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Assessment of a sub-chronic consumption of tartrazine (E102) on sperm and oxidative stress features in Wistar rat

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Abstract

Tartrazine (FD and C Yellow No. 5) is still one of the most used coloring agent in drugs, cosmetics and food industry, several toxic effects in rodent as human were described, including some disruption regarding the reproductive system function. This study was conducted in order to evaluate the effect of a sub-chronic consumption of tartrazine on sperm quality, testosterone level and oxidative stress markers in testis. Therefore, 300 mg/kg of body weight of tartrazine were daily intragastrally (i.g.) administrated to Wistar rats during 30 days. Sperm features, testosterone and cholesterol levels in plasma and testis were assessed. We evaluated lipid peroxidation (malondialdehyde (MDA)) and antioxidant enzymes activities, including Glutathion-S-Transferase (GST) and catalase in testis. Furthermore, toxicity indicators to know; lactate dehydrogenase (LDH) and acid phosphatase were measured in plasma. We showed that tartrazine consumption led to a significant decrease in body weight gain and critically altered sperm characteristics. Both testosterone and cholesterol levels were significantly decreased and a lower activity of thiol group (SH), catalase and GST was recorded. MDA level in testis, LDH and acid phosphatase rate in plasma were highly increased. Our results revealed that sub-chronic exposure to tartrazine could be extremely harmful to the reproductive function. Yet, it is mandatory to raise the awareness of health issue related to this dye.

Introduction

Food dyes are chemical or natural colouring product used to alter or confer colours to food in order to increase their attractiveness toward consumers, in particular children (Newsome, 1986). Dyes have been used for a long time in food industry, but not without controversy and disagreement regarding their health effects (Mekkawy *et al*., 1998). Indeed, food additives were reported as one of the factors responsible of various diseases such as hepatic cancer and nephritic failure (Collier *et al*., 1983; Seesuriyachan *et al*., 2007). Among these colouring agents, some of the most used are the azo dyes, including the aromatic azo compounds. Azo compounds contain an aromatic ring linked by an azo bond to the second naphthalene or benzene ring. They are reduced by azo reductase producing aromatic amines, some of which, have been found to be toxic, mutagenic, and carcinogenic (Tanaka, 2007; Türkoglu, 2007).

Tartrazine (also known as E102, Food yellow 4, FD and C yellow No. 5), is a nitrous derivative of azo compounds that can be metabolised to highly sensitizing aromatic amines such as sulphanilic acid (Maekawa *et al*., 1987; Amin *et al*., 2010; Feng *et al*., 2012). Tartrazine with the following chemical formula: 4-5-Dihydro-5-oxo-1-(4-sulfophenyl)-4-((4-sulfophenyl) azo) 1H-pyrazole-3carboxylic acid (Khera and Munro, 1979), is an orange-coloured, water soluble powder widely used in food products, such as juices, biscuits, ice-creams and sauces (Miller, 1982), also in drugs, cosmetics and pharmaceuticals. The estimated amounts of used tartrazine were approximately 985.76 tones in the USA (Ishimitsu *et al*., 1998) and 71.35 tones in Japan manufactured in 1996. The acceptable daily intake (ADI) of human is 0-7.5 mg/kg/day (Walton *et al*., 1999). The metabolism of tartrazine was well documented in animals as humans and at a range of doses, absorption of oral administration of intact tartrazine is less than 5% (Feng *et al*., 2012). Tartrazine is usually absorbed in small quantities (Nihon Shokuhin, 1999) and the absorbed amount is mostly excreted in urine, under an unchanged form. The remaining tartrazine is extensively metabolised by the gastrointestinal microflora and transformed

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into aromatic amine sulfanilic acid (Moutinho *et al*., 2007). Tartrazine was reported as one of the most controversial colouring agents. In fact, considerable studies have shown that this food dye was safe to be consumed in the acceptable daily intake, since no harmful effects were recorded in both human and experimental models (Tanaka *et al*., 2008; Poul *et al*., 2009). However, other investigations have reported that tartrazine can induce angioedema, exacerbations of asthma, urticaria in atopic patients (Miller *et al*., 1982; Babu and Shenolikar, 1995; Ram and Ardern, 2001). Tartrazine was also found to induce immunotoxic (Koutsogeorgopoulou *et al*., 1998; Guendouz *et al*., 2013), genotoxic and mutagenic effects (Sasaki *et al*., 2002; Mpountoukas *et al*., 2010; Oliveira *et al*., 2010).

Moreover, clinical studies showed some adverse reactions in children including irritability, sleep disturbance after tartrazine consumption (Bhatia *et al*., 2000) and hyperactivity (Collins-Williams, 1985; McCann *et al*., 2007). The toxicity of tartrazine was mostly related to free radicals formation that disrupt antioxidant enzymes activities or from arylamin azo reduction (Nony *et al*., 1980; Pearce *et al*., 2003). Indeed, tartrazine metabolites can generate reactive oxygen species (ROS), promoting lipid peroxidation and inhibiting endogenous antioxidant defence enzymes which, in turn, accelerate oxidative stress (Bansal *et al*., 2005) and damage most cellular components, therefore leading to cell death (Morales *et al*., 2004).

Regardless of the variety of studies conducted to explore the oxidative effect caused by tartrazine in many organs such as kidney, liver and brain (Bansal *et al.*, 2005; Amin *et al.*, 2010; Gao *et al*., 2011), there is still a lack of information concerning the oxidative damage induced by tartrazine consumption in male reproductive organs. This study was carried out in order to assess tartrazine daily consumption on sperm quality and to highlight the variation of oxidative stress status in testis. In this context, adult rats were exposed to 300 mg/kg/day i.g. of tartrazine for 30 days. Sperm features, cholesterol, testosterone, oxidative stress biomarkers and antioxidant enzymes activity were measured by the end of the sub-chronic treatment.

Materials and Methods

Chemicals

Tartrazine used in this study was a commercialized orange powder "Les Chaines", obtained from Paris (France). Spermoscan kit, Vita-Eosin kit, Total Cholesterol kit, Total Proteins kit, ELISA kit, Acid Phosphatase kit, LDH kit. All obtained from SIGMA ALDRICH (St. Louis, MO, USA).

Animals and experimental model

20 sexually mature male Wistar rats, weighting 120 ± 4 g were purchased from 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 at 12/12 h light-dark. Animals were housed by 2 into polypropylene cage. They were fed with standard pellet diet (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 institutional guidelines, in compliance with ethical standards, national and international laws and guidelines for use of animals in biomedical research.

Rats were divided randomly into two groups of ten animals each. The first group corresponded to the control group (Ctr) and was given 300 mg/kg/day i.g. distilled water. The second group received 300 mg/kg/day i.g. tartrazine diluted in distilled water, 7 days a week for 30 days. Used tartrazine dose represent 5% of the LD_{50}(tartrazine LD_{50} correspond to 6375 mg/kg, when orally administrated in rat).

Body weight gain, testes and epididymis weight

Body weight, water and food intake were determined daily during the treatment period. Testes and epididymis were carefully dissected out, made free from adherents and weighed immediately after dissection on precision balance to the nearest mg.

Sperm analyses: density, motility, viability and morphology

One single cauda epididymis was used to extract mature sperm in order to perform cytological tests. Cauda epididymis was placed in RPMI 1640 medium to ensure spermatozoa survival. After 10 min of incubation at ambient temperature, sperm well dispersed in the medium were recovered as a stock solution. Sperm solution was diluted prior to 1/5 and then counted using Malassez cell counting chamber on light microscope (G × 100). Two slides were made for each animal and averaged. Sperm concentration was expressed as total number × 10^6 ml^{-1}. Simultaneously, motility was also analyzed and sperm cells were classified as ''motile" or ''immotile" following Okamura *et al.* (2005) description. Results were averaged and recorded as percentage.

Sperm viability was assessed using a commercial Kit Vita-Eosine. A smear was performed on a slide and allowed to dry in the open air for 5 min. Sperm cells

counting was carried out under light microscope (G × 400). Counting of viable spermatozoa characterized by a white colored head was performed three times out of a total of 150 spermatozoa and averaged. Data are expressed as percentage. To highlight cell defects, spermatozoa were gently spread on glass slides and dried in the open air for 30 min. Sperm morphology was assessed by a commercial kit (Spermoscan, Ral diagnosis). A total of 400 spermatozoa per slide were examined under light microscope (G × 400). Three slides were performed for each animal and sperm abnormalities count was expressed as percentage.

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Testosterone and cholesterol assays

Venous blood samples were obtained from the orbital sinus via glass capillaries in heparinized tubes, then centrifuged at 1000 × *g* during 10 min at 4°C. Collected plasma was used to perform testosterone, cholesterol and acid phosphatase assays. Testes from each animal were excised and a portion of each testicle was homogenized in ice-cold 10% PBS (0.10 M; pH = 7.40) using a steel homogenizer (Ultra-Turrax T-25) and homogenate was centrifuged at 9000 × *g* at 4°C for 20 min. The supernatant was kept frozen at - 80°C until use. Testosterone concentration in plasma was assessed by an enzyme-linked immunosorbent assay (ELISA), according to the standard protocol supplied by the kit manufacturer. The formation of immune complexes (mAb 1-testosterone-mAb 2) was revealed by the addition of horseradish peroxidase enzyme (HRP). Absorbance at 450 nm was measured by a plate reader using a reference filter at 630 nm (Handelsman, 2006). Cholesterol level was determined using the usual colorimetric method. Briefly, 0.01 ml of the supernatant of testis homogenate and plasma was added to 1 ml of working reagent for 5 min at 37°C, and absorbance was measured at 505 nm according to the manufacturer's protocol (Trinder, 1969).

Assessment of oxidative stress and toxicity markers

Supernatant of testicular tissue was homogenized in 2 ml of ice-cold phosphate buffer saline (0.10 M; pH = 7.40) and used to measure MDA, SH and antioxidant enzymes level including catalase and GST in testis. Results were corrected to the corresponding total proteins level. LDH and acid phosphatase assays were performed in plasma.

Toxicity markers analyses: LDH and acid phosphatase assays

In order to asses LDH level in plasma, 0.05 ml of each plasma sample and 0.01 ml of NADH solution (1.25 mM) were added into duplicate wells of a 96 well-plate, bringing samples to a final volume of 0.05 ml with LDH Assay Buffer. Absorbance was measured at 450 nm. Acid phosphatase assay in plasma was performed according to Bergmeyer (1974) method. A volume of 0.90 ml of the substrate solution freshly prepared (composed of 4-nitrophenyl phosphate and 5 ml of the citrate buffer solution) was mixed with 0.10 ml of each sample and absorbance was measured at 405 nm.

Total proteins assay

To measure total proteins level in testis, the colorimetric method of (Bradford, 1976) was used. 0.02 ml of the supernatant of homogenized testis was added to 1 ml of working reagent (sodium potassium tartrate and copper sulfate) and incubated at room temperature (20-25°C) for 5 min. Absorbance was measured at a wavelength λ = 546 nm.

Oxidative stress biomarkers analysis: catalase, GST, MDA and SH assays

Testis samples were homogenized on ice in 0.50 ml of cold buffer, then centrifuged at 10 000 × *g* for 15 min at 4°C to remove insoluble material. Catalase activity was assessed according to Asru (1972). The decomposition of hydrogen peroxide (H_2O_2) was monitored following the absorbance decrease at 240 nm. 0.02 ml of each sample was mixed with 0.75 ml of phosphate buffer and 0.20 ml of H_2O_2 was added to initiate the reaction. Absorbance was measured every 30 s for 2 min, at a wavelength λ = 240 nm. GST activity was determined spectrophotometrically a 25°C in presence of 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH). Briefly, 0.05 ml of each sample (previously diluted to 1/10th) was mixed with 0.4 ml of diluted phosphate buffer (1 ×) and 0.53 ml of distilled water was added. The mixture was stirred and 0.01 ml of GSH and 0.01 ml CDNB were added to initiate the enzymatic reaction. Increased absorbance at λ = 340

nm was monitored every 30 s for 2 min as described by Habig and Jacoby (1981). MDA level was measured according to Genot *et al*. (1996). This method is based on the thiobarbituric acid (TBA) reaction. A volume of 0.50 ml from supernatant of testis homogenate was mixed with 0.20 ml of

Table 1. Incidence of tartrazine on body weight gain and both testes and epididymis weight

Organs weight and body weight gain	Ctr	Tr
Body weight gain (%)	57.20 ± 0.23	37.40 ± 0.20 **
Testes relative weight (%)	0.49 ± 0.01	0.53 ± 0.02
Epididymis relative weight (%)	0.17 ± 0.07	0.21 ± 0.08

Data are expressed as mean ± SEM. **p<0.01. Control group (Ctr) in contrast with the tartrazine treated group (Tr).

Table 2. Incidence of tartrazine on sperm characteristics.

Sperm features	Ctr	Tr
Sperm count (million/ml of sperm suspension)	16.24 ± 0.93	8.85 ± 0.32 ***
Sperm motility (%)	62.01 ± 2.15	14.13 ± 1.92 ***
Sperm viability (%)	84.90 ± 0.90	65.60 ± 3.21 ***
Sperm abnormalities (%)	8.40 ± 0.60	20.30 ± 0.82 ***

Data are expressed as mean ± SEM. ***p<0.001. Control group (Ctr) in contrast with the tartrazine treated group (Tr).

phosphate buffer with 0.50 ml of TCA-BHT, 0.16 ml of HCl and 0.64 ml of Tris. The mixture was incubated 10 min at 80°C, then centrifuged at 1000 × *g* at 4°C for 10 min. Absorbance was determined at 530 nm. SH level variation was evaluated using the method of Miao-Lin (1994). Briefly, 5,5'-dithiobis 2-nitrobenzoic acid or Ellman reagent (DTNB) was used in redox reaction, 0.05 ml of testicular supernatant was added to 1 ml of phosphate buffer, and the first absorbance at 412 nm was immediately determined. Afterwards, 0.02 ml of DTNB was added to the above mixture and incubated 15 min at room temperature, in order to perform a second absorbance reading at the same wavelength (λ = 412).

Statistical analyses

Data were expressed as mean ± SEM (standard error). Statistical test t-student was applied to find significant difference between values of various parameters recorded for control and treated groups. We used a software ''STATISTICA'' (version 10.0) to assess whether the difference is significant or not.

Table 3. Incidence of tartrazine on different sperm abnormalities, in head, middle piece and flagellum

Abnormalities locations	Ctr	Tr
Coiled flagellum (spirally coiled) (%)	25.44 ± 0.65	23.74 ± 0.05
Coiled flagellum (coiled on itself) (%)	9.39 ± 0.12	28.41 ± 0.90 **
Angled flagellum (%)	10.09 ± 1.09	32.28 ± 0.88 **
Absent flagellum (%)	51.07 ± 0.88	9.57 ± 0.41 ***
Middle piece and head (%)	4.01 ± 0.10	6 ± 0.44

Data are expressed as mean ± SEM. ***p<0.001 and **p<0.01. Control group (Ctr) in contrast with the tartrazine treated group (Tr).

p<0.05 was considered statistically significant.

Results

Tartrazine effect on Body weight gain, testes and epididymis weight

All animals survived the experimentation and no obvious signs of toxicity were observed in Tr group. However, rats exposure to tartrazine led to a significant decrease (p<0.01) in body weight gain in Tr-treated group when compared with the Ctr. No significant variation was noticed in both testes and epididymis weight, as illustrated in Table 1.

Tartrazine alter on sperm quality

Tartrazine caused a highly significant decrease in sperm density as well as sperm motility when compared with the Ctr (Table 2). Similarly, we noted a marked lower sperm viability in Tr group (Table 2) and we showed that tartrazine significantly increased sperm abnormalities particularly those affecting the flagellum, with a majority of angled and coiled tail forms (Table 3). Noting, that no significant incidence of abnormalities was observed neither in sperm middle piece nor in the head in Tr-treated rats.

Tartrazine reduce testosterone and cholesterol level

Data illustrated in Table 4, shows that tartrazine decreased plasmatic testosterone concentration in Tr group when compared with the Ctr group (p<0.05)

Table 4. Illustration of cholesterol and testosterone levels variation, in plasma and testis

Biochemical parametres	Ctr	Tr
Testosterone (µg/ml)	2.99 ± 0.17	2.38 ± 0.25 *
Total cholesterol (g/l)	0.50 ± 0.01	0. 42 ± 0.01 **
Testicular cholesterol (g/l)	0.53 ± 0.04	0.19 ± 0.02 ***

Data are expressed as mean ± SEM. **p<0.01, ***p<0.001, *p<0.05. Control group (Ctr) in contrast with the tartrazine treated group (Tr).

Table 5. Incidence of tartrazine on lipid peroxidation, antioxidants factors and toxicity indicator

Oxidative stress and toxicity indicators	Ctr	Tr
Total proteins (g/l)	30.80 ± 1.60	23.70 ± 1.40 ***
LDH (UI/ml)	20.90 ± 1.11	30.20 ± 0.80 *
Acid phosphatase (UI/l)	12.36 ± 1.28	18.79 ± 0.71 *
Catalase (µmol/min/g of total proteins)	1.50 ± 0.08	1.12 ± 0.02 **
GST (nmol/min/mg of total proteins)	2.72 ± 0.14	3.30 ± 0.18 **
SH (mM)	0.24 ± 0.02	0.13 ± 0.01 ***
MDA (nmol/g of total proteins)	0.36 ± 0.03	0.71 ± 0.06 ***

Data are expressed as mean ± SEM. ***p<0.001, **p<0.01, *p<0.05. Control group (Ctr) in contrast with the tartrazine treated group (Tr).

(Table 4). Otherwise, total cholesterol level in plasma as well as testicular cholesterol were significantly decreased in Tr-treated group in contrast with the Ctr (p<0.01).

Tartrazine increases toxicity biomarkers and affect oxidative stress homeostasis

Illustrated results in Table 5, shows a distinct increase (p<0.05) in both acid phosphatase and LDH level in plasma following tartrazine treatment (p<0.05). Total proteins level in testis was highly reduced in Tr group when compared with the Ctr (p<0.001) (Table 5). Although, data indicated a significant increase (p<0.001) in MDA concentration in testis, whereas, SH level remained considerably decrease (p<0.001) in Tr group *versus* the Ctr group. A lower catalase activity in testis was recorded, while, GST activity was significantly elevated (p<0.01) in Tr group (Table 5).

Discussion

During the last decades many controversies concerning azo synthetics dyes and mainly tartrazine were discussed. Tartrazine is a well consumed dye as well manufactured and used in food, drugs and cosmetics industry in different countries, where tartrazine require the labeling of many products. However, previous researches, still prove that tartrazine is a toxic dye. In the present study, we showed a noxious effect of tartrazine on sperm characteristics, testosterone and cholesterol levels and oxidative stress biomarkers. All along the experimentation period, all physiological parameters were measured and all animals survived the experimentation. We did not notice any significant variation of liquid or food intake (data not shown). Furthermore, tartrazine did not have any significant effect on testes or epididymis weight, whereas, it caused a considerable decrease in body weight gain. Similar results have been reported by Amin *et al.* (2010) in young rats orally exposed to 15 and 500 mg/kg of tartrazine for 30 days. Tanaka (2006) and Tanaka *et al.* (2008), have also showed that tartrazine consumption at different doses; 83, 259, 773 mg/kg/day for 37 days, induced body weight loss in male albinos mice. These previous studies corroborate the results we obtained. Body weight loss is considered as the first indicator of sensitive toxicity to tartrazine in rodents (Ezeuko *et al*., 2007), therefore, body weight decrease shows a generalized tartrazine toxicity in rat organism. Furthermore, by the present work, we observed that tartrazine can alter germ cells quality. Sperm density and motility were significantly low below normal values. These findings are in agreement with a study conducted by Visweswaran and Krishnamoorthy (2012) in Wistar rats treated with 72 mg/kg of tartrazine daily for 60 days. We suggested that the critical decline in sperm density could be related to a spermatogenesis failure or to an excessive spermatozoa apoptosis, which can also explain the highly decreased sperm survival we noticed. On the other hand, an alteration of Sertoli cell function could be also considered. Evaluation of

sperm cell motility and defects revealed an increase in flagellum abnormalities and a motility decrease. Hypothetically, we strongly suggested that the impairment of cells locomotion system is possibly the major cause of sperm decreased motility. Our results are consistent with previous studies, that reported similar effects in experimental animals treated with different tartrazine doses, to know Abdel Aziz *et al*. (1997) in albinos mice treated with 680 and 1360 mg/kg of food coloring erythtosine, following 5 daily p.oinjection, Mehedi *et al*. (2009) in rats treated with 173.9, 1767.8 and 5541.4 mg/kg/day of tartrazine for 28 days, and Gautam *et al.* (2010) who evidenced the same findings in albinos mice treated with lower tartrazine doses (0.2 and 0.4 mg/kg/day) for 30 days. Moreover, our classification of sperm abnormalities revealed that sperm abnormalities were mostly observed in sperm tail, with a majority of angeled and coiled forms. Theoretically, we proposed that tartrazine possibly induced a deterioration of flagellum development during maturation or spermatogenesis process. The involved mechanism in tartrazine deleterious effect in sperm cells could involve different molecules and trigger events such as apoptosis or autophagy which are essential for sperm survival, development and selection. Therefore, we still need more accurate in vitro studies and assays to identified some tartrazine mechanism of action in testis.

Hypoandrogenic environment can disturb epididymal function, such as sperm maturation and protection (Jarvi, 2012). These disturbances could be attributed to a spermatogenesis, as well as steroidogenesis failure. Indeed, we perceived a significant decrease in testosterone level in plasma and to the best of our knowledge, no other studies carried out tartrazine effect on testosterone level. It is a fact that testosterone is an essential factor controlling testicular development and sperm apoptosis, a lower concentration of this indispensable androgen is usually associated to a decrease in Leydig cells activity or to a lower testosterone capture in testis. As we know, testosterone is necessary to maintain a normal fertilizing ability, and sperm maturation is a testosterone dependent process (Orgebin-Crist and Tichenor, 1973). A low testosterone level in testis, principally induce an excessive sperm apoptosis and consequently diminish sperm survival. Consequently, we suggested that testosterone decreased due to tartrazine treatment is the main cause of sperm quality decline we noted. Otherwise, known as the major precursor of androgens a small variation of cholesterol level can significantly affect testosterone production. The decrease in testicular cholesterol we observed was related to an attenuation of cholesterol capture and may be to its de novo synthesis in testis, which led to a lower production of testosterone.

Our results are in agreement with those observed by Ashour and Abdelaziz (2009), who stated that albinos rats exposure to 125 mg/kg/day of Fast green, a synthetic organic food additive similar to tartrazine for 35 days, can provoke a significant decrease in cholesterol level in serum. Taken together, our findings showed that tartrazine have many harmful effects on spermatogenesis and steroidogenesis in rat. Therefore,we judged important to measure toxicity indicators in order to concretely confirm not only tartrazine gonadotoxicity but also generalized toxic effect. Known as a stable enzyme widely used to evaluate agent toxicity level in tissue and cells, LDH is generally increased in different pathological conditions like cancer or following exposure to relatively toxic product. In this context, we highlighted a considerable increase in LDH as well as acid phosphatase level in plasma. We related LDH and acid phosphatase elevation in tartrazine-treated rat to an evoked tartrazine toxicity in prostate, vesicular glands and various organs, such as liver, kidney, lung and pancreas. To our knowledge no other study carried out the measurement of LDH and acid phosphatase level after tartrazine treatment. Accordingly, tartrazine toxicity seem to be more amplified in sperm cells that appeared to be highly sensitive and fragile to this molecule.

On the other hand, sperm cells alteration, toxicity biomarkers changes as well as testosterone and cholesterol level variation, could be a result of oxidative stress enhancement through an overproduction of reactive oxygen species (ROS). Radicals are species containing one or more unpaired electrons, they are produced during normalmetabolism and perform several useful functions. Excessive production of these ROS can result in tissue damage, which often involve generation of highly reactive oxidants (Halliwell, 1992). Measurement of oxidative stress biomarkers in testis was essential to determine ROS involvement in the different previous alterations we observed. Indeed, Sweeney *et al.* (1994), mentioned that azo dyes products are generally genotoxic, yet, not through N-hydroxylation and esterification which is characteristic of many aromatic amines, but rather through a mechanism involving oxygen radicals. According to Moutinho *et al.* (2007) aromatic amines generated from tartrazine metabolism can produce ROS as a part of their metabolic products through a specific interaction of these amino groups with nitrite or nitrate, contained in foods or in the stomach. This process generally induce an imbalance

of the oxidative status in many tissues. During the last years, the interest regarding the involvement of oxidative stress in tartrazine effect has increased and a plenty of studies showed a considerable toxicity in different experimental pattern. Practically, we noticed a related tartrazine ability to disruption the redox homeostasis. Previously tartrazine has been shown to increase lipid peroxidation and to alter antioxidant enzyme activities in liver, brain and blood (Amin *et al*., 2010; Gao *et al.*, 2011; Abd El-Wahab and El-Deen Moram, 2013). In recent reports, researchers showed that ROS overproduction can be detrimental to testicular function (Aitken and Roman, 2008; Mathur and D'Cruz, 2011), however, there is very limited studies in the literature that explored oxidative damage occurrence in reproductive organs after daily exposure to tartrazine. Our oxidative stress biomarkers study revealed a significant increase in MDA level, which is the major lipid peroxidation product suggesting an increased ROS attack on phospholipid in cells membrane. SH or thiol groups which are highly sensitive to free radical attacks and represent a reliable indicator of oxidative stress induction were decrease by the end of tartrazine treatment. We supposed that lower activity of reduced GSH or a possible total proteins decrease could be the major cause of SH attenuation in testis. Our findings are in agreement with earlier studies that demonstrated that food coloring additives and more precisely azo compounds such as tartrazine can overproduce ROS and increase oxidative stress in liver, kidney and brain. An imbalance of redox status has been reported by Himri *et al.* (2011) in rats chronically exposed to 5, 7.5, and 10 mg/kg of tartrazine for 90 days, Amin *et al.* (2010) and Gao *et al.* (2011) in mice treated with 175, 350, and 700 mg/kg of tratrazine for 30 days.

Germ cells are particularly hypersensitive to oxidative stress-induced damage because of their plasma membrane which contain a large amount of polyunsaturated fatty acids (Alvarez and Storey, 1995) and a low concentration of the protective scavenging enzymes (Sharma and Agarwal, 1996). We suggested that lipid peroxidation product increase in tartrazine-treated rats can be directly related to sperm membrane damage, which led to sperm damage. Among all the well-known biological antioxidant defense, catalase and GST are still the mainly studied antioxidant enzymes, that play a major role in sperm protection against peroxidative damages (Curtis *et al.*, 2007) To prevent cell death by toxic radicals, antioxidant enzymes activity could be either increase or decrease in numerous pathological conditions. In our study, tartrazine increased GST and decreased catalase activity which justify a deterioration of the antioxidant defense system by tartrazine consumption. These results are in accordance with Visweswaran and Krishnamoorthy (2012) in tartrazine-treated rat testis.

Collectively, we suggested that the amplification of ROS attacks in testis after exposure to tartrazine is a potential mechanism that led to sperm alterations we noted. Yet, our work have several limitations and more measurement and studies to define the precise molecular process involved in tartrazine toxicity are strongly required.

Conclusion

By the present study, we concluded that tartrazine induced decrease in cholesterol and testosterone level resulted in a reduced sperm density and a higher percentage of dead, immotile and abnormal germ cells. Oxidative stress biomarkers study showed that testicular injury due to tartrazine may have a detrimental impact on sperm maturation process and consequently decrease fertility in rat. In this regard, detailed investigation including fertility examination, reproductive performance, delay of conception, number of pups and changes that possibly occur during preconception, mating periods or the lactation need to be performed. Furthermore, more clinical studies concerning tartrazine in human are necessary to evaluate the risks associated with this food dye consumption. Meanwhile, consumers especially children have to be aware about tartrazine consumption, as well as other coloring agents frequently used in large quantity in food industry.

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