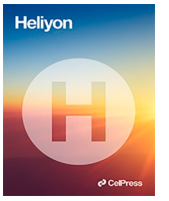
Heliyon 11 (2025) e41456

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Evaluation of bone development and organs in rat fetuses exposed to tartrazine

Osman Öztürk [a], Sümeyye Uçar [b], Züleyha Doğanyiğit [c], Aslı Okan Oflamaz [c], Evrim Suna Arıkan [d], Şükrü Ateş [e], Seher Yılmaz [e,*]

[a] Yozgat Bozok University, Faculty of Medicine, Department of Pediatric Health and Diseases, Yozgat, Turkey
[b] Erciyes University, Faculty of Medicine, Department of Anatomy, Kayseri, Turkey
[c] Yozgat Bozok University, Faculty of Medicine, Department of Histology and Embryology, Yozgat, Turkey
[d] Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Biology, Afyonkarahisar, Turkey
[e] Yozgat Bozok University, Faculty of Medicine, Department of Anatomy, Yozgat, Turkey

ARTICLE INFO

Keywords:
Tartrazine
Teratogenic effect
Double skeleton staining
Food dye
Rat fetuses

ABSTRACT

Tartrazine finds widespread application in the realms of alimentation, pharmaceuticals, cosmetic formulations, and textile manufacturing. Tartrazine has a negative effect on human health such as hyperactivity, allergies and asthma in children. Substances such as tartrazine might effect the embryo in a kind of aspects, containing physical or mental disorders, and a decrease in the child's intellectual memory. In this study, Sprague dawley female rats, 70–100 days old, weighing 250–300 g, with confirmed pregnancy, were divided into two groups of 5: control and tartrazine group. Rats were sacrificed on the 20th day and heart, lung, kidney and liver tissues were removed from the fetuses. The effect of tartrazine on fetal bone development was assessed by double skeletal staining, histological analysis on organs, and *IL-6, IL-1β, TNF-α*, and *TRPM2* gene levels. It has been observed that tartrazine, which is frequently used as a food dye, damages important fetal tissues such as liver, kidney, lung and heart. A statistically meaningful reduce was observed in the total length, total area, bone length and bone area values of the limb bones in the tartrazine group compared to the control group ($p < 0.05$). It was observed that the mRNA levels of *IL-6, IL-1β, TNF-α* and *TRPM2* genes in the livers of the fetuses changed compared to the control group ($p < 0.05$). In this study on the use of tartrazine during pregnancy, it was observed that both organs and bone development were damaged. More studies are needed on the effects of tartrazine.

1. Introduction

Food additives play a crucial role in achieving the intended hue or flavor in various food items, contributing to the processing of nearly all categories of comestibles. Some of these substances are reported to have teratogenic effects. Exposure to teratogens during pregnancy, such as chemicals, can lead to physical or functional abnormalities in the embryo or fetus [1]. The impact of substances on embryonic growth may be subject to diverse factors, including the duration and extent of exposure to teratogenic elements, as well as the developmental stage of the embryo during the exposure period [2]. Teratogens can impact the embryo through various means,

* Corresponding author.
E-mail address: seher.yilmaz@yobu.edu.tr (S. Yılmaz).

https://doi.org/10.1016/j.heliyon.2024.e41456
Received 13 February 2024; Received in revised form 16 December 2024; Accepted 23 December 2024
Available online 30 December 2024

causing physical deformities, behavioral or cognitive disorders, and impairing the child's intellectual development and memory. Leading teratogens include physical agents, metabolic conditions, infections, and newly introduced drugs and chemicals [3].

Tartrazine (TAZ) is an azo dye and its chemical formula is 3-carboxy-5-hydroxy-1 (p-sulfophenyl)-4-(sulfophenyl azo) pyrazolone salt [4]. It serves as a primary food coloring agent, imparting a yellow hue to desserts, fruit juices, jams, mustard, and carbonated beverages. Furthermore, it is widely employed for pigmenting pharmaceuticals intended for human use, including vitamins, antacids, as well as in the formulation of cosmetics and hair care products [5]. Notably, it is utilized as a saffron substitute in culinary endeavors [6]. Recent research has drawn attention to potential teratogenic effects associated with certain food additives in pregnant women, with different researchers suggesting a link between the use of food additives during pregnancy and teratogenic outcomes [7,8].

Studies in the literature on the teratogenic impact of tartrazine, which is used as a food coloring. Consumers, dietitians, and healthcare professionals are currently highly concerned about the safety limits and post-exposure health risks related to the use of food ingredient. Previous studies have shown in experimental animals that TAZ is hepatotoxic and nephrotoxic when used in doses several times higher than the acceptable daily intake dose [9]. It has also been decided that tartrazine bring about serious cellular changes in rat liver and kidney by inducing leukocyte-associated DNA damage [10]. The consequences of teratogenic substance exposure are closely related to the stage of embryogenic development. A study demonstrated that almost all drugs used throughout pregnancy will pass into the embryo through passive diffusion [11].

The embryonic development stage constitutes the most critical period in the sensitivity of the embryo to external factors and its interaction with drugs. Studies propose that the teratogenic capacity of a substance can be predicted if it has the ability to breach the placental barrier, obstruct protein synthesis, and hinder maternal enzymes [12,13]. Furthermore, studies have demonstrated that agents administered during the cleavage, the implantation day of rat pregnancy, do not induce teratogenic effects [14,15]. As tissue differentiation progresses in the embryo, sensitivity to teratogens decreases. After organogenesis is completed, the fetus becomes resistant to teratogens [16]. Previous studies on rodents have indicated that exposure to teratogenic agents during organogenesis results in various abnormalities, encompassing embryonic and fetal resorption, stunted growth, limb and skeletal malformations, and detrimental impacts on the central nervous system [17].

In this study, the rat embryo is most sensitive to internal and external stimuli and cell differentiation occurs, which is the critical organogenesis period between 6 and 15 days [18]. We applied tartrazine between days. We aimed to evaluate how tartrazine administration affects the bone/cartilage development of the limbs in embryos.

2. Materials and methods

2.1. Experimental groups

The study was approved by the Erciyes University animal experiments local ethics committee (decision 22/116) on june 01, 2022. Female Sprague Dawley rats, aged between 70 and 100 days and weighing 250–300 g, were sourced from Erciyes University DEKAM for the research. Rats were fed standard rat diet. The tartrazine (E102, Lot number: 120220908, origin; Turkey) dose to be administered to rats was calculated using an interspecies dosage conversion scheme [19]. Female rats with confirmed pregnancy were separated two groups.

Control Group (Pregnant female rat n = 5): Physiological saline was added to the standard rat diet.

Tartrazine Group (Pregnant female rat n = 5): Tartrazine was given with oral gavage dose of 4.5 mg/kg/day during their pregnancy [20].

It will include the organogenesis period, which is the most sensitive period to drugs and teratogens during pregnancy, between the 6th and 15th days [18] of pregnancy. Tartrazine and saline were given to both groups, including the following days. Rats were sacrificed on the 20th day. At least 7 fetuses were taken from each 5 pregnant rats and a total of 35 fetuses were examined. Heart, lung, kidney and liver tissues taken from fetuses and histological examinations were performed.

2.2. Histologic analyzes

After the removal of heart, lung, kidney and liver tissues taken from fetuses, it was put into 10 % formaldehyde solutions and fixed for 1 day. Following fixation, tissues were left under water flowing throughout the night. Routine histological paraffin embedding process was performed on a day [21]. 5 μm thick sections of each paraffin block samples were taken. The sections were passed through xylol and decreasing alcohol series and stained with hematoxylene Eosin. Glomerular degeneration for each animal, vacuolization observed in tubulointerstitial injuries, hemorrhage and infiltration of inflammatory cells were evaluated in kidney samples for each animal [22]. Hepatic sinusoidal dilatation, hemorrhage and apoptotic hepatocytes were evaluated in liver samples [21]. Hemorrhage and irregular myocardial fiber were evaluated in heart samples [23]. And finally, hemorrhage and mononuclear cell infiltration were evaluated in lung samples. At least 10 images from the examined tissue samples of six fetal rats from each experimental group were histologically scored for each parameter and averaged. Histopathological results in each category were scored as 0: absent, 1: mild, 2: moderate and 3: severe. Quantification was performed randomly and blindly by two researchers [21]. The tissues were histopathologically under the Olympus BX53 light microscope.

2.3. Double skeleton staining

Upon determining the fetuses' height and weight, a dual skeletal staining protocol was implemented to assess their skeletal

development. To execute the double skeleton staining, all fetuses underwent a 4–7 day immersion in 70 % ethyl alcohol to facilitate water drainage, followed by 1–3 days in pure acetone for oil purification. Subsequently, skin removal and extraction of internal organs and eyes occurred. To mitigate limb ruptures and bone damage, a sizable sample size was maintained, excluding damaged subjects from staining. Fetuses were transitioned from acetone to a dual staining solution incubating tissues for 7 days at 38–40 °C. After a 2-h rinse under running tap water, fetuses underwent a transparency phase. Stained specimens were then preserved in ascending concentrations of glycerin. For morphometric analysis, fetal extremities were photographed using a Nikon E5700 digital camera, with resulting images processed via the ImageJ program for length and area measurements. Ossification rates in long bones were determined from these measurements.

2.4. RNA expression analysis

Total RNA was extracted from tissues of the fetuses using PureZole (#7326890, Biorad, USA). Nanodrop ND-1000 spectrophotometer V3.7 was used for determining purity and amount of the RNA samples. Subsequently, all of the RNA samples were converted

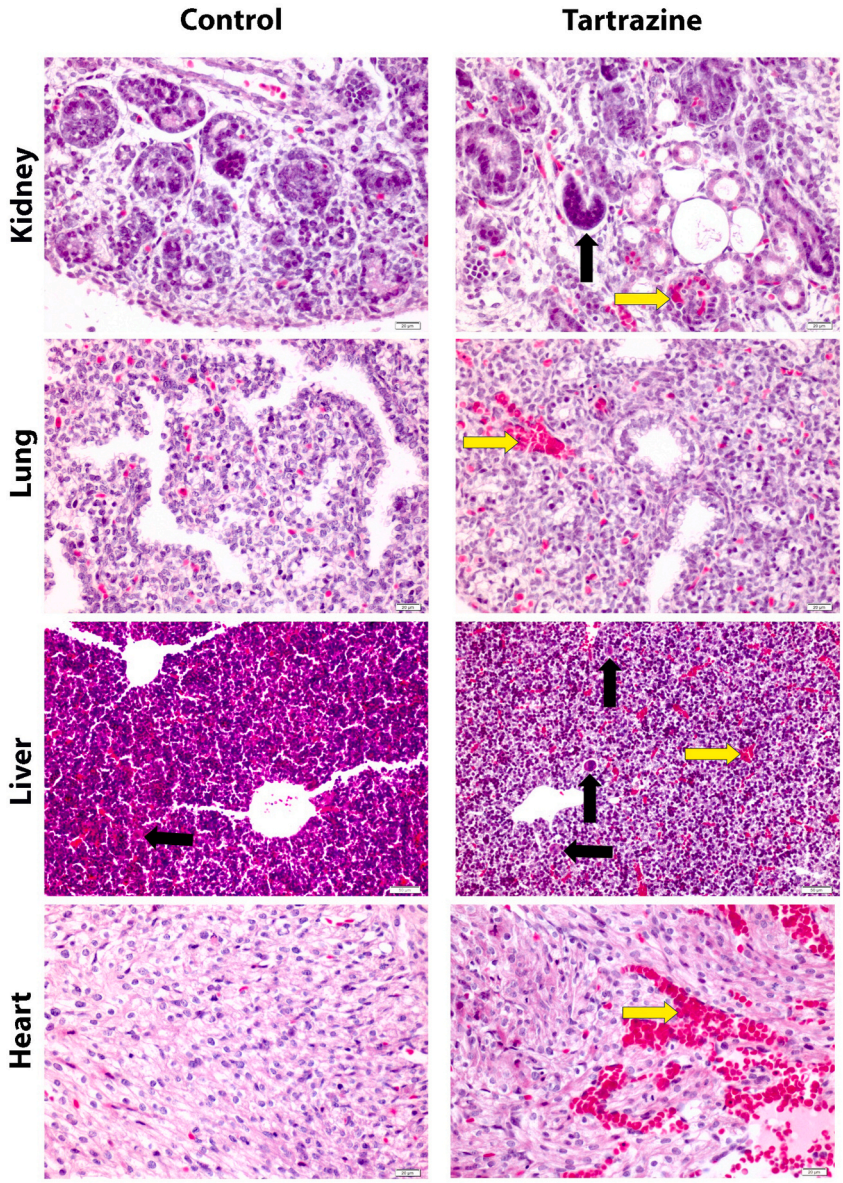


Fig. 1. Hematoxylene Eosin staining images in fetal tissues of control and tartrazine groups. The magnification rate of kidney, lung and heart images is 40× and the scale bar is 20 μm and the magnification rate of the liver images is 20× and the scale bar is 50 μm). Black arrows in the kidney show glomerular degeneration and black arrow megacaryocytes in the liver. The yellow arrow in all tissue images shows hemorrhage.

into cDNA (1708890, Biorad, USA). The expression analysis of *IL-6*, *IL-1β*, *TNF-α*, and *TRPM2* mRNA was carried out using the Real-time PCR method with the Step-One-Plus Thermocycler (Applied Biosystems). Amplifications were conducted using cDNA, site-specific primers (Oligomer Biyotechnology, Ankara), SYBR Green (1725270, Biorad, USA), and nuclease-free water. *GAPDH* was employed as the internal control. The primer sequences for *TNF-α*, *IL-1β*, and *IL-6* were designed as mentioned in Ref. [24]. The primer sequences for *TRPM2* were designed as mentioned in Ref. [25].

Rat-*IL-6* F: 5′-TCCTACCCCAACTTCCAATGCTC-3′,
Rat-*IL-6* R: 5′-TTGGATGGTCTTGGTCCTTAGCC-3′
Rat-*IL-1β* F: 5′-CACCTCTCAAGCAGAGCACAG-3′
Rat-*IL-1β* R: 5′-GGGTTCCATGGTGAAGTCAAC-3′
Rat-*TNF-α* F: 5′-AAATGGGCTCCCTCTCATCAGTTC-3′
Rat-*TNF-α* R: 5′-TCTGCTTGGTGGTTTGCTACGAC-3′
*Rat-TRPM2 F:*5′GAAGGAAAGAGGGGGTGTG-3′
Rat-TRPM2 F: 5′ CATTGGTGATGGCGTTGTAG-3’
Rat-*GAPDH* F: 5′- GAGGACCAGGTTGTCTCCTG-3′
Rat-*GAPDH* R: 5′-GGATGGAATTGTGAGGGAGA-3′,

The subsequent RT-PCR protocol was employed: for *IL-6*, *IL-1β*, *TNF-α*, 98 °C for a duration of 3 min, 40 cycles consisting of 15 s at 98 °C and 30 s at 61 °C. Additionally for *TRPM2*, 30 s at 98 °C, 40 cycles consisting of 5 s at 98 °C and 30 s at 58 °C. For confirmation of singular product amplification, a melting curve analysis was executed at the conclusion of the PCR. Each run was executed in triplicate.

2.5. Statistical analysis

GraphPad Prism (version 8.0, GraphPad Software Inc., San Diego, California) program was used for statistical analysis. Normality of the data was evaluated with the Kolmogorov-Smirnov test. For data with normal distribution, Unpaired *t*-test (parametric) was

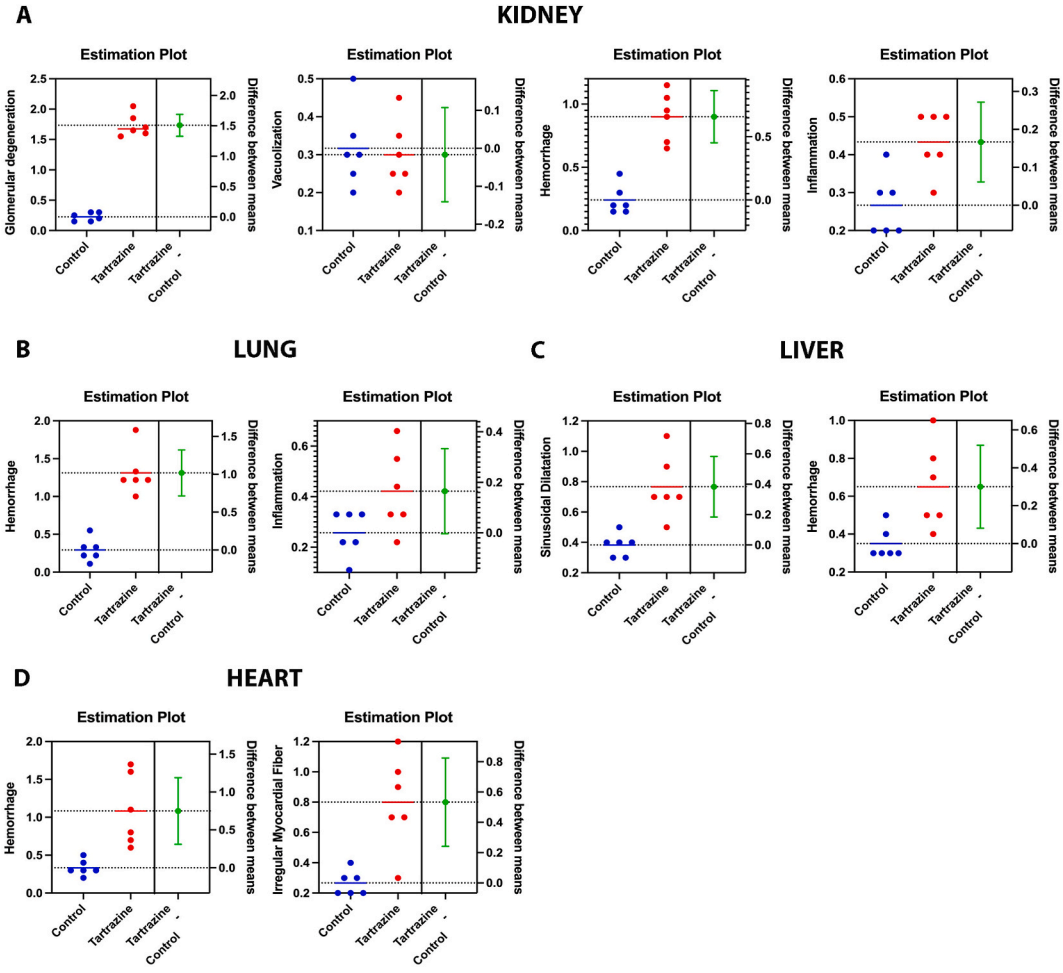


Fig. 2. Kidney (A), lung (B), liver (C) and heart (D) histology score bar charts. Unpaired *t*-test was applied.

applied to compare between groups. For data with non-normal distribution, Mann Whitney test (nonparametric) was applied for comparison between groups. Data are expressed as mean ± standard deviation. $p < 0.05$ was considered statistically significant. Genetic analysis was conducted utilizing REST 2009 V2.0.13 Software [26].

3. Results

3.1. Histological results

As shown in Fig. 1, it was observed that there was an increase in hemorrhage in all tissues of tartrazine groups (Figs. 1 and 2A-D, Tables 1 and 2). It was observed that glomerular damage increased in fetal kidney tissues of tartrazine groups (Fig. 2A) and an increase in hepatic sinusoidal dilatation in fetal liver tissues (Fig. 2C–Tables 1 and 2). In addition, it was observed that there are little irregular myocardial fibers in the heart tissues of tartrazine groups (Fig. 2D). The presence of multi -lobed megakaryocytes were observed in the liver sections of both control and tartrazine groups (Fig. 2C). The presence of hemorrhage in the lung tissues was remarkable compared to the control group (Fig. 2B–Table 2). Statistically increased hemorrhage was observed in the tartrazine group compared to the control group ($p < 0.05$). Inflammatory cell infiltration rates were also higher in the tartrazine group than in the control group, although this was not statistically significant ($p > 0.05$).

3.2. Double skeleton staining results

The weight of the fetus samples taken was measured. In addition, fore-aft length, biparietal length and occipitofrontal length measurements were made to evaluate the development of the offspring. When the data were evaluated, the weights of the fetus in the tartrazine-treated group decreased significantly compared to the control group. Fore-aft, biparietal and occipitofrontal length values were found to be significantly lower than the tartrazine values compared to the control group (Table 3).

Total length, total area, bone length and bone area measurements were made on the scapula, humerus, radius, ulna (Fig. 3A), femur, tibia and fibula (Fig. 3B) bones stained with the double skeleton staining method. In Fig. 3A and B and Fig. 4, tissue samples painted with the double skeleton staining method are shown. It was observed that the total length, total area, bone length and bone area values in the scapula were significantly lower in the tartrazine group than in the control group (Fig. 3A–Table 4). A statistically significant decrease was observed in the total length, total area, bone length and bone area values in the humerus, radius and ulna in the tartrazine group compared to the control group (Fig. 3A–Table 4). A statistically significant decrease was observed in the total length, total area, bone length and bone area values of the femur, tibia and fibula bones in the tartrazine group compared to the control group (Fig. 3B–Table 5).

3.3. RNA expression analysis of IL-6, IL-1β, TNF-α, and TRPM2

Alteration of *IL-6, IL-1β, TNF-α* and *TRPM2* mRNA levels of each tissues were determined according to the mRNA levels of related control tissues (Fig. 5A–D). The mRNA levels of the *IL-6, IL-1β, TNF-α* and *TRPM2* gene were altered in hearts of fetuses compared to the control (0.14; 0.9; 0.69; 0.9; fold change, respectively) (Fig. 5A). The mRNA levels of the *IL-6, IL-1β, TNF-α* and *TRPM2* gene were altered in lungs of fetuses compared to the control (0.19; 0.1; 0.19; 0.82; fold change, respectively) (Fig. 5B). The mRNA levels of the *IL-6, IL-1β, TNF-α* and *TRPM2* gene were altered in kidneys of fetuses compared to the control (0.35; 0.43; 0.68; 0.84; fold change; respectively) (Fig. 5C). The mRNA levels of the *IL-6, IL-1β, TNF-α* and *TRPM2* gene were altered in livers of fetuses compared to the control (14,93*; 1.09; 0.92; 0.67; fold change, respectively, *$p < 0.001$) (Fig. 5D).

4. Discussion

Tartrazine, an azoic dye, is extensively utilized across various industries, including cosmetics, textiles, pharmaceuticals, and food. In the cosmetics realm, it finds its way into soaps, hair products, while in pharmaceuticals, it's present in vitamins, medicines, and drug capsules. Its usage spans a wide array of processed food products. Recent studies have shed light on potential hazards associated with tartrazine, such as food allergies, as well as carcinogenic and mutagenic effects [27–31].

Several studies have highlighted significant adverse effects of tartrazine on various physiological parameters. In one study, high-dose exposure to tartrazine led to a notable increase in offspring body weight and adverse effects on behavioral parameters [31]. It is reported that external substances such as drugs and food dyes taken during pregnancy can affect embryonic development during organogenesis in mammals and cross the placental barrier [32,33]. Hashem et al. reported that administration of tartrazine to pregnant

Table 1
Histology scores of fetal kidney tissues.

Groups	Glomerular Degeneration	Vacuolization	Hemorrhage	Infiltration of inflammatory cells
Control	0.22 ± 0.06	0.31 ± 0.10	0.24 ± 0.11	0.26 ± 0.08
Tartrazine	1.73 ± 0.79****	0.30 ± 0.08	0.90 ± 0.19****	0.41 ± 0.06

Data shown in the table are expressed as mean ± SD. Unpaired *t*-test was applied. ****$p < 0.0001$.

Table 2
Histology scores of fetal lung, liver and heart tissues.

Tissues	Lung		Liver		Heart	
Groups	Hemorrhage	Infiltration of inflammatory cells	Sinusoidal dilatation	Hemorrhage	Hemorrhage	Irregular myocardial fiber
Control	0.29 ± 0.15	0.25 ± 0.09	0.38 ± 0.07	0.35 ± 0.08	0.33 ± 0.10	0.26 ± 0.08
Tartrazine	1.33 ± 0.29****	0.42 ± 0.16	0.76 ± 0.20**	0.65 ± 0.22 *	1.08 ± 0.47**	0.80 ± 0.30**

Data shown in the table are expressed as mean ± SD. Unpaired *t*-test was applied (In lung tissue ****$p < 0.0001$. In liver tissue, for hepatic sinusoidal dilatation **$p = 0.0016$, for hemorrhage *$p = 0.0122$. In heart tissue, for hemorrhage **$p = 0.0034$, for irregular myocardial fiber **$p = 0.0022$).

Table 3
Fetus weights and length measurements.

Measured parameters	Control	Tartrazine	*p*
Fetus weight (g)	2.19 ± 0.165	1.79 ± 0.210**	0.0018
Fore–aft length (mm)	26.23 ± 1.290	24.03 ± 1.456*	0.0113
Biparietal lengths (mm)	7.40 ± 0.310	6.82 ± 0.367**	0.0077
Occipitofrontal lengths (mm)	8.57 ± 0.884	7.36 ± 0.151*	0.0379

Data were expressed as mean ± std., and $p < 0.05$ was considered statistically significant.

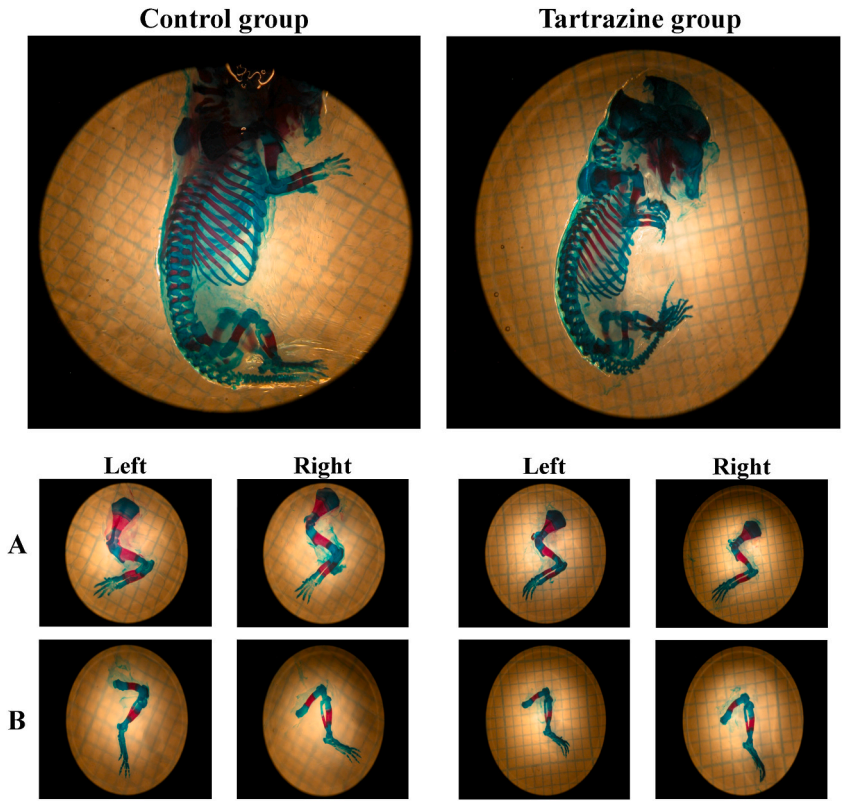


Fig. 3. Bone samples stained with the double skeleton staining method. A: Forelimb bones, B: Hindlimb bones.

rats, even at a dose as low as 4.5 mg/kg, caused retardation of fetal organ development in the heart, lung and bone and impaired fetal growth compared to the control group [20]. In our double Skeleton Staining results, there was a decrease in the weights of the fetuses in the tartrazine-applied group, as well as in the fore-aft, biparietal and occipitofrontal length values. In addition, it was observed that the total length, total area, bone length and bone area values of the bones forming the skeleton were reduced by tartrazine exposure.

Severe histopathological and cellular changes in cardiovascular system, liver and kidney tissues, DNA damage, and changes in antioxidant enzymes, lipid peroxidation, and lipid profile have been reported in experimental animal models [10,34,35]. Another investigation demonstrated that tartrazine induced pancreatic damage through histopathological alterations and changes in serum and biochemical parameters, resulting in oxidative stress [29]. It is known that excessive production of reactive oxygen species (ROS)

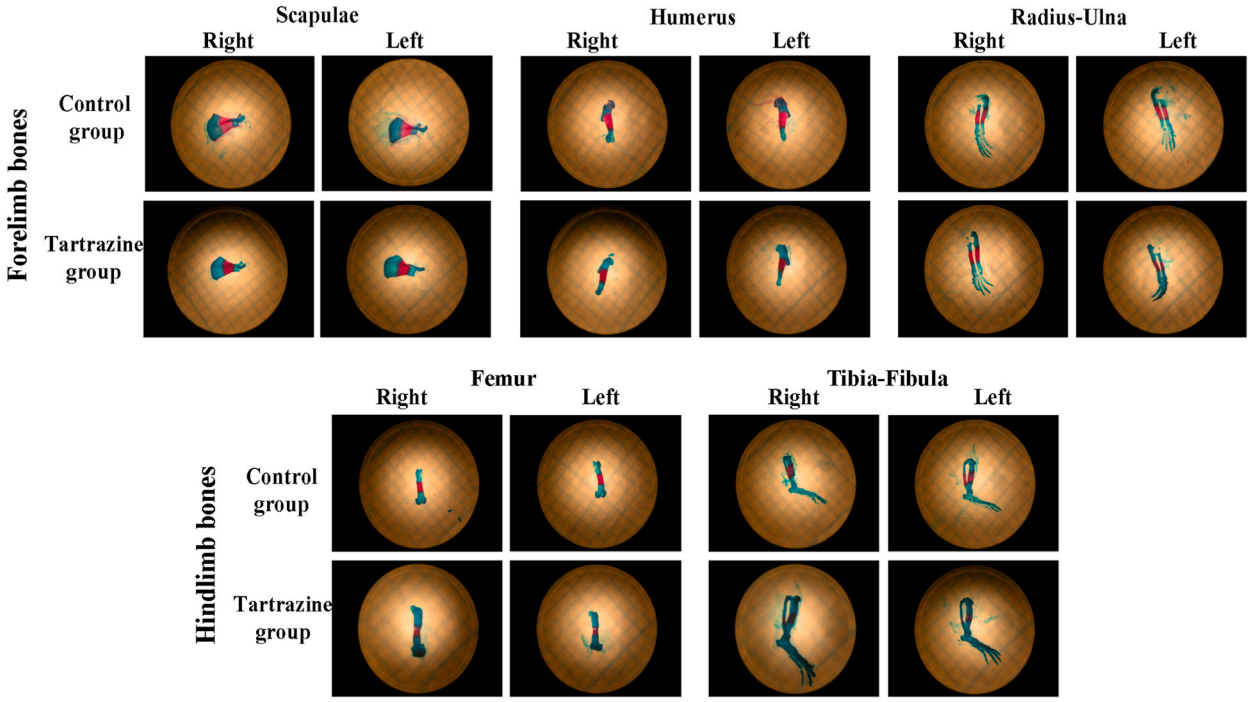


Fig. 4. Examples of forelimb and hindlimb bones painted with the double skeleton staining method.

Table 4
Length and area measurements made on the forelimb bones.

Bones	Measured parameters	Control	Tartrazine	*p*
Scapulae	**Total length (mm)**	3.52 ± 0.020	3.17 ± 0.036****	<0.0001
	Total area (mm^2)	4.46 ± 0.384	4.27 ± 0.185*	0.0219
	Bone length (mm)	1.30 ± 0.159	1.12 ± 0.104**	0.0034
	Bone area (mm^2)	1.52 ± 0.179	1.24 ± 0.086****	<0.0001
Humerus	**Total length (mm)**	4.09 ± 0.065	3.89 ± 0.071****	<0.0001
	Total area (mm^2)	3.57 ± 0.121	3.35 ± 0.278****	<0.0001
	Bone length (mm)	1.42 ± 0.094	1.22 ± 0.058****	<0.0001
	Bone area (mm^2)	1.18 ± 0.058	1.06 ± 0.026****	<0.0001
Radius	**Total length (mm)**	2.95 ± 0.148	2.85 ± 0.080*	0.0397
	Total area (mm^2)	1.25 ± 0.035	1.12 ± 0.043****	<0.0001
	Bone length (mm)	1.07 ± 0.058	0.98 ± 0.037****	<0.0001
	Bone area (mm^2)	0.35 ± 0.031	0.27 ± 0.030****	<0.0001
Ulna	**Total length (mm)**	4.01 ± 0.105	3.83 ± 0.114***	0.0002
	Total area (mm^2)	1.76 ± 0.104	1.66 ± 0.084*	0.0104
	Bone length (mm)	1.23 ± 0.120	1.15 ± 0.053*	0.0294
	Bone area (mm^2)	0.38 ± 0.147	0.27 ± 0.035*	0.0138

Data were expressed as mean ± std., and $p < 0.05$ was considered statistically significant.

causes oxidative stress, which can impair bone formation and cause bone loss [36,37], and it has been reported that Tartrazine can also trigger oxidative stress by causing an increase in ROS [27,38–40].

Exposure to tartrazine increases necrosis and inflammation in liver and kidney tissues, resulting in organ damage [41,42]. Desoky et al. studied that tartrazine exposure was found to cause abnormalities in liver and kidney histology, including tubular dilatation, thickened basement membrane, glomerular capillary dilatation, and tubular degeneration in the kidneys, as well as liver histopathological changes such as blood sinusoid enlargement, central vein bleeding, and necrosis [41].

Additionally, tartrazine administration in rats resulted in liver parenchymal necrosis with inflammatory cell infiltration, inflammation parameters in apoptotic hepatocytes [42], and degenerated Purkinje cells with shrunken shape and deeply stained cytoplasm, along with gray matter edema and nuclear pyknosis. These findings collectively underscore the potential harmful effects of tartrazine on various organ systems and physiological processes [43]. According to our findings, exposure to tartrazine causes increased hemorrhage in tissues. In fetal organs the tartrazine was observed to increase glomerular damage in kidneys, to cause hepatic sinusoidal dilatation in the liver, and to increase irregular myocardial fibers.

It is reported that immunosuppression and inflammatory changes are up-regulated by *IL-6*, *IL-1β*, *TNF-α* and *TRPM2* [44–46]. It is

Table 5
Length and area measurements made on the hindlimb bones.

Bones	Measured parameters	Control	Tartrazine	*p*
Femur	**Total length (mm)**	3.47 ± 0.244	3.01 ± 0.067**	0.0037
	Total area (mm^2)	2.21 ± 0.186	1.93 ± 0.218**	0.0010
	Bone length (mm)	0.71 ± 0.167	0.57 ± 0.051**	0.0053
	Bone area (mm^2)	0.34 ± 0.143	0.23 ± 0.043*	0.0101
Tibia	**Total length (mm)**	2.96 ± 0.205	2.70 ± 0.186**	0.0021
	Total area (mm^2)	1.68 ± 0.204	1.46 ± 0.179***	0.0007
	Bone length (mm)	0.71 ± 0.134	0.47 ± 0.079**	0.0018
	Bone area (mm^2)	0.43 ± 0.052	0.26 ± 0.041****	<0.0001
Fibula	**Total length (mm)**	3.04 ± 0.022	2.76 ± 0.114****	<0.0001
	Total area (mm^2)	0.82 ± 0.133	0.64 ± 0.095**	0.0064
	Bone length (mm)	0.61 ± 0.134	0.47 ± 0.090**	0.0030
	Bone area (mm^2)	0.14 ± 0.038	0.10 ± 0.023***	0.0006

Data were expressed as mean ± std., and $p < 0.05$ was considered statistically significant.

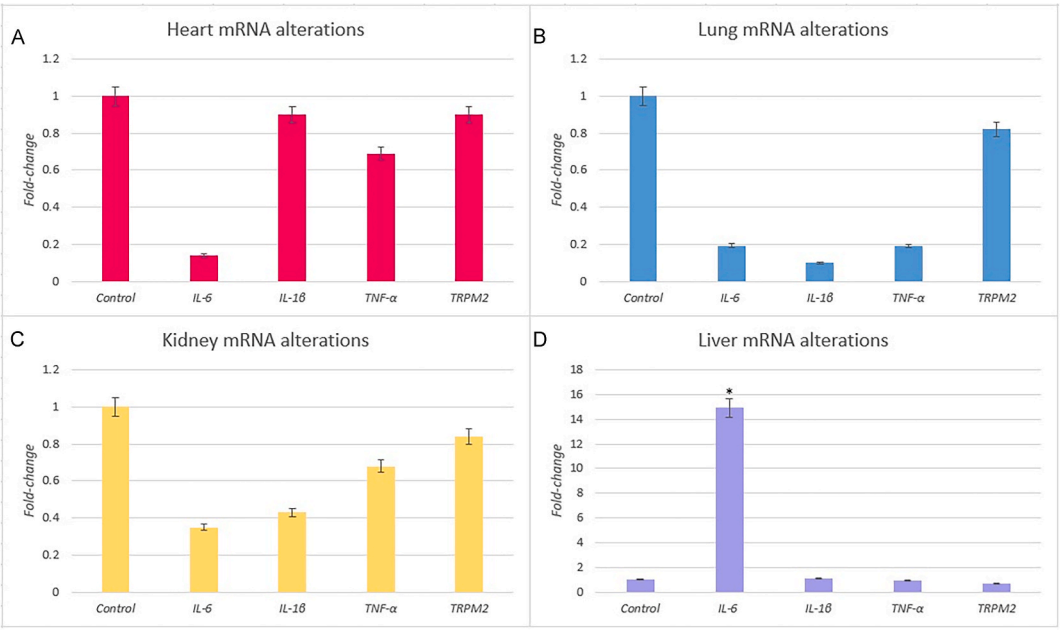


Fig. 5. The results of real-time PCR analysis. Relative mRNA expression of *IL-6, IL-1β, TNF-α,* and *TRPM2* genes in heart (A), lung (B), kidney (C) and liver (D) tissues exposed to tartrazine were given as fold change compared to the control. *Represents the significance of $p < 0.001$ compared to control. *GAPDH* is reference gene for normalization.

stated that tartrazine may mediate inflammation through its direct effect on the macrophage cell population and thus cytokine production [46,47]. We showed that tartrazine, which is frequently used as a food coloring, causes damage to major fetal tissues such as liver, kidney, lung and heart. Tartrazine group compared to the control group statistically significant decrease was observed in the total length, total area, bone length and bone area values of the limb bones ($p < 0.05$). The mRNA levels of the *IL-6, IL-1β, TNF-α* and *TRPM2* gene were altered in livers of fetuses compared to the control group ($p < 0.05$).

5. Conclusions

Our research investigates the impact of tartrazine on fetal bone development using double skeleton staining, histological analysis, and examination of *IL-6*, *IL-1β*, *TNF-α*, and *TRPM2* gene levels. Our findings indicate that tartrazine detrimentally influences growth and development, as revealed by the double skeleton staining method. Specifically, our examination of long bones within limb bones led us to conclude that tartrazine exhibits a teratogenic effect, reducing the ossification rate during bone development. Histopathologically, tartrazine was observed to increase hemorrhage in fetal organs. The mRNA levels of the *IL-6*, *IL-1β*, *TNF-α* and *TRPM2* gene were altered in livers of fetuses compared to the control group. Consequently, we infer that tartrazine possesses embryotoxic and teratogenic potential in rats, suggesting that its use during pregnancy can have harmful effects on fetal development. We anticipate that our study will provide valuable insights for future research endeavors in this field.

CRediT authorship contribution statement

Osman Öztürk: Methodology, Formal analysis. **Sümeyye Uçar:** Writing – original draft, Methodology, Data curation. **Züleyha Doğanyiğit:** Methodology, Investigation, Writing – original draft. **Aslı Okan Oflamaz:** Methodology, Data curation, Conceptualization. **Evrim Suna Arıkan:** Methodology, Data curation. **Şükrü Ateş:** Investigation, Data curation. **Seher Yılmaz:** Writing – review & editing, Writing – original draft.

Ethical statement

The study was approved by the Erciyes University animal experiments local ethics committee (decision 22/116). Experimental animal care and all procedures performed were performed according to the European Communities Council Directive 2010/63/EU.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding statement

Financial support for the “Double Skeleton Staining” methods part of this project was provided by Yozgat Bozok University Scientific Research Projects Coordination Unit (Grant **number**: **THD-2022–1054**).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Osman Ozturk reports financial support was provided by Yozgat Bozok University Scientific Research Projects Coordination Unit. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank Yozgat Bozok University Scientific Research Projects Coordination Unit for its financial support in the Double Staining Methods part of the project (THD-2022-1054).

References

[1] S. Sambu, U. Hemaram, R. Murugan, A.A. Alsofi, Toxicological and teratogenic effect of various food additives: an updated review, BioMed Res. Int. 2022 (2022). https://www.hindawi.com/journals/bmri/2022/6829409/. (Accessed 14 January 2024).
[2] V.A. Vaclavik, E.W. Christian, Essentials of Food Science, Springer, New York, NY, 2014, https://doi.org/10.1007/978-1-4614-9138-5.
[3] C. Tantibanchachai, Retinoids as teratogens. Embryo Project Encyclopedia, 2014, pp. 2–28.
[4] T. Tanaka, O. Takahashi, S. Oishi, A. Ogata, Effects of tartrazine on exploratory behavior in a three-generation toxicity study in mice, Reprod. Toxicol. 26 (2008) 156–163.
[5] K.V. Allam, G.P. Kumar, Colorants-the cosmetics for the pharmaceutical dosage forms, Int. J. Pharm. Pharmaceut. Sci. 3 (2011) 9.
[6] N. Mehedi, S. Ainad-Tabet, N. Mokrane, S. Addou, C. Zaoui, O. Kheroua, D. Saidi, Reproductive toxicology of tartrazine (FD and C Yellow No. 5) in Swiss albino mice, Am. J. Pharmacol. Toxicol. 4 (2009) 130–135.
[7] J.F. Borzelleca, J.B. Hallagan, Multigeneration study of FD & C Red No. 3 (erythrosine) in Sprague-Dawley rats, Food Chem. Toxicol. 28 (1990) 813–819.
[8] T.F.X. Collins, T.N. Black, M.W. O'Donnell Jr., M.E. Shackelford, P. Bulhack, Teratogenic potential of FD & C Red No. 3 when given in drinking water, Food Chem. Toxicol. 31 (1993) 161–167.
[9] H.M.F.A. El-Wahab, G.S.E.-D. Moram, Toxic effects of some synthetic food colorants and/or flavor additives on male rats, Toxicol. Ind. Health 29 (2013) 224–232, https://doi.org/10.1177/0748233711433935.
[10] L. Khayyat, A. Essawy, J. Sorour, A. Soffar, Tartrazine induces structural and functional aberrations and genotoxic effects in vivo, PeerJ 5 (2017) e3041.
[11] M.R. Syme, J.W. Paxton, J.A. Keelan, Drug transfer and metabolism by the human placenta, Clin. Pharmacokinet. 43 (2004) 487–514, https://doi.org/10.2165/00003088-200443080-00001.
[12] K. Anita, V.L. Mehta, U. Gupta, S. Prabhu, J.S. Bapna, Methods for teratogenicity testing-existing and future models, Indian J. Pharmacol. 27 (1995) 204.
[13] E. Raymond, D. Sun, S.-F. Chen, B. Windle, D.D. Von Hoff, Agents that target telomerase and telomeres, Curr. Opin. Biotechnol. 7 (1996) 583–591.
[14] M.-K. Chung, W.-J. Yu, J.-S. Lee, J.-H. Lee, Embryotoxicity and toxicokinetics of the antimalarial artesunate in rats, Toxicol. Res. 29 (2013) 27–34, https://doi.org/10.5487/TR.2013.29.1.027.
[15] I.W. Selderslaghs, A.R. Van Rompay, W. De Coen, H.E. Witters, Development of a screening assay to identify teratogenic and embryotoxic chemicals using the zebrafish embryo, Reprod. Toxicol. 28 (2009) 308–320.
[16] E.G. McQueen, Teratogenicity of drugs, N. Z. Vet. J. 20 (1972) 156–159, https://doi.org/10.1080/00480169.1972.34038.
[17] I. Chahoud, S.N. Kuriyama, F.J. Paumgartten, Maternal protein-and-energy restriction reduces the developmental toxicity of cyclophosphamide and hydroxyurea in rats, Toxicology 179 (2002) 137–149.
[18] Z. Erisgin, Y. Tekelioglu, Flow cytometric examination of apoptotic effect on brain tissue in postnatal period created by intrauterine oxcarbazepine and gabapentin exposure, Bratisl. Lek. Listy 118 (2017) 107–111, https://doi.org/10.4149/BLL_2017_022.
[19] A.B. Nair, S. Jacob, A simple practice guide for dose conversion between animals and human, J. Basic Clin. Pharm. 7 (2016) 27.
[20] M.M. Hashem, Y.M. Abd-Elhakim, K. Abo-El-Sooud, M.M.E. Eleiwa, Embryotoxic and teratogenic effects of tartrazine in rats, Toxicol. Res. 35 (2019) 75–81, https://doi.org/10.5487/TR.2019.35.1.075.
[21] Z. Doğanyiğit, A. Okan, E. Kaymak, D. Pandır, S. Silici, Investigation of protective effects of apilarnil against lipopolysaccharide induced liver injury in rats via TLR 4/HMGB-1/NF-κB pathway, Biomed. Pharmacother. 125 (2020) 109967.

[22] N. Inandiklioglu, Z. Doganyigit, A. Okan, E. Kaymak, S. Silici, Nephroprotective effect of apilarnil in lipopolysaccharide-induced sepsis through TLR4/NF-κB signaling pathway, Life Sci. 284 (2021) 119875.
[23] E. Akyuz, Z. Doğanyiğit, A. Okan, S. Yılmaz, S. Uçar, A.T. Akin, Immunoreactivity of Kir3. 1, muscarinic receptors 2 and 3 on the brainstem, vagus nerve and heart tissue under experimental demyelination, Brain Res. Bull. 197 (2023) 13–30.
[24] Y. Iwashita, E. Zhang, J. Maruyama, A. Yokochi, Y. Yamada, H. Sawada, Y. Mitani, H. Imai, K. Suzuki, K. Maruyama, Thrombomodulin protects against lung damage created by high level of oxygen with large tidal volume mechanical ventilation in rats, J Intensive Care 2 (2014) 57, https://doi.org/10.1186/s40560-014-0057-0.
[25] N.L. Cook, T.J. Kleinig, C.V.D. Heuvel, R. Vink, Reference genes for normalising gene expression data in collagenase-induced rat intracerebral haemorrhage, BMC Mol. Biol. 11 (2010) 7, https://doi.org/10.1186/1471-2199-11-7.
[26] M.W. Pfaffl, G.W. Horgan, L. Dempfle, Relative expression software tool (REST\copyright) for group-wise comparison and statistical analysis of relative expression results in real-time PCR, Nucleic Acids Res. 30 (2002) e36–e36.
[27] K.A. Amin, H.A. Hameid II, A.H. Abd Elsttar, 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 (2010) 2994–2999.
[28] P. Barciela, A. Perez-Vazquez, M.A. Prieto, Azo dyes in the food industry: features, classification, toxicity, alternatives, and regulation, Food Chem. Toxicol. 178 (2023) 113935.
[29] Z. Erdemli, E. Altinoz, M.E. Erdemli, M. Gul, H.G. Bag, S. Gul, Ameliorative effects of crocin on tartrazine dye–induced pancreatic adverse effects: a biochemical and histological study, Environ. Sci. Pollut. Res. 28 (2021) 2209–2218, https://doi.org/10.1007/s11356-020-10578-6.
[30] K. Rovina, S. Siddiquee, S.M. Shaarani, A review of extraction and analytical methods for the determination of tartrazine (E 102) in foodstuffs, Crit. Rev. Anal. Chem. 47 (2017) 309–324, https://doi.org/10.1080/10408347.2017.1287558.
[31] T. Tanaka, Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet, Food Chem. Toxicol. 44 (2006) 179–187.
[32] E. Atay, T. Ertekin, H. Yılmaz, H.S. Güler, Ö. Al, M. Nisari, A. Yay, E. Unur, S. Özdamar, B. Yalçın, Impact of prenatal exposure to bisphenol A on pregnant rats: fetal bone development and immunohistochemistry implications, Toxicol. Ind. Health 35 (2019) 119–135, https://doi.org/10.1177/0748233718823146.
[33] M. Booth, N. Powell, C. Corfield, J.M. French, An automated technique for double staining of bone and cartilage in fetal mouse skeletal specimens using alizarin red S and Alcian blue, Biotech. Histochem. 97 (2022) 222–227, https://doi.org/10.1080/10520295.2021.1933179.
[34] A. El-Hakama, E. Fatma, I.M. Farrag, Tartrazine: potential hepatorenal and cardiovascular toxicity and the possible protective effect of vitamin E in Wistar rats, J. Recent Advan. Med, 3 (2022) 96–107.
[35] U.A. Adele, G. Iroh, O.N. Briggs, H.A. Waribo, I. Elekima, Evaluation of anti-oxidant enzymes, lipid peroxidation, lipid profile and liver function in albino rats orally administered tartrazine, Int. J. Biochem. Res. Rev. 29 (2020) 19–29.
[36] J.-R. Chen, O.P. Lazarenko, M.L. Blackburn, K.E. Mercer, T.M. Badger, M.J. Ronis, p47phox-Nox2-dependent ROS signaling inhibits early bone development in mice but protects against skeletal aging, J. Biol. Chem. 290 (2015) 14692–14704.
[37] E. Cicek, E. Cakmak, Hydrogen peroxide induced oxidative damage on mineral density and mechanical properties of bone, Braz. Arch. Biol. Technol. 61 (2018). https://www.scielo.br/j/babt/a/gg7DgS9xkxzysdKpSDgzdss/?lang=en. (Accessed 14 January 2024).
[38] G. Albasher, N. Maashi, S. Alfarraj, R. Almeer, T. Albrahim, F. Alotibi, M. Bin-Jumah, A.M. Mahmoud, Perinatal exposure to tartrazine triggers oxidative stress and neurobehavioral alterations in mice offspring, Antioxidants 9 (2020) 53.
[39] N. El Golli, Toxicity induced after subchronic administration of the synthetic food dye tartrazine in adult rats, role of oxidative stress, Recent Adv Biol Med 2 (2016) 652.
[40] B. Visweswaran, G. Krishnamoorthy, Oxidative stress by tartrazine in the testis of Wistar rats, J. Pharm. Biol. Sci. 2 (2012) 44–49.
[41] G.E. El-Desoky, S.M. Wabaidur, Z.A. AlOthman, M.A. Habila, Evaluation of Nano-curcumin effects against Tartrazine-induced abnormalities in liver and kidney histology and other biochemical parameters, Food Sci. Nutr. 10 (2022) 1344–1356, https://doi.org/10.1002/fsn3.2790.
[42] N. Demircigil, M. Gul, N. Gokturk, E.K. Kustepe, H.G. Bag, M.E. Erdemli, Thymoquinone played a protective role against tartrazine-induced hepatotoxicity, Iran. J. Basic Med. Sci. 26 (2023) 99.
[43] M.A. El-sakhawy, D.W. Mohamed, Y.H. Ahmed, Histological and immunohistochemical evaluation of the effect of tartrazine on the cerebellum, submandibular glands, and kidneys of adult male albino rats, Environ. Sci. Pollut. Res. 26 (2019) 9574–9584, https://doi.org/10.1007/s11356-019-04399-5.
[44] Ö. Öcal, A. Coşar, M. Nazıroğlu, Amantadine attenuated hypoxia-induced mitochondrial oxidative neurotoxicity, apoptosis, and inflammation via the inhibition of TRPM2 and TRPV4 channels, Mol. Neurobiol. 59 (2022) 3703–3720, https://doi.org/10.1007/s12035-022-02814-6.
[45] W. Iheanyichukwu, A.O. Adegoke, O.G. Adebayo, M. Emmanuel U, A.P. Egelege, J.T. Gona, F.M. Orluwene, Combine colorants of tartrazine and erythrosine induce kidney injury: involvement of TNF-α gene, caspase-9 and KIM-1 gene expression and kidney functions indices, Toxicol. Mech. Methods 31 (2021) 67–72, https://doi.org/10.1080/15376516.2020.1828523.
[46] Y.M. Abd-Elhakim, M.M. Hashem, A.E. El-Metwally, A. Anwar, K. Abo-El-Sooud, G.G. Moustafa, H.A. Ali, Comparative haemato-immunotoxic impacts of long-term exposure to tartrazine and chlorophyll in rats, Int. Immunopharm. 63 (2018) 145–154, https://doi.org/10.1016/j.intimp.2018.08.002.
[47] M. Guendouz, N. Mehedi, C. Zaoui, D. Saidi, O. Khéroua, Immune response after Tartrazine subchronic ingestion in swiss albino mice, Int. J. Pharm. Pharmaceut. Sci. 5 (2013) 584–592.