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RESEARCH ARTICLE

Effect of Azo-Class Food Additive Tartrazine on Tooth Development in Mice

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Abstract

Tartrazine (Tz) is a chemically synthesized food dye that is being used widely as a coloring agent in different foods. Growing pieces of evidence are reporting that these food additives are genotoxic. Since colored foods are particularly attractive to children, they are the most vulnerable to the adverse effects of food additives. Thus, this study was designed to elucidate the effect of Tz on tooth development in mice models. For this study, we administered Tz at 0.75 mg/Kg, 1.5 mg/Kg, and 3.0 mg/Kg intraperitoneally into two-week-old mice for 14 consecutive days. Then, the mice were euthanized, and mandibles were separated for removing molar (M) teeth and collecting tissue samples from the jaw. We assessed expressions of *Adipoq*, *Bsp*, *Nqo1*, and *Sirt6* genes by polymerase chain reaction (PCR) in the tissue samples and measured the size of molar 1 (M1), molar 2 (M2), and molar 3 (M3) tooth. While there was no noticeable change in length, we observed that M3 tooth became thinner in Tz treated animal. Similarly, mice body weight was decreasing dose dependently, with a significant decline at the highest dose. Additionally, *Bsp* and *Sirt6* gene expression were enhanced in Tz administration in mice. Overall, our data indicates that Tz has a negative effect on tooth development, and further research is needed for elucidating its mechanism of action.

Keywords: Tartrazine, Tooth Development, Toxicity, *Bsp*, Mice, *Sirt6.*

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Introduction

Tartrazine (Tz) is a well-known azo dye, being used widely as a coloring agent in food products (Amin et al. 2010). The acceptable daily intake (ADI) amount of Tz is 7.5 mg/Kg body weight (Bhatt et al. 2018). A variety of consumer goods including cotton candy, soft drinks, flavored chips (Doritos, Nachos, etc.), cereals (corn flakes, muesli, etc.), cake mixes, soups, sauces, some rice, ice cream, candy, chewing gum, marzipan, jam and jelly, and some of medical preparations, such as vitamins, antiacids, medicinal capsules, and certain prescription drugs are being processed by using contain Tz as a coloring agent (Amin et al. 2010, El-Desoky et al. 2017). Unfortunately, Tz has been reported to increase alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, serum albumin, and malondialdehyde (MDA); and decrease glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (*GPx*) and catalase (CAT) (Amin et al. 2010, El-Desoky et al. 2017). As a whole, Tz adversely alters biochemical markers in human vital organs (Amin et al. 2010, El-Desoky et al. 2017). Tz not only changes hepatic and renal biochemical parameters but also induces oxidative stress at higher doses (Amin et al. 2010, El-Desoky et al. 2017). Tz has been reported with its genotoxic effect by inducing micronuclei in the lymphocytes of mice (Vega-Cabanillas et al. 2021). Muntjac fibroblasts developed chromosomal abnormalities due to exposure to Tz *in vitro* (Patterson and Butler 1982). Bhatt and colleagues (2018) reported oxidative damage of the brain in rat that were administered Tz at an ADI dose for humans (Bhatt et al. 2018). Tz administration significantly impaired SOD, CAT, GST, GR (glutathione reductase), and GPx in rat brain (Bhatt et al. 2018). Moreover, sexual maturation in female rats was hampered due to Tz administration (Mindang et al. 2022). Tz exposure accelerated aging in *Caenorhabditis elegans* and decreased their lifespan in

addition to altered movement patterns and dopamine receptor production (Guerrero-Rubio et al. 2023). Though the effect of Tz on different organs including brain, liver, and kidney in mammal, and different living organisms from chordate to nematode have been studied; effect of Tz on tooth development is elusive since children are the major consumers of colored food, placing them at a higher risk because of possibility of exceeding the prescribed ADI (Rao et al. 2004, Husain et al. 2006). Therefore, this study was designed to evaluate the effect of Tz on tooth development in mice model.

Materials and Methods

Animal breeding and care

To have baby mice (*Mus musculus*), breeding of mice was done according to previous report (Bruce 1947). Animals were housed in a well-ventilated room (25°C ± 2°C temperature and ~70% humidity) of the Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh. They were provided with food and drinking water *ad libitum*. This experiment was approved [Ref. No. 249(35)/320/IAMEBBC/IBSc] by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) of IBSc, University of Rajshahi, Bangladesh.

Experimental design

Male animals (14-days-old) were grouped into 4 groups (n = 3). One group was left as a control. Another 3 groups were served as Tartrazine (Tz) treatment groups based on Tz doses (0.75 mg/Kg, 1.5 mg/Kg, and 3.0 mg/Kg). Since root development of the first molar (M1) starts from day 8th of birth (Radlanski et al. 2015), administration of Tz from day 7 or 8 could be the best. But intraperitoneal administration of Tz could be injurious to the animals, hence we avoided to start introduce Tz at that stage. Second molar (M2) and third molar (M3) tooth development starts from the 14th and 21st day of birth, respectively (Radlanski et al. 2015); therefore, Tz administration was started from the 15th day of birth to study the possible effect of Tz on the root growth of M2 and M3. Tz was administered intraperitoneally for 2 weeks.

Animal body weight

The body weight of animals was measured on the day (day 15th of birth) of the first treatment, and 24 h later of the last treatment (day 29th of birth).

Tooth extraction and evaluation

The animals were sacrificed by cervical dislocation after 24h of the last treatment (29th day of birth) and their molar teeth were extracted and the length of the root was measured and compared with the control.

RNA isolation and polymerase chain reaction (PCR)

RNA was isolated using TRIzol according to the manufacturer's protocol. Isolated total RNA was quantified using a nanodrop-spectrophotometer. One microgram of total RNA was reverse transcribed using TIANScript M-MLV (TIANGEN, China) reverse transcription kit as per the protocol provided with the kit. PCR was done by mixing 1 µL cDNA with 5.5 µL nuclease-free water, 0.4 µL dNTPs (5 mM), 1µL $MgCl_2$ (25 mM), 0.5 µL forward primer (10 pmoL), 0.5 µL reverse primer (10 pmoL), 1µL *Taq* polymerase buffer (10x), and 0.1 µL *Taq* polymerase. PCR was performed with SureCycler 8800 (Agilent Technologies, USA). Cycling conditions were initial PCR activation step for 3 min at 95°C, followed by 40 cycles of 95°C for 30s, 51-55°C (according to Table 1) for 30s, 72°C for 30s and a final extension of 72°C for 10 min. Finally, PCR reactions were analyzed on a 1.5% agarose gel using a gel documentation system (Red™ Imaging System, Alpha Innotech's, USA).

Table 1: List of primers.

Genes	Primers	Sequences	Annealing temperatures (℃)
Sirt6	Forward	5'-CTGAGAGACACCATTCTGGACT-3′	55
	Reverse	5'-GGTTGCAGGTTGACAATGACC-3′	
Bsp	Forward	5'-ACCCCAAGCACAGACTTTTGA-3′	53
	Reverse	5'-TTTCTGCATCTCCAGCCTTCT-3′	
Adipoq	Forward	5'-ATCTGGAGGTGGGAGACCAA-3′	51
	Reverse	5'-GGGCTATGGGTAGTTGCAGT-3′	
Nqo1	Forward	5'-TTCTGTGGCTTCCAGGTCTT-3′	53
	Reverse	5'-AGGCTGCTTGGAGCAAAATA-3′	
Gapdh	Forward	5'-GTGGAAGGACTCATGACCACAG-3′	52
	Reverse	5'-CTGGTGCTCAGTGTAGCCCAG-3′	

Statistical analysis

Student's test was performed between control and each Tz administered group separately on GraphPad Prism for calculating statistical difference in body weight of animals. Data are presented as mean±SD (standard deviation) of three independent replicates and statistical significance was tested at $p<0.5$.

Results

Effect of Tz on mice body weight

According to the data presented in Table 2, the mice before starting treatment were almost the same in weight. After completing treatment, 0.75mg/kg Tz, 1.5mg/kg Tz, and 3.0 mg/kg Tz treatment groups were also the same as the control group as they are not significantly different, though weight was decreased compared to control. But the decreasing trend was dose dependent.

Table 2: Effect of Tz on body weight.

Groups		Body weight (g) (n=3)	
		Age (15 days)	Age (29 days)
Control	-	11.97 ± 1.93	26.06 ± 2.29
Tartrazine (Tz)	0.75 mg/kg	13.06 ± 1.81	25.00 ± 3.08
	1.5 mg/kg	12.48 ± 1.78	24.01 ± 3.32
	3.0 mg/kg	12.93 ± 1.67	20.01 ± 2.76*

*$p<0.5$

Effect of Tz on tooth development

According to Fig. 1, there was no difference among groups in terms of size (2 mm) of first molar (M1) and second molar (M2). But molar 3 (M3) in all Tz treated groups became thinner than the control group. Besides, M1, M2, and M3 at 3.0 mg/Kg treated group became yellowish.

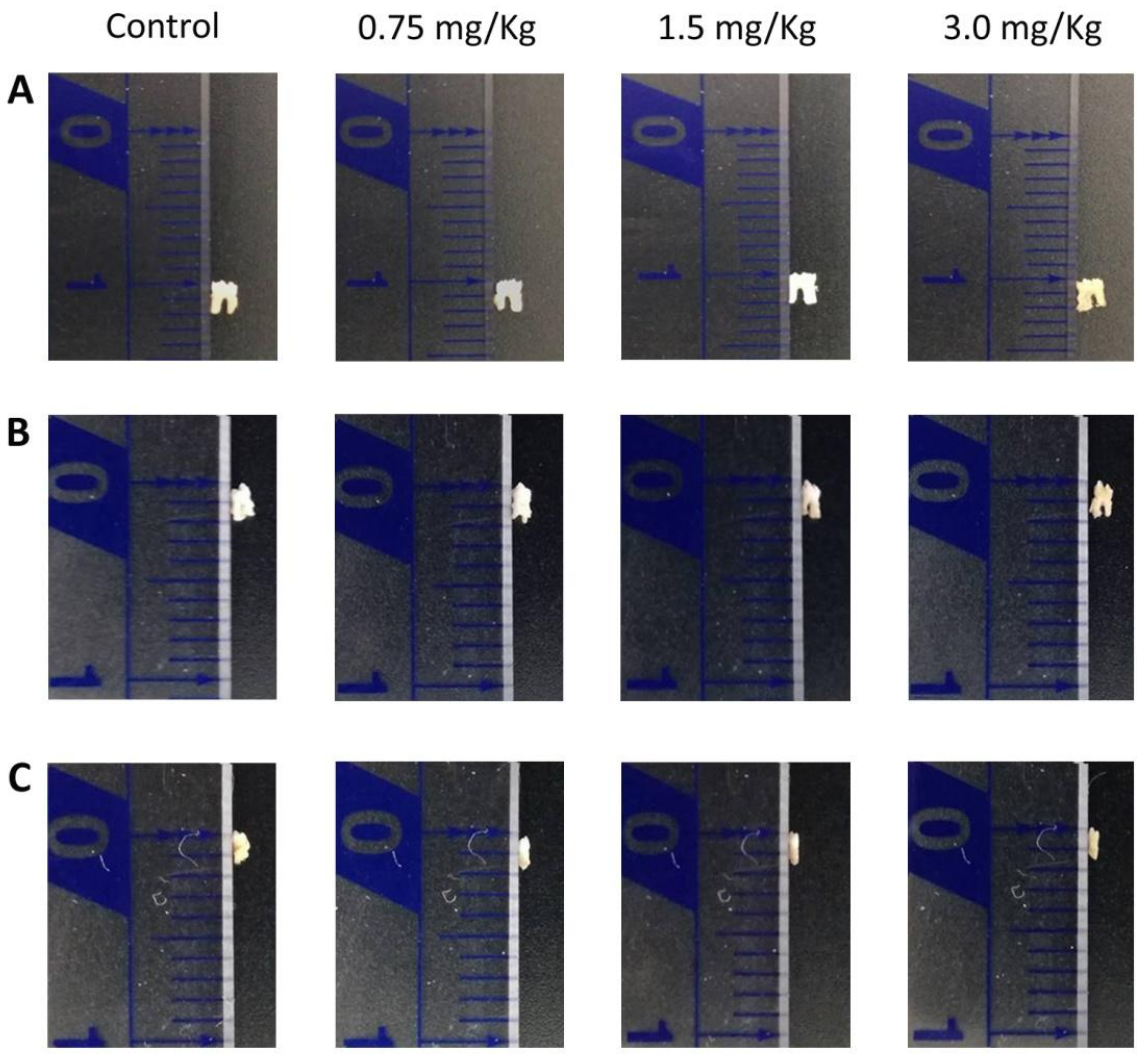

Fig. 1: Effect of Tz on molar tooth development. A = M1, B = M2, C = M3.

Effect of Tz on gene expression

Qualitative expressions of *Adipoq*, *Nqo1*, *Bsp*, and *Sirt6* genes were normalized to housekeeping gene *Gapdh* (Fig. 2). Equal intensities of *Gapdh* express across all groups suggest that equal amount of total RNA was used for cDNA synthesis (Fig. 2E). There was no change in expression of *Adipoq* (Fig. 2A) and *Nqo1* (Fig. 2C) genes. Interestingly, expression of *Bsp* increased dose-dependently (Fig. 2B). Additionally, expression of *Sirt6* was enhanced, but only at 1.5 mg/Kg and 3.0 mg/Kg doses (Fig. 2D).

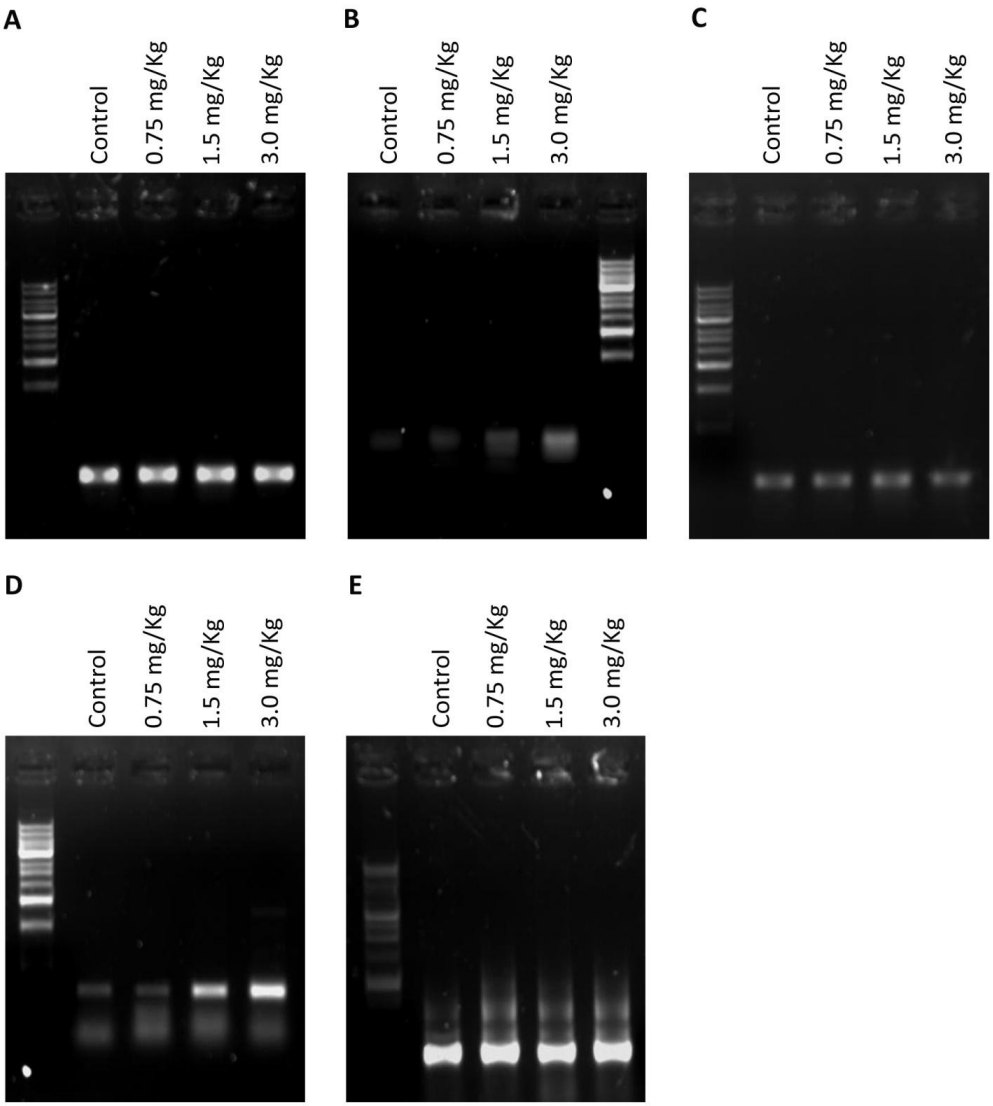

Fig. 2: Effect of Tz on gene expression. A = *Adipoq,* B = *Bsp*, C = *Nqo1*, D = *Sirt6*, E = *Gapdh.*

Discussion

Though the acceptable daily intake (ADI) amount is 7.5 mg/Kg body weight[2] for human, adverse effects of Tz at the ADI level have been reported in animal models as shown in different studies (Bhatt et al. 2018). In this study, we administered a lower ADI level of Tz in mice and found a noticeable adverse effect. We recorded a decreasing trend in body weight of all Tz treated group; however, only the highest dose treated group had significantly decreased body weight. Our data in terms of body weight is contradictory with a prior report (Vega-Cabanillas et al. 2021) showing no change in body weight of mice after Tz administration. Nevertheless, we assumed that body weight of animals in our study decreased since they were administered Tz at an earlier age (2 weeks) and for a longer duration (2 weeks) compared to the animal's age and duration of experiment in the study reported by Vega-Cabanillas et al. (2021). However, the decreased body weight is indicative to the damage to any vital organ of mice. We evaluated and compared the size of molar (M) teeth. Though no change was observed in the length of teeth, we found that the third molar (M3) became thinner in Tz administered groups compared to the control. Besides, we observed that all molar teeth in 3.0 mg/Kg Tz treated group became yellowish, which might be a sign of deposition of Tz in teeth.

In a recently published study (Zand et al. 2023), a group showed enhancement in expression of several epigenetic modifier genes, including *Dnmt1, Dnmt3a, Dnmt3b, Hdac2, Hdac3,* and *Hdac8* in mice treated with Tz and correlated their data to carcinogenesis. Interestingly, we found increased expression of *Bsp* and *Sirt6* genes in a Tz administered animal. A previous report showed that increased *Bsp* expression induces receptor activator of nuclear factor kappa-β ligand (RANKL) mediated osteoclast differentiation and bone resorption (Valverde et al. 2005), hence, we anticipated that the thinner M3 in Tz-treated group was due to the influence of increased *Bsp* expression. Dental mesenchymal cells (DMCs) play a profound role in tooth development as well as in the repair and regeneration of dental tissue (Liao et al. 2017). *Sirt6* is a gene that plays an important role in DMC differentiation, tooth root development, and eruption of the tooth (Liao et al. 2017). *Sirt6* belongs to the sirtuin (SIRT) family of nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases. It maintains genome stability and telomere function, regulates glucose and lipid metabolism, and controls inflammation. Moreover, this gene has been reported as a tumor suppressor in addition to its role in determining the lifespan (Liao et al. 2017). Expression of *Sirt6* has been identified in muscles, brain and heart tissue in adult mice (Mostoslavsky et al. 2006). Expression of *Sirt6* decreases in an age-dependent manner (Hu et al. 2018). *Sirt6* has no effect on tooth development before birth, but deletion of *Sirt6* gene in mice has two post-natal impacts: a delay in tooth eruption and sluggishness in the development of dental roots (Liaog et al. 2017). Here we found increased *Sirt6* expression, which might play a role in maintaining stability of the genome from the damaging effect of Tz or in response to inflammation induced by Tz (Patterson and Butler 1982, Amin et al. 2010, El-Desoky et al. 2017, Liaog et al. 2017, Bhatt et al. 2018, Vega-Cabanillas et al. 2021, Guerrero-Rubio et al. 2023).

In conclusion, Tz-administration did not severely impair tooth development in mice, but it had a detrimental effect to some extent. Though the effect of Tz in mice may not be reproducible in humans because of the completely different tooth development process, for example, the primary teeth in humans are replaced with permanent teeth which do not happen in mice. Nonetheless, awareness should be exercised when adding this food additive, especially to products being formulated aiming at children.

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Author's Contribution: MAH designed the experiment, supervised the study, and finally corrected the article. MST, IS, DR, MMHM and ASMFAR conducted experiments, collected data statistical analysis procedure and completed the draft of the article.

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Data availability: The data were analyzed during the study period are available from the corresponding author upon request.

References

Amin KA, Hameid II HA and Abd Elsttar AH (2010). Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicology 48(10): 2994-2999.

Bhatt D, Vyas K, Singh S, John PJ and Soni I (2018). Tartrazine induced neurobiochemical alterations in rat brain sub-regions. Food and Chemical Toxicology 113: 322-327.

Bruce HM (1947). The feeding and breeding of laboratory animals. VI. The breeding of mice. Epidemiology and Infection 45(4): 420-430.

El-Desoky GE, Abdel-Ghaffar A, Al-Othman ZA, Habila MA, Al-Sheikh YA, Ghneim HK, Giesy JP and Aboul-Soud MAM (2017). Curcumin protects against tartrazine-mediated oxidative stress and hepatotoxicity in male rats. European Review for Medical and Pharmacological Sciences 21(3): 635-645.

Guerrero-Rubio MA, Hernández-García S, García-Carmona F and Gandía-Herrero F (2023). Consumption of commonly used artificial food dyes increases activity and oxidative stress in the animal model Caenorhabditis elegans. Food Research International 169: 112925.

Hu X, Zhu S, Liu R, Miller JD, Merkley K, Tilton RG and Liu H (2018). Sirt6 deficiency impairs corneal epithelial wound healing. Aging (Albany NY) 10(8): 1932. https://doi.org/10.18632/aging.101513.

Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N and Al-Sinan M (2006). Estimates of dietary exposure of children to artificial food colours in Kuwait. Food Additives and Contaminants 23(3): 245-251.

Liao X, Feng B, Zhang D, Liu P, Zhou X, Li R and Ye L (2017). The *Sirt6* gene: Does it play a role in tooth development? PloS One 12(3): e0174255.

Mindang ELN, Awounfack CF, Ndinteh DT, Krause RWM and Njamen D (2022). Effects of tartrazine on some sexual maturation parameters in immature female wistar rats. International Journal of Environmental Research and Public Health 19(16): 10410.

Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S and Murphy MM (2006). Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124(2): 315-329.

Patterson RM and Butler JS (1982). Tartrazine-induced chromosomal aberrations in mammalian cells. Food and Chemical Toxicology 20(4): 461-465.

Radlanski R J, Renz H, Zimmermann CA, Mey R and Matalova E (2015). Morphogenesis of the compartmentalizing bone around the molar primordia in the mouse mandible during dental developmental stages between lamina, bell-stage, and root formation (E13–P20). Annals of Anatomy-Anatomischer Anzeiger 200: 1-14.

Rao P, Bhat RV, Sudershan RV, Krishna TP and Naidu N (2004). Exposure assessment to synthetic food colours of a selected population in Hyderabad, India. Food Additives and Contaminants 21(5): 415-421.

Valverde P, Tu Q and Chen J (2005). BSP and RANKL induce osteoclastogenesis and bone resorption synergistically. Journal of Bone and Mineral Research 20(9): 1669-1679.

Vega-Cabanillas R, Sisniegas M and Zavala F (2021). Tartrazine induces genotoxicity in lympocytes of BALB/c Mus musculus. Revista Peruana de Medicina Experimentaly Salud Pública 38(4): 587-594.

Zand AS, Enkhbilguun JM, Macharia F Budán, Gyöngyi Z and Varjas T (2023). Tartrazine modifies the activity of DNMT and HDAC genes- Is this a link between cancer and neurological disorders? Nutrients 15(13): 2946.