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Embryotoxic and Teratogenic Effects of Tartrazine in Rats

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Abstract

Tartrazine (TAZ) is one of the most commonly used artificial dyes for foods and drugs. We determined the effect of TAZ on fetal development by examining morphological, visceral, and skeletal malformations in rat fetuses following daily oral administration of TAZ to pregnant Wistar rats at the 6th-15th day of gestation. TAZ at 0.45 and 4.5 mg/kg induced 6.0 and 7.1% fetal resorptions, as well as 10.0 and 10.5% fetal mortality, respectively. Fetal body weight and length were significantly lower in the groups treated with TAZ at 0.45 (3.97 ± 0.21 g and 27.3 ± 0.54 mm, respectively) and 4.5 mg/kg (3.48 ± 0.15 g and 23.22 ± 1.02 mm, respectively) than in the control group (4.0 ± 0.15 g and 30.01 ± 0.42 mm, respectively). TAZ at 0.45 and 4.5 mg/kg induced hepatic damage (20 and 33.3%, respectively), dark brown pigmentation due to hemosiderin in the splenic parenchyma (16.7 and 21.7%, respectively), as well as destructed and necrotic renal tubules (16.7 and 26.7%, respectively) in the fetuses. Moreover, TAZ at 0.45 and 4.5 mg/kg caused one or more missing coccygeal vertebrae (20 and 40%, respectively), missing sternebrae (6 and 10%, respectively), missing hind limbs (24 and 4%, respectively), and irregular ribs (16 and 20, respectively) in the fetuses. We concluded that TAZ has embryotoxic and teratogenic potentials in rats.

***Key words*:** Food additives, Tartrazine, Azo dye, Teratogenicity, Embryotoxicity

INTRODUCTION

Tartrazine (TAZ), also known as E 102, FD & C Yellow No. 5, is an azo dye and salt of chemical formula 3-carboxy-5-hydroxy-1 (p-sulfophenyl)-4-(sulfophenyl azo) pyrazolone (1). It is the most frequently used food colorants to achieve yellow color in sweets, juices, jams, mustard, and sodas. In addition, it has been widely used to color human pharmaceuticals, such as vitamins, antacids, cosmetics, and hair products (2). This food colorant is also used in cooking as a substitute for saffron (3).

Existing data on the toxicity of TAZ are contradictory (4). Some reports confirmed that TAZ exerts no dangerous effect in both human and experimental models (5-8). However, other studies reported that TAZ induces neurotoxic (9,10), immunotoxic (11), reprotoxic (12,13), genotoxic, and mutagenic effects (4), as well as irritability, restlessness, and sleep disturbance (14-16). Recently, researchers' attention has focused on the potential teratogenic effects of some food additives in pregnant women.

Several studies have suggested possible teratogenic effects of the use of food additives during pregnancy (17-19). However, few studies have examined the teratogenic potential of TAZ. Collins *et al*. (20) reported that TAZ exhibits no maternal or developmental toxicity in Osborne-Mendel rats when administered by gavage at doses of 0, 60, 100, 200, 400, 600, or 1,000 mg/kg body weight/day on gestational days 0-19. A recent study reported that TAZ up to 10 mM is not embryotoxic nor teratogenic to zebrafish embryos (21). However, no previous study has examined the teratogenic potential of TAZ when administered at several folds its acceptable daily intake (ADI). Therefore, the present study aimed to investigate the susceptibility of rat embryos at the stage of active organogenesis, to anomalies caused by TAZ at doses of 2.5- or 25-fold its ADI.

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MATERIALS AND METHODS

Chemicals. TAZ powder (E 102, C.I. 19140) of 86.7% purity was obtained from Courtex International, France. All other chemicals and reagents used in this study were of analytical grade.

Experimental animals and treatments. The experimental procedures were in accordance with the guideline of the care and use of laboratory animals in scientific investigations and approved by the Ethics of Animal Use in Research Committee of Cairo University, Egypt.

We chose to use albino rats, the most frequently used mammals in teratology experiments, in our study because of their stable genetic constitution, high availability, and frequent use in previous studies.

Female Wistar rats, aged 70-100 days and weighing 130-200 g, were fed Purina rat diet (Relation Purina Company, CA, USA) *ad libitum*. The appropriate TAZ dosage for rats was determined by using the inter-species dosage conversion scheme (22).

This experiment was based on a previous drug teratogenicity testing (23). Vaginal smears were taken periodically to examine the rats' estrus cycle (24). Each female rat was then mated with a proven fertile male rat. On the morning following mating, vaginal smear was taken and examined microscopically. The presence of sperm indicated pregnancy, and the day of this confirmation was marked as the first day of pregnancy (25). Confirmed pregnant female rats were caged separately.

Pregnant rats were divided into three groups consisting of 30 rats each. The 1st and 2nd groups were administered TAZ 0.45 and 4.5 mg/kg, respectively. The 3rd group, the control, was administered saline. TAZ and saline were administered orally each day from the 6th to the 15th day of gestation, which was considered to be the period of organogenesis, when fetal organs are most sensitive to the potential effects of drugs (24).

Morphological examination. All rats were sacrificed by cervical dislocation on the 20th day of pregnancy. The uterus was opened and fetal swellings, sites of resorption, number of viable fetuses, and number of dead fetuses in each uterine horn were counted. All fetuses in each group were subjected to an external morphological examination according to the method of Manson and Jeanne (26).

Visceral examination. One third of fetuses in each group were preserved in Bouin's solution after intra-abdominal injection of 0.2 mL Bouin's solution. After fixation, visceral abnormalities in the fetuses were examined (27).

Skeletal examination. The other two-thirds of the fetuses were fixed in 95% ethyl alcohol and stained with alizarin red. Skeletal malformations in the fetuses were then examined (25).

Statistical analysis. The data were tested for normality by the Shapiro-Wilk W-test (28). Data are expressed as the means ± standard error (SE). The statistical analyses were conducted by one-way analysis of variance (ANOVA) followed by the Duncan's Multiple Range test using the (Version 2; GraphPad Software Company, CA, USA). A probability level of 0.05 was chosen for statistical significance.

RESULTS

Morphology of uterus and fetuses. In the present study, fetal resorption and mortality in the rats treated with 0.45 mg/kg TAZ at were 6 and 7.1%, whereas those in the TAZ 4.5 mg/kg-treated rats were 10.0 and 10.5%, respectively (data not significant). Fetal body weight and length in the groups treated with TAZ 0.45 (3.97 ± 0.21 g and 27.3 ± 0.54 mm, respectively) and 4.5 mg/kg (3.48 ± 0.15 g and 23.22 ± 1.02 mm, respectively) were significantly lower than those in the control group (4.0 ± 0.15 g and 20.01 ± 0.42 mm, respectively) (Table 1, Fig. 1, 2).

Visceral examination findings. The viscera of rat fetuses treated with TAZ 0.45 and 4.5 mg/kg revealed cardiomegaly (16.7 and 25%, respectively) and hepatic damage (20 and 33.3%, respectively). Moreover, TAZ 0.45 and 4.5 mg/kg-treated fetuses showed dark brown pig-

Table 1. Morphological malformations in rat uterus and fetuses after maternal oral administration of tartrazine

Drug	Dose (mg/kg)	Number of implantation sites	Fetuses						Fetal weight (g)	Fetal length (mm)
			Resorbed		Dead		Viable			
			No.	%	No.	%	No.	%		
Control	-	220	5	2.3	4	1.8	211	59.9	4.0 ± 0.15	30.01 ± 0.42
TAZ	0.45	200	12	6	20	10.0	168	84.0	3.97 ± 0.21	27.3 ± 0.54**
	4.5	210	15	7.1	22	10.5	173	82.4	3.48 ± 0.15*	23.22 ± 1.02**

TAZ, tartrazine. *Significant ($p < 0.05$), **Highly significant ($p < 0.01$). Differences in the numbers of resorbed, dead, and viable fetuses were not significant at $p < 0.05$. Fetal weight and length are expressed as the mean ± SE.

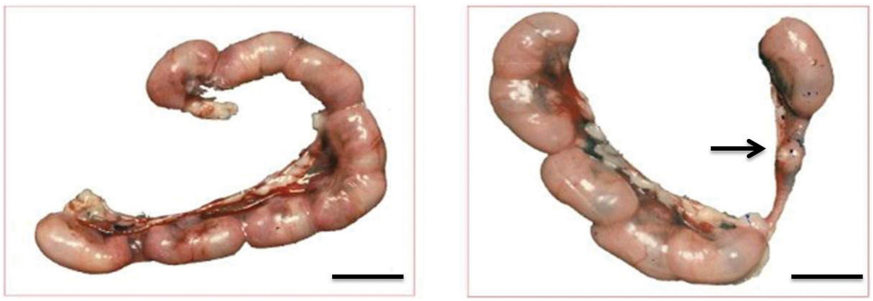

Fig. 1. Rat uterus showing fetal resorption (arrow) after daily oral administration of tartrazine (4.5 mg/kg) to pregnant rats, from the 6th to 15th day of gestation (scale bar: 100 mm). Left side: control, scale bar: 100 mm.

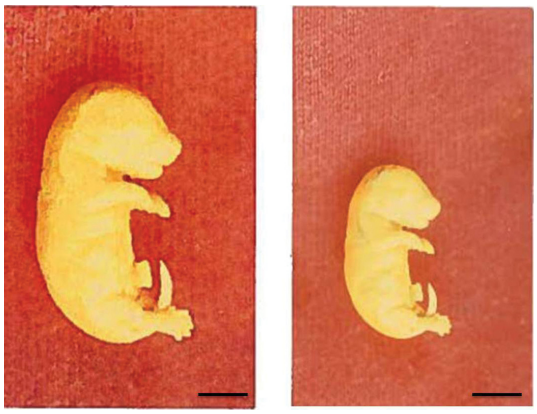

Fig. 2. Fetal body weight and length were decreased by daily oral administration of tartrazine (4.5 mg/kg) to pregnant rats at the 6th-15th day of gestation (scale bar: 50 mm). Left side: control, scale bar: 50 mm.

mentation due to hemosiderin in the splenic parenchyma (16.7 and 21.7%, respectively), as well as destructed and necrotic renal tubules (16.7 and 26.7%, respectively) (Table 2, Fig. 3-5).

Skeletal examination findings. The rat fetuses treated with TAZ 0.45 and 4.5 mg/kg showed skeletal malformations, including one or more missing coccygeal vertebrae (20 and 40%, respectively), missing sternebrae (6 and 10%, respectively), missing hind limbs (24 and 4%, respectively), and irregular ribs (16 and 20%, respectively) (Table 3, Fig. 6).

DISCUSSION

Currently, safety limits and post-exposure health hazards associated with the consumption of food additives are of high concern to consumers, nutritionists, and toxicolo-

Table 2. Visceral malformations in rat fetuses after maternal oral administration of tartrazine

Drug	Dose (mg/kg)	Number of fetuses examined	Malformations											
			Brain		Palate		Heart		Spleen		Liver		Kidney	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	-	60	-	-	-	-	-	-	-	-	-	-	-	-
Tartrazine	0.45	60	0	0	0	0	10	16.7	10	16.7	12	20.0	10	16.7
	4.5	60	14	23.3	0	0	15	25	13	21.7	20	33.3	16	26.7

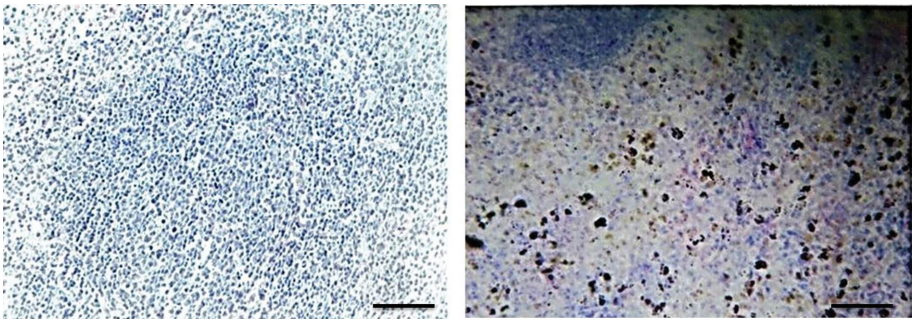

Fig. 3. Rat spleen showing dark brown pigmentation due to hemosiderin in the splenic parenchyma after daily oral administration of tartrazine (4.5 mg/kg) to pregnant rats at the 6th-15th day of gestation (scale bar: 100 μm). Left side: control, scale bar: 200 μm.

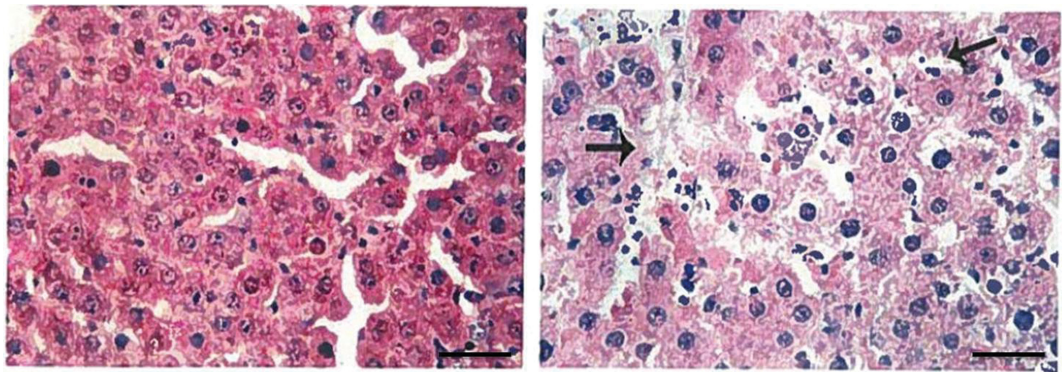

Fig. 4. Rat liver showing hepatic damage and parenchymal necrosis after daily oral administration of tartrazine (4.5 mg/kg) to pregnant rats at the 6th-15th day of gestation (scale bar: 25 μm). Left side: control, scale bar: 25 μm.

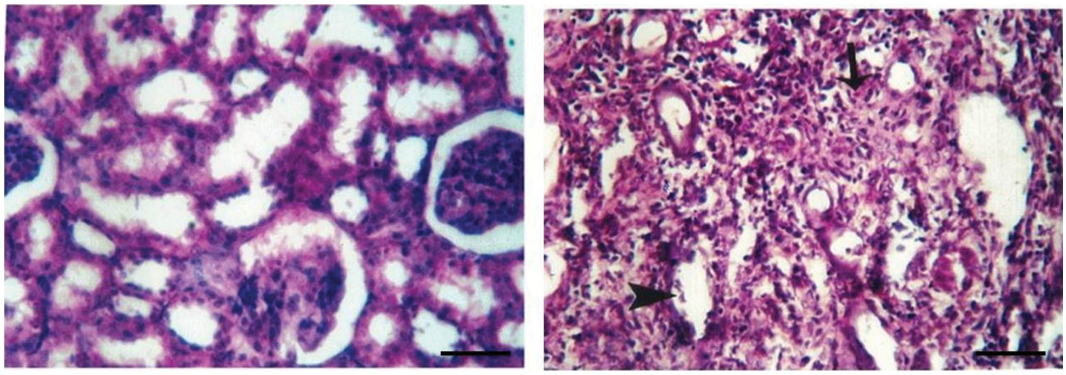

Fig. 5. Rat kidney tissues showing focal fibrosis (arrow) in the destructed and necrotic renal tubules (arrowhead) after daily oral administration of tartrazine (4.5 mg/kg) to pregnant rats at the 6th-15th day of gestation (scale bar: 100 μm). Left side: control, scale bar: 100 μm.

Table 3. Skeletal malformations in rat fetuses after maternal oral administration of tartrazine

Drug	Dose (mg/kg)	Number of fetuses examined	Malformations									
			Skull		Vertebral column		Ribs		Sternebrae		Limbs	
			No.	%	No.	%	No.	%	No.	%	No.	%
Control	-	50	-	-	-	-	-	-	-	-	-	-
Tartrazine	0.45	50	0	0	10	20	8	16	3	6	2	4
	4.5	50	0	0	20	40	10	20	9	18	12	24

gists (29-32). Hence, a complete and precise assessment of the toxicity of food additives is highly needed. Among azo dye food colorants, TAZ is the most widely used. Previous studies revealed that TAZ at doses of several times higher than its ADI for human exhibits hepatonephrotoxicity and alters various metabolic aspects in experimental animals (33). Moreover, administration of TAZ induces leucocyte DNA damage associated with severe cellular alterations in rat liver and kidneys, which may lead to adverse health effects (4).

The stage of embryonic development is critical in determining the sensitivity of the embryo and the pattern of embryonic to the drug. A previous study showed that nearly all drugs administered during pregnancy will, to some degree, enter fetal circulation via passive diffusion (34). The most significant factors affecting placental diffusion are lipid solubility and molecular size. The greater the lipid solubility and molecular size, the greater is the placental transfer (35).

It has been repeatedly shown that agents administered during the cleavage and blastula stages of mammalian embryonic development and before implantation, which in rats occurs on the 6th day of gestation, typically elicit no teratogenic response, even though the same agents elicit

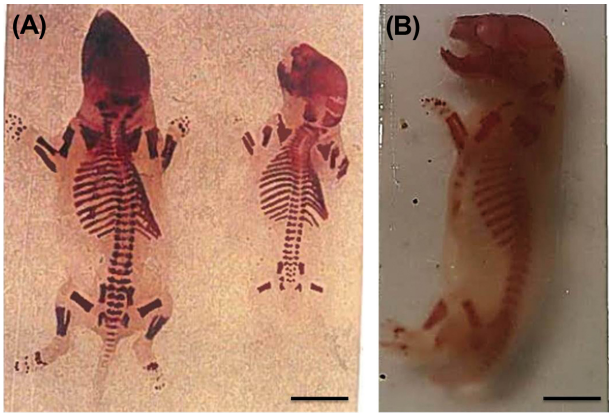

Fig. 6. Missing coccygeal vertebrae, hind limb, and sternebrae, as well as irregular ribs caused by daily oral administration of tartrazine (4.5 mg/kg) to pregnant rats at the 6th-15th day of gestation (A) (scale bar: 50 mm). (B) Control, scale bar: 50 mm.

marked responses when administered at high doses at a later stage of embryo development (36,37).

Embryo susceptibility to teratogenesis decreases as tissue differentiation proceeds, and the embryo as a whole is resistant to teratogens once organogenesis is completed (38). Therefore, TAZ administration in this study was restricted to the critical period of organogenesis, which is the 6th-15th day of gestation, during which cellular differentiation occurs and the rat embryo is most sensitive to various external and internal factors.

In the present study, TAZ was administered at 0.45 and 4.5 mg/kg, which were equal to 2.5 and 25 times of its ADI, respectively. FDA recommends an ADI of 3.75 mg/kg for TAZ. Moreover, the World Health Organization suggests that TAZ ADI be limited to 2.5 mg/kg (39). An ADI of 0-7.5 mg/kg/day for TAZ was established by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and has been maintained by the European review committees (40). In 2016, JECFA increased the previously established ADI of TAZ to 0-10 mg/kg (41). In the current study, the tested doses, which were several folds the ADI established by JECFA (41), could be relevant to humans because humans may be exposed to TAZ from multiple sources at the same time. For example, TAZ is now extensively used as an ingredient in personal care and cosmetics products (16). In addition, various types of medications, primarily for easy identification, include TAZ to achieve a yellow, orange, or green hue in a liquid, capsule, pill, lotion, or gel. Types of pharmaceutical products that may contain TAZ include vitamins, antacids, cold medications, lotions, and prescription drugs (42). Hence, humans may be exposed to TAX from sources other than food; thus, such sources should be taken into account in the risk assessment of food additives. Consequently, we used TAX at several folds the ADI for humans and converted the doses to those for rats according to Paget and Barnes (43).

In this study, TAZ administration caused fetal resorption and mortality, and significantly decreased fetal weight and length. Additionally, cardiomegaly, hepato-renal damage, and splenic pigmentation were observed in the TAZ-treated groups. In this regard, exposure to food additives is strongly related to mutagenicity in the form of gene mutation and chromosomal aberration (44).

The present study compared the effect of TAZ in developing fetuses during the gestation period with that in the control group. It has been stated that the teratogenic effect of a drug can be predicted if the drug can cross the placental barrier (36) and/or inhibit protein synthesis (45) and further inhibit the material enzymes activity (46).

A previous study (47) showed similar findings to ours, in which oral administration of amaranth sunset yellow and curcumin to pregnant females caused growth retardation, hypoplasia of the heart and lungs, and skeletal abnormalities in the metacarpal and metatarsal bones and caudal vertebrae.

A 13-week subchronic oral toxicity study showed several hepatic and renal parameters were altered in rats fed diet containing 5 mg/kg TAZ (48). On the contrary, in a study by Tanaka (8), mice were fed diet containing TAZ at 83, 259, and 773 mg/kg/day from the age of 5 weeks in the F0 generation until 9 weeks of age in the F1 generation. There was no significant change in food intake and body weight in the F0 and F1 generations. There was also no significant change in the number of pregnant females, number of litters, litter size, litter weight, total sex ratio, and average sex ratio. This discrepancy could be attributed to the difference in animal species tested; thus, further studies using different animal species are needed.

The effect of TAZ on fetal growth and development could be attributed to several factors. Intestinal microflora metabolize TAZ into two metabolites, sulfanilic acid and aminopyrazolone (49). These metabolites are degraded very slowly or not at all, and can generate reactive oxygen species, which could induce embryonic malformations (48). Oxidative stress is involved in the toxicity of many xenobiotics (50-53). In addition, it was reported that synthetic food coloring agents, such as TAZ, inhibit mitochondrial respiration in rat liver and kidneys (54), and disrupt the integrity of mitochondrial membranes, which is vital for maintaining mitochondrial functions and inducing cellular apoptosis. Hence, TAZ-induced embryonic malformations could be associated with an increase in apoptosis in the embryos.

In summary, TAZ at 0.45 and 4.5 mg/kg increased fetal resorptions and mortality, as well as cardiomegaly, hepato-renal damage, and splenic pigmentation in the fetuses. The treatment also induced skeletal malformations in the fetuses, including missing coccygeal vertebrae, missing sterne-

brae, missing hind limbs, and irregular ribs. Thus, we concluded that TAZ has embryotoxic and teratogenic potentials in rats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Tanaka, T., Takahashi, O., Oishi, S. and Ogata, A. (2008) Effects of tartrazine on exploratory behavior in a three-generation toxicity study in mice. *Reprod. Toxicol.*, **26**, 156-163.
2. Allam, K.V. and Kumar, G.P. (2011) Colorants-the cosmetics for the pharmaceutical dosage forms. *Int. J. Pharm. Pharm. Sci*., **3**, 9.
3. Mehedi, N., Ainad-Tabet, S., Mokrane, N., Addou, S., Zaoui, C., Kheroua, O. and Saidi, D. (2009) Reproductive toxicology of tartrazine (FD and C Yellow No. 5) in Swiss albino mice. *Am. J. Pharmacol. Toxicol.*, **4**, 130-135.
4. Khayyat, L., Essawy, A., Sorour, J. and Soffar, A. (2017) Tartrazine induces structural and functional aberrations and genotoxic effects *in vivo*. *PeerJ*, **5**, e3041.
5. Amin, K., Abdel Hameid, H., 2nd. and Abd Elsttar, A.H. (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.
6. Diacu, E. and Ene, C.P. (2009) Simultaneous determination of tartrazine and sunset yellow in soft drinks by liquid chromatography. *Rev. Chim.*, **60**, 745-749.
7. Mpountoukas, P., Pantazaki, A., Kostareli, E., Christodoulou, P., Kareli, D., Poliliou, S., Mourelatos, C., Lambropoulou, V. and Lialiaris, T. (2010) Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. *Food Chem. Toxicol*., **48**, 2934-2944.
8. Tanaka, T. (2006) Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. *Food Chem. Toxicol*., **44**, 179-187.
9. Ardern, K.D. and Ram, F.S. (2001) Tartrazine exclusion for allergic asthma. *Cochrane Database Syst. Rev*., **(4)**, CD000460.
10. Mohamed, A.A.R., Galal, A.A. and Elewa, Y.H. (2015) Comparative protective effects of royal jelly and cod liver oil against neurotoxic impact of tartrazine on male rat pups brain. *Acta Histochemica*, **117**, 649-658.
11. Abd-Elhakim, Y.M., Hashem, M.M., El-Metwally, A.E., Anwar, A., Abo-El-Sooud, K., Moustafa, G.G. and Ali, H.A. (2018) Comparative haemato-immunotoxic impacts of long-term exposure to tartrazine and chlorophyll in rats. *Int. Immunopharmacol*., **63**, 145-154.
12. Boussada, M., Lamine, J., Bini, I., Abidi, N., Lasrem, M., El-Fazaa, S. and El-Golli, N. (2017) Assessment of a sub-chronic consumption of tartrazine (E102) on sperm and oxidative stress features in Wistar rat. *Int. Food Res. J.*, **24**, 1473-1481.
13. Yang, S., Jia, Z.C., Chen, J.Y., Hu, J.X. and Zhang, L.S. (2014) Toxic effects of atrazine on reproductive system of male rats. *Biomed. Environ. Sci.*, **27**, 281-288.
14. Rowe, K.S. and Rowe, K.J. (1994) Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated-measures study. *J. Pediatr*., **125**, 691-698.
15. Schab, D.W. and Trinh, N.-H.T. (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**, 423-434.
16. Stevens, L.J., Burgess, J.R., Stochelski, M.A. and Kuczek, T. (2014) Amounts of artificial food colors in commonly consumed beverages and potential behavioral implications for consumption in children. *Clinic. Pediatr*., **53**, 133-140.
17. Borzelleca, J., Goldenthal, E., Wazeter, F. and Schardein, J. (1987) Evaluation of the potential teratogenicity of FD & C Blue No. 2 in rats and rabbits. *Food Chem. Toxicol.*, **25**, 495-497.
18. Borzelleca, J. and Hallagan, J. (1990) Multigeneration study of FD & C Red No. 3 (erythrosine) in Sprague-Dawley rats. *Food Chem.Toxicol.*, **28**, 813-819.
19. Collins, T., Black, T., O'Donnell Jr., M., Shackelford, M. and Bulhack, P. (1993) Teratogenic potential of FD & C Red No. 3 when given in drinking water. *Food Chem. Toxicol.*, **31**, 161-167.
20. Collins, T.F.X., Black, T.N., Brown, L.H. and Bulhack, P. (1990) Study of the teratogenic potential of FD & C Yellow No. 5 when given by gavage to rats. *Food Chem. Toxicol.*, **28**, 821-827.
21. Joshi, V. and Katti, P. (2018) Developmental Toxicity Assay for Food Additive Tartrazine Using Zebrafish (Danio rerio) Embryo Cultures. *Int. J. Toxicol.*, **37**, 38-44.
22. Nair, A.B. and Jacob, S. (2016) A simple practice guide for dose conversion between animals and human. *J. Basic Clin. Pharm.*, **7**, 27-31.
23. McElhatton, P. (1999) Principles of teratogenicity. *Curr. Obstet. Gynaecol.*, **9**, 163-169.
24. Garry, V.F. and Truran, P. (2017) Chapter 62 - Teratogenicity A2 in Reproductive and Developmental Toxicology (2nd Edition) (Gupta, R.C. Ed.). Academic Press, pp. 1167-1181.
25. Ariyuki, F., Ishihara, H., Higaki, K. and Yasuda, M. (1982) A study of fetal growth retardation in teratological tests: relationship between body weight and ossification of the skeleton in rat fetuses. *Teratology*, **26**, 263-267.
26. Manson, J.M. and Kang, Y.J. (1994) Test methods for assessing female reproductive and developmental toxicology in Principles and Methods of Toxicology (3rd edition) (Hayes, A.W. Ed.). Raven Press, New York, pp. 989-1034.
27. Fisher, B., Rose, N.C. and Carey, J.C. (2008) Principles and

practice of teratology for the obstetrician. *Clin. Obstet. Gynecol*., **51**, 106-118.
28. Shapiro, S.S. and Wilk, M.B. (1965) An analysis of variance test for normality (complete samples). *Biometrika*, **52**, 591-611.
29. Abd-Elhakim, Y.M., Hashem, M.M., Anwar, A., El-Metwally, A.E., Abo-El-Sooud, K., Moustafa, G.G., Mouneir, S.M. and Ali, H.A. (2018) Effects of the food additives sodium acid pyrophosphate, sodium acetate and citric acid on hemato-immunological pathological biomarkers in rats: Relation to PPAR-α, PPAR-γ and tnfα signaling pathway. *Environ. Toxicol. Pharmacol.*, **62**, 98-106.
30. Abd-Elhakim, Y.M., Anwar, A., Hashem, M.M., Moustafa, G.G. and Abo-El-Sooud, K. (2018) Sodium acetate, sodium acid pyrophosphate, and citric acid impacts on isolated peripheral lymphocyte viability, proliferation, and DNA damage. *J. Biochem. Mol. Toxicol.*, **32**, e22171.
31. Abo-EL-Sooud, K., Hashem, M.M., Badr, Y.A., Eleiwa, M.M., Gab-Allaha, A.Q., Abd-Elhakim, Y.M. and Bahy-EL-Dien, A. (2018) Assessment of hepato-renal damage and genotoxicity induced by long-term exposure to five permitted food additives in rats. *Environ. Sci. Pollut. Res. Int.*, **25**, 26341-26350.
32. Abo-EL-Sooud, K., Hashem, M.M., ElHakim, Y.M.A., Kamel, G.M. and Gab-Allaha, A.Q. (2018) Effect of butylated hydroxyl toluene on the immune response of Rift Valley fever vaccine in a murine model. *Int. Imunopharmacol.*, **62**, 165-169.
33. El-Wahab, H.M. and Moram, G.S. (2013) Toxic effects of some synthetic food colorants and/or flavor additives on male rats. *Toxicol. Ind. Health*, **29**, 224-232.
34. Syme, M.R., Paxton, J.W. and Keelan, J.A. (2004) Drug transfer and metabolism by the human placenta. *Clin. Pharmacokinet*., **43**, 487-514.
35. Briggs, G.G., Freeman, R.K. and Yaffe, S.J. (2012) Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk, Lippincott Williams & Wilkins.
36. Selderslaghs, I.W., Van Rompay, A.R., De Coen, W. and Witters, H.E. (2009) Development of a screening assay to identify teratogenic and embryotoxic chemicals using the zebrafish embryo. *Reprod. Toxicol.*, **28**, 308-320.
37. Chung, M.-K., Yu, W.-J., Lee, J.-S. and Lee, J.-H. (2013) Embryotoxicity and toxicokinetics of the antimalarial artesunate in rats. *Toxicol. Res.*, **29**, 27-34.
38. McQueen, E. (1972) Teratogenicity of drugs. *New Zealand Vet. J.*, **20**, 156-159.
39. He, Q., Liu, J., Liu, X., Li, G., Deng, P., Liang, J. and Chen, D. (2018) Sensitive and selective detection of tartrazine based on TiO_2-electrochemically reduced graphene oxide composite-modified electrodes. *Sensors*, **18**, 1911.
40. Walton, K., Walker, R., Van de Sandt, J., Castell, J., Knapp, A., Kozianowski, G., Roberfroid, M. and Schilter, B. (1999) The application of *in vitro* data in the derivation of the acceptable daily intake of food additives. *Food Chem. Toxicol.*, **37**, 1175-1197.
41. World Health Organization & Joint FAO/WHO Expert Committee on Food Additives (2016) Evaluation of Certain Food Additives: Eighty-Second Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 1000. World Health Organization. Available from: http://www.who.int/iris/handle/10665/250277/.
42. Stevenson, D.D. (2008) Tartrazine, azo, and non-azo dyes in Food Allergy. Adverse Reactions to Foods and Food Additives (4th edition) (Metcalfe, D.D., Sampson, H.A. and Simon, R.D. Eds.). Blackwell Publishing, New York, pp. 377-385.
43. Paget, G. and Barnes, G. (1964) Evaluation of Drug Activities (vol. 1), Academic Press, London.
44. Ishidate, M., Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M. and Matsuoka, A. (1984) Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.*, **22**, 623-636.
45. Anita, K., Mehta, V., Gupta, U., Prabhu, S. and Bapna, J. (1995) Methods for teratogenicity testing-existing and future models. *Indian J. Pharmacol*., **27**, 204.
46. Raymond, E., Sun, D., Chen, S.-F., Windle, B. and Von Hoff, D.D. (1996) Agents that target telomerase and telomeres. *Curr. Opin. Biotechnol*., **7**, 583-591.
47. Hashem, M.M., Atta, A.H., Arbid, M.S., Nada, S.A. and Asaad, G.F. (2010) Immunological studies on Amaranth, Sunset Yellow and Curcumin as food colouring agents in albino rats. *Food Chem. Toxicol.*, **48**, 1581-1586.
48. Himri, I., Bellahcen, S., Souna, F., Belmekki, F., Aziz, M., Bnouham, M., Zoheir, J., Berkia, Z., Mekhfi, H. and Saalaoui, E. (2011) A 90-day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats. *Int. J. Pharm. Pharm. Sci.*, **3**, 159-169.
49. Chung, K.T., Stevens, S.E., Jr. and Cerniglia, C.E. (1992) The reduction of azo dyes by the intestinal microflora. *Crit. Rev. Microbiol.*, **18**, 175-190.
50. Rotimi, O.A., Rotimi, S.O., Oluwafemi, F., Ademuyiwa, O. and Balogun, E.A. (2018) Oxidative stress in extrahepatic tissues of rats co-exposed to aflatoxin B1 and low protein diet. *Toxicol. Res.*, **34**, 211.
51. Salimi, A., Talatappe, B.S. and Pourahmad, J. (2017) Xylene induces oxidative stress and mitochondria damage in isolated human lymphocytes. *Toxicol. Res.*, **33**, 233.
52. Fallahzadeh, A.R., Rezaei, Z., Rahimi, H.R., Barmak, M.J., Sadeghi, H., Mehrabi, S., Rabani, S.M., Kashani, I.R., Barati, V. and Mahmoudi, R. (2017) Evaluation of the effect of pentoxifylline on cisplatin-induced testicular toxicity in rats. *Toxicol. Res.*, **33**, 255.
53. Amraoui, W., Adjabi, N., Bououza, F., Boumendjel, M., Taibi, F., Boumendjel, A., Abdennour, C. and Messarah, M. (2018) Modulatory role of selenium and vitamin E, natural antioxidants, against bisphenol A-induced oxidative stress in wistar albinos rats. *Toxicol. Res.*, **34**, 231.
54. Reyes, F.G., Valim, M.F. and Vercesi, A.E. (1996) Effect of organic synthetic food colours on mitochondrial respiration. *Food Addit. Contam.*, **13**, 5-11.