 1.7[c]	51.3 ± 1.0[c]	8.8 ± 0.3[a]
Female						
0.0%	101.1 ± 2.5[a]	1.1 ± 0.0[a]	5.6 ± 0.3[a]	31.6 ± 6.8[a]	33.2 ± 1.2[a]	1.4 ± 0.1[a]
0.1%	103.6 ± 2.7[a]	1.1 ± 0.1[a]	7.8 ± 0.4[a]	42.5 ± 6.3[b]	27.1 ± 2.2[a]	5.6 ± 0.4[c]
0.45%	75.0 ± 4.9[c]	1.1 ± 0.1[a]	13.4 ± 0.4[c]	24.1 ± 3.4[a]	32.1 ± 5.4[a]	5.9 ± 0.4[c]
1%	108.2 ± 0.1[c]	1.3 ± 0.1[a]	13.5 ± 0.6[a]	33.4 ± 2.1[a]	28.9 ± 2.1[a]	4.2 ± 0.2[b]
2.5%	124.2 ± 0.0[c]	1.9 ± 0.2[c]	13.9 ± 0.2[c]	43.9 ± 2.3[c]	57.1 ± 1.6[c]	6.7 ± 0.1[c]

Values are means ± SE for six animals. Means which have different letters are significantly different ($p < 0.05$).

Table 5a. Absolute and relative organ weights of male mice treated with tartrazine for 13 weeks.

Group	Brain (g)	Thymus (g)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Kidney (g)
Absolute							
Control	0.41 ± 0.01[a]	0.04 ± 0.01[a]	0.21 ± 0.02[a]	0.27 ± 0.01[a]	1.95 ± 0.13[a]	0.29 ± 0.03[a]	0.49 ± 0.04[a]
0.1%	0.43 ± 0.01[a]	0.03 ± 0.01[a]	0.21 ± 0.01[a]	0.27 ± 0.02[a]	2.29 ± 0.11	0.45 ± 0.06[b]	0.55 ± 0.05[a]
0.45%	0.44 ± 0.01[b]	0.04 ± 0.01[a]	0.25 ± 0.02[a]	0.24 ± 0.01[b]	2.34 ± 0.14[b]	0.25 ± 0.02[a]	0.63 ± 0.02[b]
1%	0.43 ± 0.01[a]	0.04 ± 0.01[a]	0.22 ± 0.01[a]	0.30 ± 0.03[a]	2.17 ± 0.19[a]	0.26 ± 0.05[a]	0.59 ± 0.05[a]
2.5%	0.41 ± 0.01[a]	0.03 ± 0.01[a]	0.21 ± 0.01[a]	0.24 ± 0.01[b]	1.91 ± 0.13[a]	0.21 ± 0.02[a]	0.62 ± 0.02[b]
Relative							
Control	1.16 ± 0.06[a]	0.12 ± 0.02[a]	0.58 ± 0.04[a]	0.78 ± 0.06[a]	5.49 ± 0.41[a]	0.82 ± 0.12[a]	1.34 ± 0.06[a]
0.1%	1.18 ± 0.06[a]	0.10 ± 0.03[a]	0.57 ± 0.03[a]	0.73 ± 0.03[a]	6.29 ± 0.31[a]	1.22 ± 0.18[a]	1.50 ± 0.09[a]
0.45%	1.04 ± 0.03[a]	0.09 ± 0.01[a]	0.58 ± 0.04[a]	0.57 ± 0.03[a]	5.49 ± 0.33[a]	0.59 ± 0.05[a]	1.47 ± 0.05[a]
1%	1.11 ± 0.03[a]	0.11 ± 0.01[a]	0.56 ± 0.02[a]	0.77 ± 0.07[a]	5.59 ± 0.44[a]	0.67 ± 0.13[a]	1.50 ± 0.10[a]
2.5%	1.05 ± 0.02[a]	0.10 ± 0.02[a]	0.52 ± 0.02[a]	0.60 ± 0.03[b]	4.86 ± 0.34[a]	0.52 ± 0.06[b]	1.58 ± 0.04[b]

Values are means ± SE for 10 animals. Relative weight is expressed per gram of organ per 100 grams of body weight. Means which have different letters are significantly different ($p < 0.05$).

2/6 of mice treated with 0.45% tartrazine dose (Figure 1b). In addition, a gliale cell hyperplasia with congestion of blood vessels was observed in two animals (one male and one female) of 2.5% tartrazine groups (Figure 1d). Indeed, Gao et al. (2011) showed a decline in the activities of catalase, glutathione peroxydase (GSH-Px) and superoxide dismutase (SOD) as well as a rise in the level of malonaldehyde (MDA) in brain of tartrazine treated rats and these changes were associated with brain oxidative damage. The mechanisms might be attributed to promoting lipid peroxidation products and reactive oxygen species, inhibiting endogenous antioxidant defense enzymes and brain tissue damage. In addition, the data of Park et al. (2009) indicated that combinations of excessively high dose of tartrazine and Brilliant Blue FCF may adversely affect both developmental and adult hippocampal neurogenesis affecting the central nervous system.

The hepatocellular damages are characterized by a mild hydropic degeneration of the centrilobular heaptocytes, as well sinusoidal and centrolobular vena compressions were observed in all mice that ingested 1 and 2.5% tartrazine doses (6/6 and 6/6, respectively) (Figure 2b). Also, condensed chromatin material in the nucleus of male and female 2.5% tartrazine groups cells were noted in all these groups (Figure 2b). A slight hydropic degeneration appeared in 4/6 of female treated with 0.1 and 0.45% and in 3/6 of male treated with 0.45%

Table 5b. Absolute and relative organ weights of male mice treated with tartrazine for 13 weeks.

Group	Testes (g)	Epididymis (g)	Seminal vesicle (g)	Salivary gland (g)	Adrenal gland (g)	Bladder (g)
Absolute						
Control	0.18 ± 0.01[a]	0.08 ± 0.01[a]	0.35 ± 0.02[a]	0.29 ± 0.02[a]	0.01 ± 0.01[a]	0.03 ± 0.01[a]
0.1%	0.20 ± 0.01[a]	0.10 ± 0.01[a]	0.27 ± 0.03[b]	0.27 ± 0.02[a]	0.01 ± 0.01[a]	0.04 ± 0.01[a]
0.45%	0.24 ± 0.01[b]	0.08 ± 0.01[a]	0.39 ± 0.03[a]	0.31 ± 0.02[a]	0.01 ± 0.01[a]	0.04 ± 0.01[a]
1%	0.20 ± 0.00[a]	0.08 ± 0.01[a]	0.34 ± 0.03[a]	0.27 ± 0.02[a]	0.01 ± 0.01[a]	0.04 ± 0.01[a]
2.5%	0.20 ± 0.01[a]	0.08 ± 0.01[a]	0.32 ± 0.02[a]	0.21 ± 0.02[a]	0.01 ± 0.01[a]	0.03 ± 0.01[a]
Relative						
Control	0.51 ± 0.04[a]	0.21 ± 0.03[a]	0.95 ± 0.05[a]	0.81 ± 0.03[a]	0.03 ± 0.00[a]	0.10 ± 0.01[a]
0.1%	0.54 ± 0.03[a]	0.27 ± 0.03[a]	0.73 ± 0.07[b]	0.76 ± 0.05[a]	0.02 ± 0.00[a]	0.11 ± 0.01[a]
0.45%	0.56 ± 0.02[a]	0.20 ± 0.02[a]	0.91 ± 0.07[a]	0.74 ± 0.04[a]	0.03 ± 0.01[a]	0.10 ± 0.01[a]
1%	0.52 ± 0.02[a]	0.21 ± 0.02[a]	0.89 ± 0.09[a]	0.71 ± 0.04[a]	0.04 ± 0.02[a]	0.10 ± 0.02[a]
2.5%	0.51 ± 0.02[a]	0.19 ± 0.02[a]	0.8 ± 0.06[a]	0.52 ± 0.05[b]	0.03 ± 0.10[a]	0.08 ± 0.01[a]

Values are means±SE for 10 animals. Relative weight is expressed per gram of organ per 100 grams of body weight. Means which have different letters are significantly different ($p < 0.05$).

Table 5c. Absolute and relative organ weights of female treated with tartrazine for 13 weeks.

Group	Brain (g)	Thymus (g)	Heart (g)	Lung (g)	Liver (g)	Spleen (g)	Kidney (g)
Absolute							
Control	0.45 ± 0.01[a]	0.06 ± 0.01[a]	0.16 ± 0.01[a]	0.24 ± 0.01[a]	1.76 ± 0.06[a]	0.17 ± 0.01[a]	0.39 ± 0.02[a]
0.1%	0.42 ± 0.02[a]	0.06 ± 0.01[a]	0.16 ± 0.00[a]	0.24 ± 0.01[a]	1.67 ± 0.11[a]	0.17 ± 0.01[a]	0.36 ± 0.02[a]
0.45%	0.43 ± 0.01[a]	0.07 ± 0.01[a]	0.14 ± 0.01[a]	0.21 ± 0.01[a]	1.53 ± 0.09[a]	0.13 ± 0.01[a]	0.31 ± 0.01[b]
1%	0.42 ± 0.01[b]	0.06 ± 0.01[a]	0.17 ± 0.01[a]	0.23 ± 0.01[a]	1.49 ± 0.06[b]	0.18 ± 0.01[a]	0.34 ± 0.01[b]
2.5%	0.41 ± 0.02[b]	0.06 ± 0.01[a]	0.17 ± 0.01[a]	0.21 ± 0.01[a]	1.64 ± 0.05[a]	0.18 ± 0.04[a]	0.35 ± 0.02[a]
Relative							
Control	1.36 ± 0.03[a]	0.19 ± 0.02[a]	0.48 ± 0.02[a]	0.71 ± 0.03[a]	5.30 ± 0.14[a]	0.52 ± 0.03[a]	1.15 ± 0.03[a]
0.1%	1.16 ± 0.16[a]	0.19 ± 0.02[a]	0.51 ± 0.02[a]	0.75 ± 0.05[a]	5.24 ± 0.34[a]	0.52 ± 0.03[a]	1.12 ± 0.05[a]
0.45%	1.36 ± 0.03[a]	0.22 ± 0.02[a]	0.45 ± 0.02[a]	0.67 ± 0.03[a]	4.79 ± 0.17[b]	0.42 ± 0.02[a]	1.00 ± 0.03[b]
1%	1.38 ± 0.04[a]	0.19 ± 0.02[a]	0.55 ± 0.04[a]	0.77 ± 0.05[a]	4.90 ± 0.14[a]	0.59 ± 0.05[a]	1.11 ± 0.03[a]
2.5%	1.29 ± 0.04[a]	0.19 ± 0.02[a]	0.52 ± 0.03[a]	0.68 ± 0.03[a]	5.08 ± 0.13[a]	0.57 ± 0.09[a]	1.11 ± 0.04[a]

Values are means±SE for 10 animals.Relative weight is expressed per gram of organ per 100 grams of body weight. Means which have different letters are significantly different ($p < 0.05$).

tartrazine doses. This anomaly was characterized by the ballooning of hepatocytes enriched with watery materials. The plasma membrane surrounds the hepatocyte and is critically important in maintaining the ion balance between the cytoplasm and the external environment. This ion balance can be disrupted by damage to plasma membrane ion pumps, or by loss of membrane integrity causing ions to leak in or out of the cell following their concentration gradients. Loss of ionic control can cause a net movement of water into the cell, resulting in cell swelling (Stevens et al., 2004). However, these morphologic abnormalities in response to tartrazine still recover. These data are well correlated with biochemical analysis because a rise of liver enzymes in serum was due to liver damage. The present findings are less severe compared to those of Mekkawy et al. (1998) where two doses of synthetic dyes (low or high doses) (Ponceau, Carmoisine, Erythrosine, Sunset Yellow, tartrazine, Fast Green, Indigotine, Brilliant Blue and Brilliant Black) showed a hepatocellular damage caused by the toxic effects of these synthetic dyes which were indicated by vacuolation, swelling, necrosis and pyknosis of the liver cells. On the other hand, a mild hydropic degeneration of hepatocytes caused by tartrazine and Sodium Benzoate were observed in rats (Upadhyay, 1997). The histopathological studies showed by Aboel-Zahab et al. (1997) are a brown pigment deposition in the portal tracts and Van Küpffer cells of the liver, in addition congested blood

Table 5d. Absolute and relative organ weights of female mice treated with tartrazine for 13 week.

Group	Ovary (g)	Uterus (g)	Salivary gland (g)	Adrenal gland (g)	Bladder (g)
Absolute					
Control	0.19 ± 0.01^{a}	0.17 ± 0.01^{a}	0.19 ± 0.01^{a}	0.01 ± 0.01^{a}	0.03 ± 0.01^{a}
0.1%	0.19 ± 0.01^{a}	0.21 ± 0.05^{a}	0.23 ± 0.01^{b}	0.01 ± 0.01^{a}	0.02 ± 0.01^{b}
0.45%	0.27 ± 0.02^{a}	0.20 ± 0.02^{a}	0.19 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}
1%	0.17 ± 0.04^{a}	0.15 ± 0.01^{a}	0.22 ± 0.03^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}
2.5%	0.18 ± 0.03^{a}	0.19 ± 0.03^{a}	0.17 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}
Relative					
Control	0.57 ± 0.03^{a}	0.51 ± 0.05^{a}	0.58 ± 0.04^{a}	0.04 ± 0.01^{a}	0.09 ± 0.05^{a}
0.1%	0.60 ± 0.04^{a}	0.67 ± 0.16^{a}	0.73 ± 0.04^{b}	0.05 ± 0.01^{a}	0.08 ± 0.05^{a}
0.45%	0.85 ± 0.06^{c}	0.63 ± 0.07^{a}	0.60 ± 0.02^{a}	0.06 ± 0.04^{a}	0.07 ± 0.04^{b}
1%	0.53 ± 0.11^{a}	0.48 ± 0.04^{a}	0.73 ± 0.09^{a}	0.05 ± 0.01^{a}	0.14 ± 0.06^{a}
2.5%	0.53 ± 0.06^{a}	0.59 ± 0.07^{a}	0.57 ± 0.05^{a}	0.06 ± 0.01^{a}	0.08 ± 0.01^{a}

Values are means ± SE for 10 animals. Relative weight is expressed per gram of organ per 100 g of body weight. Means which have different letters are significantly different ($p < 0.05$).

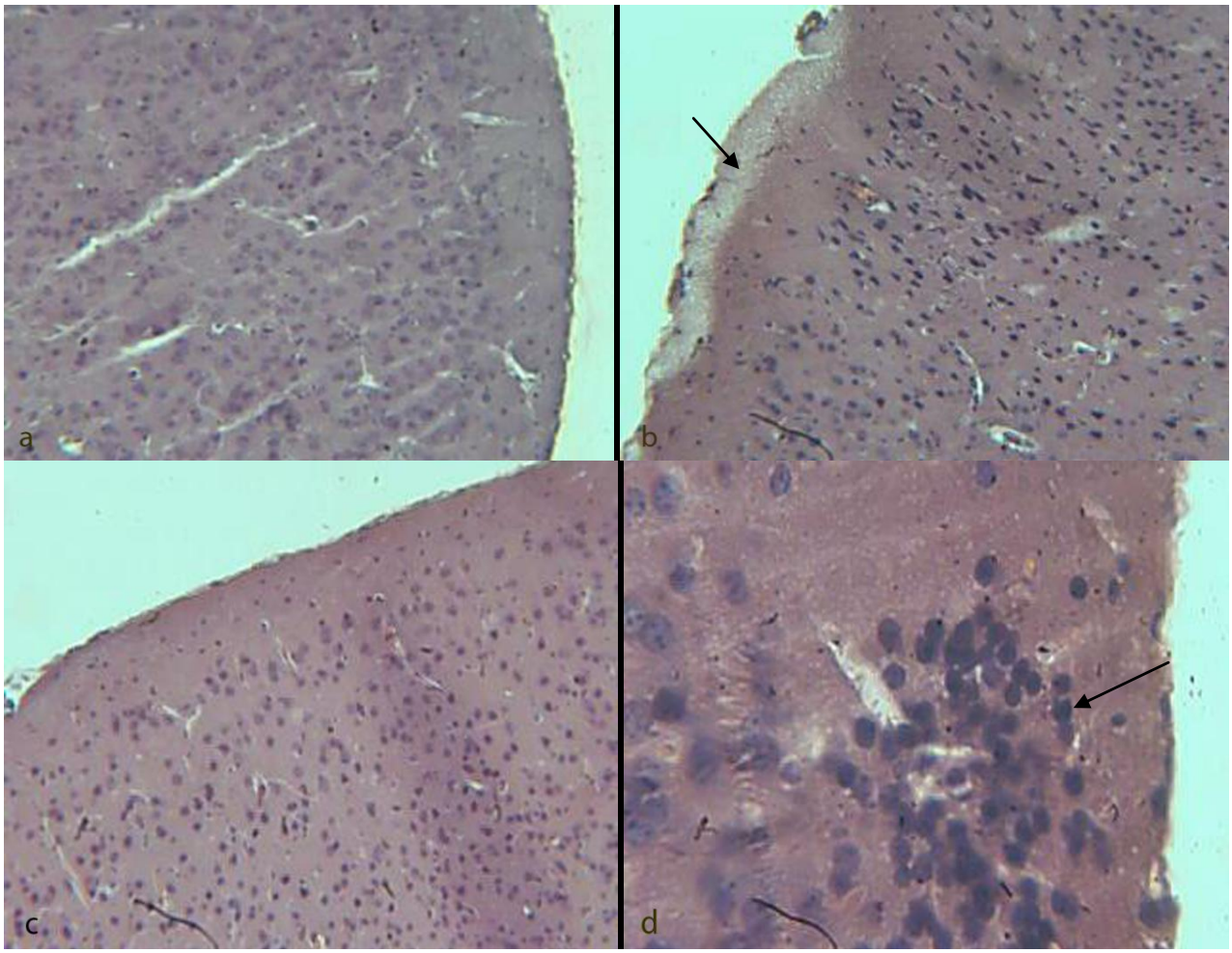

Figure 1. Histological brain examination. **a.** Histology of male control brain. **b.** Histology of brain of 2.5% tartrazine treated male mice, sub cortical edema. **c.** Histology of female control brain. **d.** Histology of brain of 2.5% tartrazine treated female mice, gliale cell hyperplasy. Haematoxyline-eosine stain, a-c, 250x, d, 1000x.

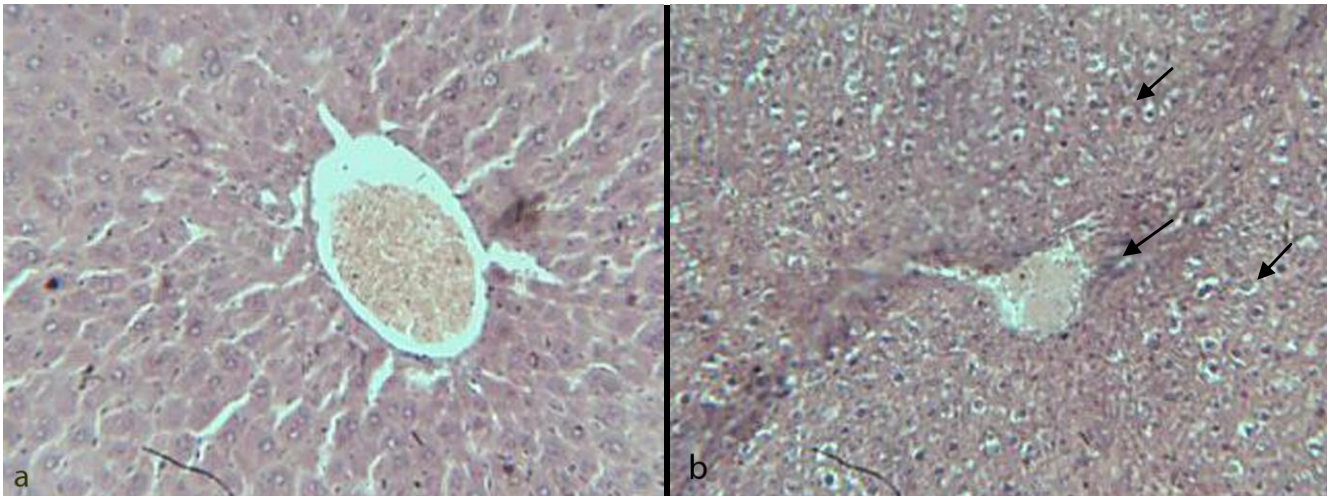

Figure 2. Histological liver examination. **a.** Histology of male control liver. **b.** Histology of liver of 2.5% tartrazine treated male mice, intracellular vacuolation, sinusoids and centrolobular vena compression, chromatine condensation. Haematoxyline-eosine stain, 250x.

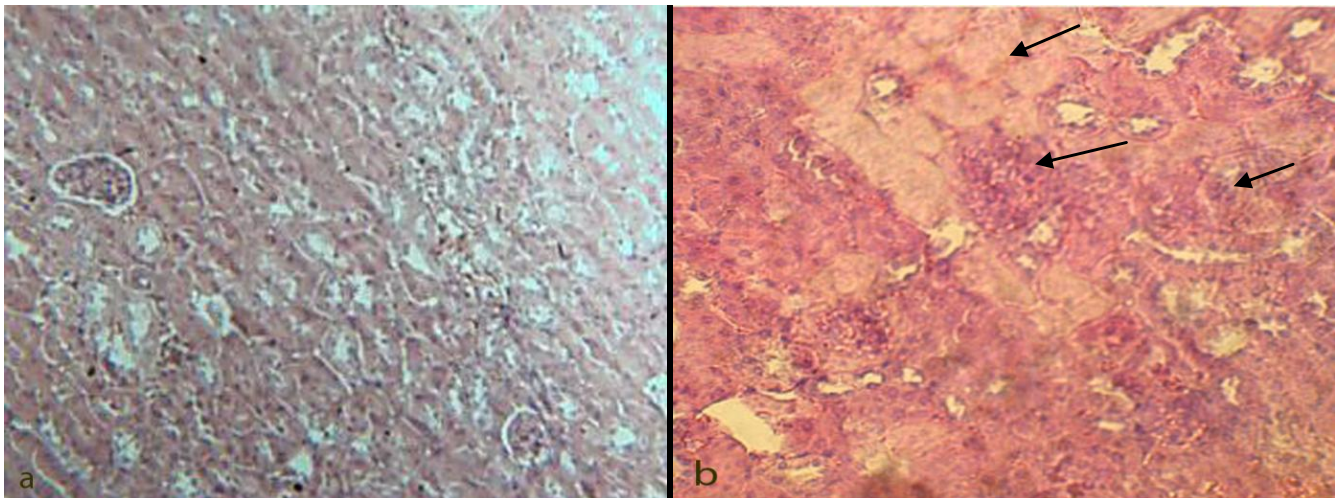

Figure 3. Histological kidney examination. **a.** Histology of male control kidney. **b.** Histology of kidney of 2.5% tartrazine treated male mice, tubular lumen compression, glomerular damage, edema per region. Haematoxyline-eosine stain, a, 100x, b, 250x.

vessels and areas of haemorrhage in both liver and renal sections were revealed in those rats given colorants B and C (sunset yellow, tartrazine, carmoisine and brilliant blue in varying concentrations).

The histopathologic findings of renal section showed a lumen compression in tubular cells with an interstitial lymphocytes cell infiltration, edema and glomerular damages in 5/6 of male mice treated with 1% and in 6/6 of 2.5% treated tartrazine doses (Figure 3b). These data concord with an increase of creatinine, urea and uric acid in the serum. In the present study, massive doses of tartrazine (1 and 2.5%) induced renal dysfunction. Also, Himri et al. (2011) showed tubular dilatation, tubular degeneration, dilatation of the glomerular capillaries, intercapillary sclerosis and atrophy of glomerulus when wistar rats received orally tartrazine at doses of 7.5 mg and 10 mg/Kg/day for 90 days.

Conclusion

Our results indicate clearly that tartrazine subchronic ingestion in albino Swiss mice at dose of 1% and 2.5% leads to hepatocellular, brain and renal damages. Therefore, excessive intake of food colorants in children may cause a risk health.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Higher Education and Scientific Research (MESRS, Algeria). Mr Houdjedj Touati is gratefully acknowledged for his corrections of the manuscript.

REFERENCES

Aboel-Zahab H, El-Khyat Z, Sidhom G, Awadallah R, Abdel-al W, Mahdy K (1997). Physiological effects of some food coloring additives on rats. Boll. Chim. Farm. 136(10):615-627.

Amin KA, Abdel Hameid H, Abd Elsttar AH (2010). Effect of food azo dyes tartrazine and Carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem. Toxicol. 48:2994-2999.

Ashour AA, Abdelaziz I (2009). Role of fast green on the blood of rats and the therapeutic action of vitamins C or E. Int. J. Integr. Biol. 6(1):6-11.

Balcells A (1998). Laboratory tests. French edition, Masson, First edition, Paris.

Bateman B, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald **C**, Stevenson J (2004). The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch. Dis. Child. 89:506-511.

Biou D (2007). Qualitative and quantitative plasma proteins anomalies. In: Vaubourdolle M, ed. Biochimistry and Hematology. French edition, Le moniteur, 3th edition, Paris. pp. 583-613.

Borzelleca JF, Hallagan JB (1988a). Chronic toxicity/carcinogenicity studies of FD&C Yellow No. 5 (tartrazine) in rats. Food Chem. Toxicol. 26:179-187.

Borzelleca JF, Hallagan JB (1988b). A chronic toxicity/carcinogenicity study of FD&C Yellow No. 5 (tartrazine) in mice. Food Chem. Toxicol. 26:189-194.

Brissot P, Ropert-Bouchet M, Troadec MB, Lorho R, Guyader D, Loréal O (2007). Liver biological exploration. EMC (Elsevier Masson SAS, Paris), Hepatology 7-007-B-10.

Das A, Mukherjee A (2004). Genotoxicity testing of the food colours Amaranth and tartrazine. Int. J. Hum. Genet. 4: 277-280.

Devlin J, David TJ (1992). tartrazine in atopic eczema. Arch. Dis. Child. 67:709-711.

Dixit S, Purshottam SK, Gupta SK, Khanna SK, Das M (2010). Usage pattern and exposure assessment of food colours in different age groups of consumers in the State of Uttar Pradesh, India. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 27(2):181-9.

Dixit S, Purshottam SK, Khanna SK, Das M (2011). Usage pattern of synthetic food colours in different states of India and exposure assessment through commodities preferentially consumed by children. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 28(8):996-1005.

Durnev AD, Oreshchenko AV, Kulakova AV, Beresten NF (1995). Analysis of cytogenetic activity of food dyes. Vopr. Med. Khim. 41:50-53.

Ezeuko VC, Nwokocha CR, Mounmbegna PE, Nriagu CC (2007). Effect of Zingiber officinale on liver function of mercuric chloride induced hepatotoxicity in adult male Wistar rats. Electron J. Biomed. 3:40-45.

Gao Y, Li C, Shen J, Yin H, An X, Jin H (2011). Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved. J. Food Sci. 76(6):T125-9.

Giri AK, Das SK, Talukder G, Sharma A (1990). Sister chromatid exchange and chromosome aberrations induced by curcumin and tartrazine on mammalian cells in vivo. Cytobios. 62: 111-117.

Hannuksela M, Haahtela T (2009). Food additive hypersensitivity--near myth. Duodecim 125:527-532.

Hashem MM, Atta AH, Arbid MS, Nada SA, Asaad GF (2010). Immunological studies on Amaranth, Sunset Yellow and Curcumin as food colouring agents in albino rats. Food Chem. Toxicol. 48:1581-1586.

Himri I, Bellahcen S, Souna F, Belmekki F, Aziz M, Bnouham M, Zoheir J, Berkia Z, Mekhfi H, Saalaoui E (2011). A 90-day oral toxicity of tartrazine, A synthetic food dye, in wistar rats. Int. J. Pharm. Pharm. Sci. 3(3):159-169.

Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N et al. (2006). Estimates of dietary exposure of children to artificial food colours in Kuwait. Food Addit. Contam. 23:245-251.

Inomata N, Osuna H, Fujita H, Ogawa T, Ikezawa Z (2006). Multiple chemical sensitivities following intolerance to azo dye in sweets in a 5-year-old girl. Allergol. Int. 55:203-205.

Ishidate M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22:623-636.

Ishidate M, Sofuni J, Yoshikawa K (1981). Chromosomal aberration tests *in vitro* as a primary screening tool for environmental mutagens and/or carcinogens. Gann. Monogr. Cancer Res. 27:95-108.

Joint FAO/WHO Expert Committee on Food Additives [JECFA] (1996). Summary of evaluations performed by the joint FAO/WHO expert committee on food additives (JECFA) 1956-1995 (first through 44th meetings); International Life Sciences Institute (ILSI) Press Washington DC. p T-3.

Maekawa A, Matsuoka C, Onodera H, Tanigawa H, Furuta K, Kanno J et al. (1987). Lack of carcinogenicity of tartrazine (FD & C Yellow No. 5) in the F344 rat. Food Chem. Toxicol. 5:891-896.

McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K et al. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. Lancet 370:1560-1567.

Mehedi N, Ainad-Tabet S, Mokrane N, Addou S, Zaoui C, Kheroua O et al. (2009). Reproductive Toxicology of tartrazine (FD and C Yellow No. 5) in Swiss Albino Mice. Am. J. Pharmacol. Toxicol. 4(4):128-133.

Mekkawy HA, Ali MO, El-Zawahry AM (1998). Toxic effect of synthetic and natural food dyes on renal and hepatic functions in rats. Toxicol. Lett. 95(1):155.

Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S (2010). Cytogenetic evaluation and DNA interaction studies of the food colorants Amaranth, Erythrosine and tartrazine. Food Chem. Toxicol. 48: 2934-2944.

Nettis E, Colanardi MC, Ferrannini A, Tursi A (2003). Suspected tartrazine-induced acute urticaria/angioedemais only rarely reproducible by oral rechallenge. Clin. Exp. Allergy 33: 1725-1729.

Osman MA, Afifi A, Hussein RM, Kamilia B, Abdel Aziz I, Salah SH (1995). Long-term biochemical and genotoxicity studies of four synthetic food and drug colorants in mice. Bull. Fac. Pharm. 33:13-21.

Ould Elhkim M, He´raud F, Bemrah N, Gauchard F, Lorino T, Lambre´C et al.(2007). New considerations regarding the risk assessment on tartrazine: An update toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. Regul. Toxicol. Pharmacol. 47:308-316.

Park M, Park HR, Kim SJ, Kim MS, Kong KH, Kim HS et al. (2009). Risk assessment for the combinational effects of food color additives: neural progenitor cells and hippocampal neurogenesis. J. Toxicol. Environ. Health A 2(21-22):141-223.

Patterson RM, Butler JS (1982). Tartrazine-induced chromosomal aberrations in mammalian cells. Food Chem. Toxicol. 20: 461-465.

Poul M, Jarry G, Ould Elhkim M, Poul JM (2009). Lack of genotoxic effect of food dyes amaranth, sunset yellow and tartrazine and their metabolites in the gut micronucleus assay in mice. Food Chem. Toxicol. 47:443-448.

Rao P, Bhat RV (2003). A comparative study on the artificial food colours usage in foods procured from urban and rural areas of Hyderabad. Nutr. Food Sci. 33: 230-234.

Rao P, Bhat RV, Sudershan RV, Krishna TP, Naidu N (2004). Exposure assessment to synthetic food colours of a selected population in Hyderabad, India. Food Addit. Contam. 21(5):415-21.

Robert A, Budinsky JR (2000). Hematotoxicity: Chemically induced toxicity of the blood. In: Williams PL, James RC, Roberts SM, ed. Principles of toxicology, environmental and industrial applications. New York: 2nd edition, Wiley-Interscience Publication. pp. 87-109.

Roberts, SM, Robert CJ, Franklin MR (2000). Hepatotoxicity: Toxic

effects on the liver. In: Williams PL, James,RC, Roberts SM, ed. Principles of toxicology, environmental and industrial applications. New York: 2nd edition, Wiley-Interscience Publication. pp. 111-128.
Rowe KS, Rowe KJ (1994). Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated measures study. J. Pediat. 125:691-698.
Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama, K et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat. Res. 519:103-119.
Schab DW, Trinh NH (2004). Do artificial food colors promote hyperactivity in children with hyperactive syndromes? A meta-analysis of double-blind placebo-controlled trials. J. Dev. Behav. Pediatr. 25(6):423-434.
Stevens A, Lowe JS, Young B (2004). Anatomy pathology, Atlas de Whlater. 4th edition, De Boeck, Bruxelle.
Tanaka T (2006). Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. Food Chem. Toxicol. 44:179-187.
Tanaka T, Takahashi O, Oishi S, Ogata A (2008). Effects of tartrazine on exploratory behavior in a three-generation toxicity study in mice. Reprod. Toxicol. 26(2):156-163.
Timbrell JA (2009). Principles of biochemical toxicology. 4th edition, Informa Healthcare, New York.
Upadhyay RR (1997). Mild hydropic degeneration of hepatocytes by tartrazine and sodium benzoate. Bionat. 17(1):43-44.
Ward NI (1997). Assessment of chemical factors in relation to child hyperactivity. J. Nutri. Env. Med. 7 (4):333-342.