Effect of Food Azo Dye Tartrazine on Learning and Memory Functions in Mice and Rats, and the Possible Mechanisms Involved

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Abstract: Tartrazine is an artificial azo dye commonly used in human food and pharmaceutical products. The present study was conducted to evaluate the toxic effect of tartrazine on the learning and memory functions in mice and rats. Animals were administered different doses of tartrazine for a period of 30 d and were evaluated by open-field test, step-through test, and Morris water maze test, respectively. Furthermore, the biomarkers of the oxidative stress and pathohistology were also measured to explore the possible mechanisms involved. The results indicated that tartrazine extract significantly enhanced active behavioral response to the open field, increased the escape latency in Morris water maze test and decreased the retention latency in step-through tests. The decline in the activities of catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) as well as a rise in the level of malonaldehyde (MDA) were observed in the brain of tartrazine-treated rats, and these changes were associated with the brain from oxidative damage. The dose levels of tartrazine in the present study produced a few adverse effects in learning and memory functions in animals. The mechanisms might be attributed to promoting lipid peroxidation products and reactive oxygen species, inhibiting endogenous antioxidant defense enzymes and the brain tissue damage.

Keywords: learning and memory, oxidative stress, pathological lesion, tartrazine

Practical Application: Tartrazine is an artificial azo dye commonly used in human food and pharmaceutical products. Since the last assessment carried out by the Joint FAO/WHO Expert Committee on Food Additives in 1964, many new studies have been conducted. However, there is a little information about the effects on learning and memory performance. The present study was conducted to evaluate the toxic effect of tartrazine on the learning and memory functions in animals and its possible mechanism involved. Based on our results, we believe that more extensive assessment of food additives in current use is warranted.

Introduction

Food additives play a vital role in today's bountiful and nutritious food supply, they allow our growing population to enjoy a variety of safe, wholesome, and tasty foods year round (Amin and others 2010). Especially, color additives are defined as any dye, pigment, or other substance that imparts color to a food, drug, or cosmetic (Park and others 2009). Tartrazine (otherwise known as E102 or FD&C Yellow 5) is a synthetic lemon yellow azo dye used as a food coloring. It is water soluble and has a maximum absorbance in an aqueous solution. Its formal chemical name is trisodium-5- hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazole-3-carboxylate. Tartrazine consists of an azo (–N=N–) group, which is very harmful to living things (Wan Ngah and others 2010).

As regard to toxicological studies of tartrazine in mammals, Davis and others (1964) have reported that the incidence of tumors and the common incidental diseases were unaffected by tartrazine in the diet (0.5% to 5.0%) in a chronic toxicity study (2 y) of rats. Maekawa and others (1987) have found that tartrazine in drinking water (1.0% to 2.0%) showed no carcinogenic effects in 2 y toxicity study of rats. Borzelleca and Hallagan (1988a,b) also have reported that the no observed adverse effect levels (NOAEL) of tartrazine were both 5.0% (2641 and 3348 mg/kg/day for males and females, respectively) in rats and mice (8103 and 9735 mg/kg/day for males and females, respectively). However, Amin has concluded that tartrazine affected adversely and altered biochemical markers (for example, glutathione peroxidase [GSH-Px], superoxide dismutase [SOD], and catalase) in vital organs for example, liver and kidney (Amin and others 2010). Tartrazine also inflamed the stomach lining (increased the number of lymphocytes and eosinophils) of rats when given in the diet for a prolonged period of time (Moutinho and others 2007).

In addition, a variety of immunologic responses have been attributed to tartrazine ingestion, including anxiety, migraines, clinical depression, blurred vision, itching, general weakness, heat waves, feeling of suffocation, purple skin patches, and sleep disturbance (Rowe and Rowe 1994). Certain people who are exposed to the dye experience symptoms of tartrazine sensitivity even at

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extremely small doses, some experience this for periods up to 72 h after exposure. In children, asthma attacks and hives have been claimed, as well as supposed links to thyroid tumors, chromosomal damage, and hyperactivity (Ward 1997). The present study was to study the effects of tartrazine as coloring agent widely used in food products, drugs, and cosmetics on the learning and memory functions in animals. Furthermore, the effects of tartrazine on the biomarkers of the oxidative stress and pathohistology were also measured to explore the possible mechanisms involved.

Materials and Methods

Chemicals

Tartrazine was obtained from Guangzhou Sanxiong Food Trading Co., Ltd., Guangzhou, China. The purity of the chemical was more than 85.0% (w/w).

Animals and treatments

KunMing mouse (20 ± 2 g) and Sprague–Dawley rats (70 ± 10 g) were obtained from the Experimental Animal Center of Shandong Luye Pharmaceutical Co. Ltd. (SPF grade, Certificate NO.SYXK 20090009). The animals (50% male and 50% female) were housed individually in cages under hygienic conditions and placed in a controlled environment with a 12-h light/dark cycle at 23 ± 3 °C and 40% to 70% humidity for 7 d before the experiment. The animals were allowed a commercial standard rat cube diet and water *ad libitum*. All procedures involved in the use of the laboratory animals were in accordance with the Guidelines of the Animal Care and Use of Laboratory Animals set by the Association of Laboratory Animal Science and the Center for Laboratory Animal Science of Yantai Univ.

Morris water maze test

A total of 40 mice were used in the present study. Animals received a daily dose of 0 (control group), 175, 350, and 700 mg/kg body mass tartrazine once a day for 30 d, 7 d a wk by gavage. The Morris water maze test was performed as described previously (Morris 1984) with some modifications. The water maze used was a circular swimming pool measuring 100 cm in diameter and 40 cm in height, filled with water to a depth of 20 cm. The water was kept at 22 ± 1 °C and colored black with a nontoxic dye to make the platform invisible. Four equally spaced points around the edge of the pool were designed as: east (E), south (S), west (W), and north (N). An escaping platform (diameter is 7 cm) was set 1 cm below the surface of the water and placed in a constant position in the middle of the SW quadrant. The mouse in the pool was trained to find the platform using a variety of extra maze cues, including the desk, wall, window, experimenter, and so on. The experimenter always set at the same position and was blind to medication status.

The trials were started 24 h after the administration of tartrazine and vehicle to different groups of mice everyday. Each mouse was trained 4 times daily at intervals of 30 sec for 6 consecutive days. A trial was started by placing a mouse by hand into the water facing the wall of the circular pool, at the sign of E, S, W, and N around the edge of the pool. Mice were allowed to swim to the hidden platform and the escape latency (time to find the hidden platform) was recorded. After reaching the platform, the mice were allowed to remain on it for 20 sec and were then removed to a holding cage for a 30-sec intertribal interval. If the platform was not found within 60 sec, the mouse was manually placed onto the platform and given a maximum score of 60 sec. The escape latencies of 4 times daily of each mice were recorded. The mean escape latency of 4 times daily was calculated (Chen and others 2002). Mice were randomly assigned to each group. Each group consisted of 10 mice.

Step-through test

A total of 40 mice were used in the present study. Animals received a daily dose of 0 (control group), 175, 350, and 700 mg/kg body mass tartrazine once a day for 30 d, 7 d a wk by gavage. The passive avoidance behavior of mouse was tested according to the methods of Introini-Collison and others (1994). The apparatus consisted of 2 compartments, an illuminated box and a dark box separated by a guillotine door. The size of both boxes was 20 × 10 × 15 cm. During the training, the mouse was placed in the illuminated compartment and allowed to enter the dark compartment through the door. Immediately after entry, a scrambled foot shock (36 V, 55 Hz) was delivered through the grid floor. The mouse could escape from the shock only by stepping back into the safe illuminated compartment. Twenty-four hours after the training, the mouse was again placed in the safe illuminated compartment. The response latency to enter the dark compartment and the numbers of errors (enter the dark compartment) in 5 min were measured. The latency of not entering the dark room during the 5-min observation period was regarded as 300 sec.

Open-field test

The rats were divided into 4 groups, for the administration of tartrazine (125, 250, and 500 mg/kg body mass) or vehicle (control group) for 30 d, 7 d a wk by gavage. Each group consisted of 10 rats. The ambulatory activity of rats was measured by an open-field apparatus. The floor of a square plastic board (100 × 100 cm) with plastic sides (30-cm high) is divided into 16 squares. The rats are individually placed in one corner of the open field and allowed to explore the area freely. The activity level is expressed as the total number of squares crossed, whereas exploratory activity is expressed as the total number of rearings and fear is expressed as the total number of fecal boli during a 5-min testing period (Stafstrom and others 1993; Harro and others 1999; Volke and others 1997; Huang and others 2002). The increase in locomotor response and rearings and a decrease in defecation, and the decrease in locomotor response and rearings and an increase in defecation can be interpreted as less-emotional activity and more emotional activity in rats, respectively (Mechan and others 2002). The open-field apparatus was cleaned using alcohol before introducing the next animal, to preclude possible cueing effects of odors left by previous subjects (Phillips 1982).

Measurement of oxidative stress state and pathohistology

At the end of the open-field test, the brain was isolated from the skull immediately. The activities of catalase, GSH-Px, SOD, and the levels of the lipid peroxidation product MDA in brain were determined according to the manufacturer's instructions (Nanjing Jiancheng Bioeng Inst., China). To prepare tissue extracts, the brain was cut longitudinally. One-half of brain was fixed with 10% formalin for subsequent hematoxylin-eosin (H&E) staining, the other half was ground with liquid nitrogen in a mortar, and the ground tissues were then treated with 4.5 mL of phosphate buffered solution (PBS) buffer. The mixtures were homogenized using a glass homogenizer for 1 min on ice. The homogenates were then filtered and centrifuged using a refrigerated centrifuge at 4 × *g*. Then, these supernatants were used to determine the enzymes' activities by enzyme-linked immunosorbent assay (Amin and others 2010).

Statistical analysis

Statistical analysis was performed using the SPSS 11.5 software for Windows. All data are expressed as mean ± SEM. Analysis of variance (ANOVA) was carried out with Newman-Keuls or Tukey's HSD *post hoc* test for multiple comparisons. Differences were considered to be significant at $P < 0.05$.

Results and Discussion

Morris water maze test

The Morris water maze test allows the parallel measurement of responses related to both conditioned and unconditioned fear in the same subject. It also permits simultaneous assessment of memory and learning of these behaviors (Gargiulo and others 1996; Harro and others 1999). In the present study, the effects of tartrazine on learning and memory were examined in mice by the Morris water maze test. The results indicated that, in the vehicle-treated group, the escape latencies were 12.7 ± 2.2 sec, 10.8 ± 2.1 sec, and 11.0 ± 2.8 sec on the 4th, 5th, and 6th day, respectively. However, chronic oral administration of tartrazine significantly increased the escape latency as compared with the vehicle-treated group. At dose of 350 mg/kg, it showed a significant increase in the escape latency on the 5th and 6th day (13.4 ± 2.8 sec and 13.5 ± 2.3 sec, respectively). Furthermore, at dose of 700 mg/kg, it showed a significant increase in the escape latency on the 4th, 5th, and 6th day (18.7 ± 4.2 sec, 19.3 ± 4.7 sec, and 18.0 ± 3.4 sec, respectively). No significant effect was observed with tartrazine at dose of 175 mg/kg as compared with the vehicle-treated control group (Figure 1). These results suggested that tartrazine could induce remarkable learning and memory impairment in mice when given for a prolonged period of time.

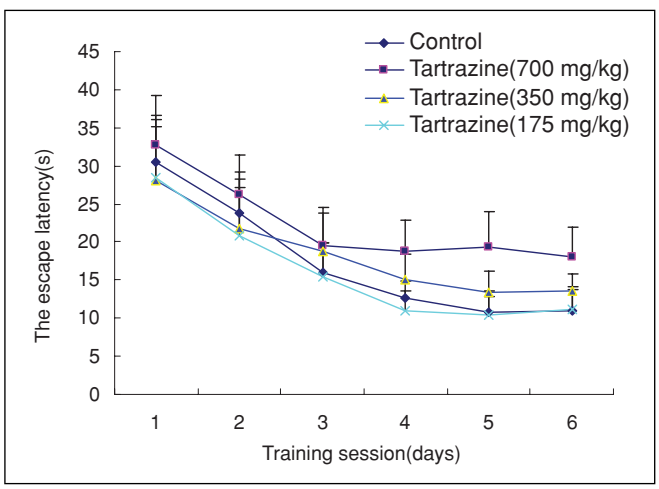

Figure 1–Effects of tartrazine on learning and memory abilities by water maze test in mice.

Step-through test

Figure 2 shows the performance in step-through test. In the acquisition trial, the step-through latencies of the tartrazine-treated mice received daily 350 and 700 mg/kg for 30 d were significantly shortened versus to the vehicle control ($P < 0.05$, $P < 0.01$), but no statistical difference was found by given tartrazine at dose of 175 mg/kg. Furthermore, there was no significant difference between tartrazine treatment groups and control group in the number of errors (data not shown).

Decreased step-through latency and increased shuttle avoidance responses suggest hyperactivity, decreased exploratory behavior, and impairment in learning and memory (Zhang and others 2010). Tanaka and others have reported that tartrazine could produce a few adverse effects on neurobehavioral parameters throughout generations in mice. Furthermore, it also seemed that tartrazine might have different effects on the behavioral development in sex

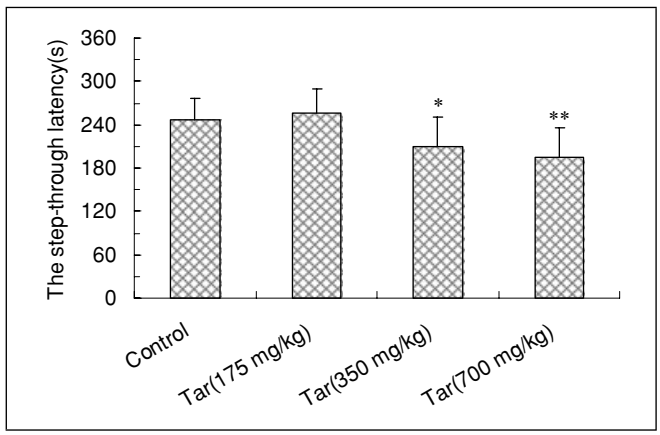

Figure 2–Effects of tartrazine on learning and memory in step-through test in mice. Each column represents the mean ± S.E.M. of 10 animals. Tar = Tartrazine. *$P < 0.05$, **$P < 0.01$ compared with Control.

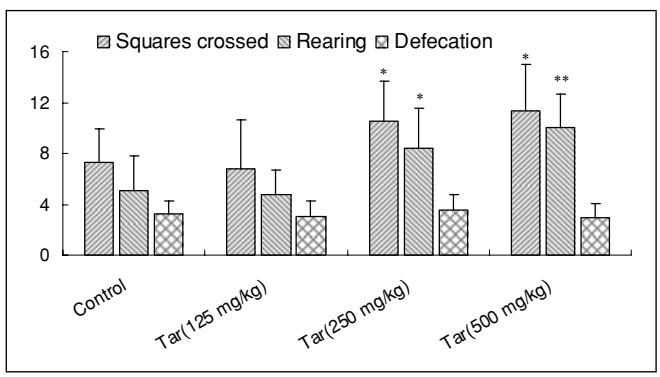

Figure 3–Effects of tartrazine on locomotor activity in rats. Each column represents the mean ± S.E.M. of 10 animals. Rearings = exploratory activity; squares crossed = motor activity; defecation = fear score. Tar = Tartrazine. *$P < 0.05$, **$P < 0.01$ compared with Control.

Table 1–Effect of tartrazine on the levels of catalase, GSH-Px, SOD, and MDA.

Group	Dose (mg/kg)	Catalase (U/mg protein)	GSH-Px (U/mg protein)	SOD (U/mg protein)	MDA (nmol/mg protein)
Control	-	40.00 ± 8.55	70.10 ± 15.01	551.30 ± 113.94	4.10 ± 0.99
Tartrazine	500	29.60 ± 6.74**	46.00 ± 8.86**	417.00 ± 95.27*	5.50 ± 1.27*
	250	31.10 ± 7.20*	55.40 ± 9.79*	445.60 ± 58.85*	5.50 ± 1.35*
	125	44.10 ± 8.02	65.60 ± 10.51	570.60 ± 111.93	3.90 ± 1.37

Values are expressed as the mean ± S.E.M. of 10 animals. *$P < 0.05$, **$P < 0.01$ compared with Control.

in reproductive toxicity testing (Tanaka 2006; Tanaka and others 2008). However, in the present study, the impairments of tartrazine were observed in both sex animals in subchronic toxicity study, and further investigation is needed to clarify these questions.

Open-field test

To determine the safety of chemicals for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a "safe" dose in humans (Hamid and others 2008; Li and others 2009). In this study, we subjected another animal, rat, to open-field test to study the long-term effects of tartrazine on behavior and the behavioral changes, and further to explore the possible mechanisms involved. Namely, squares crossed, rearings, and fecal boli were counted in 5-min periods. Figure 3 illustrates open-field activity by chronic tartrazine administration. Tartrazine-treated rats were more active than control rats in the unfamiliar open field. The total number of squares crossed by tartrazine (250 and 500 mg/kg) groups was markedly higher than that of control group ($P < 0.05$). Long-term tartrazine treatment also increased the number of rearing in rats in the novel environment ($P < 0.05$, $P < 0.01$). However, there was no statistical difference between tartrazine treatments and control group in the number of fecal pellets.

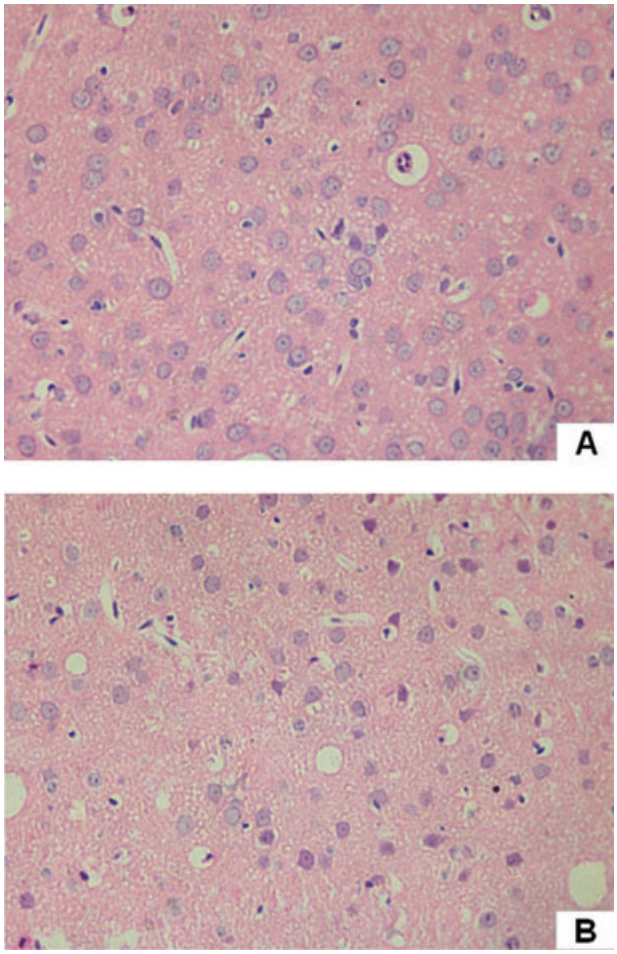

Figure 4–Histopathological changes in rat brain tissue (H&E, ×400). (A) Control rats and (B) rats treated with tartrazine 500 mg/kg. Tartrazine exerts histopathological effects on the brain tissue of the rats, indicated by swelling, vacuolar degeneration, and karyopyknosis, as well as the morphological characteristics of apoptosis.

The open-field apparatus are applied to analyze spontaneous exploratory activity and curiosity of animals to a novel environment (Ramos and Mormede 1998; Chen and others 2009). The activity level is expressed as the total number of squares crossed, whereas exploratory activity is expressed as the total number of rearings. In this study, the number of squares crossed and rearings increased in both sexes after chronic oral administration of tartrazine in the open-field test. These parameters indicated that in tartrazine-treated rats, fear and anxiety were significantly increased.

Quantification of catalase, GSH-Px, and SOD activities as well as MDA level in brain homogenate

Catalase, GSH-Px, and SOD constitute a mutually supportive enzyme system of the 1st line cellular defense against oxidative injury, decomposing O_2 and H_2O_2 before their interaction to form the more harmful hydroxyl radical (Panda and others 2008). MDA, the degradation product of the oxygen-derived free radicals and lipid oxidation, reflects the damage caused by reactive oxygen species (Qin and others 2009). Table 1 showed the levels of catalase, GSH-Px, SOD, and MDA in brain tissue of normal and experimental groups of rats. Oral administration of tartrazine (250 and 500 mg/kg) caused the significant ($P < 0.05$, $P < 0.01$) decline in the activities of catalase, GSH-Px, and SOD in brain tissue as compared with the control rats. However, the level of MDA was raised in these groups ($P < 0.05$).

Because tartrazine is from the group of azo dye food colorants, it is metabolized into aromatic amines by intestinal flora and the formed aromatic amines can generate reactive oxygen species as part of their metabolic products by interaction of these amino groups with nitrite or nitrate contained in foods or in the stomach (Moutinho and others 2007). In the present study, the decrease of catalase, GSH-Px, and SOD activities, and the increase of MDA level in the brain tissue of tartrazine-treated rats was an indication of the severity of the tartrazine-induced oxidative stress condition. Taking together, we hypothesize that tartrazine-induced oxidative stress condition might be attributed to its metabolism in animals.

Histopathological examination of brain tissue

Reactive oxygen species play an important role in pathological changes in the brain (Doverhag and others 2008). In this study, neurocyte morphological change was observed by H&E staining, which illustrated that the control cells exhibited uniform cytoplasmic distribution, normal structure, and intact cell membrane (Figure 4A). Tartrazine (500 mg/kg) exerted histopathological effects on the brain tissue of the rats, indicated by swelling, vacuolar degeneration, karyopycnosis, and nucleoli disappear, as well as the morphological characteristics of apoptosis (Figure 4B). These results indicated that oxidative stress induced brain histopathological damages. Mekkawy and others (1998) also have reported that tartrazine exerted histopathological effects on the hepatic and renal tissues of the rats, indicated by vacuolation, swelling, necrosis, and pyknosis of their cells. In addition, tartrazine not only caused changes in hepatic and renal parameters but also its effect became more risky at higher dose, because it could induce oxidative stress by formation of free radicals (Amin and others 2010). In light of the previous findings and our study, we hypothesize that oxidative stress may play an important role in tartrazine-induced harm to living things, and further study are needed to clear this question.

Conclusions

Tartrazine has been reported that exposure to tartrazine could cause asthma, migraines, eczema, thyroid cancer, and lupus (Mittal

and others 2007). Importantly, it is highly toxic and can act as catalyst in hyperactivity and other behavioral problems. In the present study, the dose levels (high and middle) of tartrazine produced neurotoxicity and deficits in learning and memory in animals, and these abilities might be attributed to promoting lipid peroxidation products and reactive oxygen species, inhibiting endogenous antioxidant defense enzymes and the brain histopathological damage. These dosages, if extrapolated on a body weight basis to humans, corresponds to 80 and 40 mg/kg bw, respectively. Although these dosages (80 and 40 mg/kg bw) were in excess of the acceptable daily intake (ADI) of tartrazine (0 to 7.5 mg/kg bw, Joint FAO/WHO Expert Committee on Food Additives 1996), many researchers have reported that some children who ingested tartrazine showed hyperactivity, and those effects showed dose-related (Rowe and Rowe 1994; Ward 1997). In addition, because of the complexity of the effects of environmental exposures on human health, it has been proposed that the study of the exposure to the combination of several toxicants might be a more relevant area of research, which could uncover new mechanisms by which environmental exposures regulate neurodegenerative diseases. In this way, it has been demonstrated that exposure to tartrazine together with other dyes (ponceau, carmoisine, erythrosine, sunset yellow, tartrazine, fast green, indigotine, brilliant blue, and brilliant black) exerted their toxicity by mechanisms involving synergistic processes or the activation of completely different signal transduction pathways. Indeed, this is an area of research whose results certainly have great potential to be translated into important advances in public health.

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