MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 1, 2026, Pages 6205-6207

Study of the toxic effects of tertrazine on some functional parameters in albino rats

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Abstract:
Objective: The present study aims to investigate the adverse effects of tartrazine dye on various functional parameters in laboratory albino rats Rattus norvegicus. The present study was conducted from September (2024) to November (2024) at the University of Karbala / College of Education for Pure Sciences / Department of Life Sciences / Graduate Laboratory. (12) Adult male albino rats were used in this experiment, with weights ranging from 190 to 230 grams and ages ranging from 11 to 13 weeks. They were systematically classified into two groups, each containing six animals. The initial group (G1) served as a negative control, as it received unrestricted access to water and feed, while the subsequent group (G2) served as a positive control, as it was given an aqueous solution of tartrazine at a dose of 400 mg/kg of total body weight. After the end of the test period (30 days), the animals were anesthetized with chloroform in a closed manner, blood was drawn directly from the heart, and tests were performed for the studied functional parameters. The results indicated a significant increase ($P \leq 0.05$) in the concentrations of liver enzymes (ALP, ALT, AST) and kidney parameters including urea and creatinine, in addition to a decrease in antioxidant levels (SOD, GSH, CAT), an increase in malondialdehyde (MDA), an increase in lipid concentrations (LDL, TC, TG) and a decrease in (HDL) when comparing group (G2) with group (G1).

Introduction

In the midst of the population census and the great development in the field of trade, industry and marketing, food has entered with the rest of the commercial goods in the field of development, marketing and improvement, and even surpasses them because it is the most popular and consumed in the world of other goods. Therefore, the old methods are no longer useful to keep pace with the development in the food industry and marketing, which prompted those working in its industry to invent new ways to improve, develop and store it until it is consumed. Processed foods are widely consumed in the food chain, as the development in the field of food monitoring represents the entry of many types of foods into the local market that meet the market's need by improving them with various food additives. However, at the same time, previous studies have shown that food additives added to processed foods, including emulsifiers, sweeteners, colors and fine particles, affect human health (Whelan *et al*., 2024).

The majority of food additives are colorants, which are compounds employed to impart color to food and beverages. They are utilized in the food sector to augment the visual appeal of products, rendering them more enticing to consumers (Şenol *et al*., 2024).

Conversely, food dyes, referred to as edible dyes or coloring substances, are chemicals extensively utilized across several industrial sectors. For example, current legislations in the United States and the European Union allow the use of (915) synthetic food dyes, respectively (Diao *et al*., 2024). Food dyes are among the most important food and pharmaceutical additives that are widely used today, as there are more than 2,500 types of them. Food dyes are divided into synthetic and natural. Synthetic dyes are manufactured industrially and are typically more vivid and stable than natural dyes. Examples of these are tartrazine (E102, yellow) and Allura Red (E129, red). Natural dyes originate from organic sources, including flora, fauna, and minerals. This includes beet juice (red), turmeric (yellow), and spirulina (green) (von HellFeld *et al*., 2024).

AZO dye is one of the dyes that are widely used in the field of food and pharmaceutical solutions, but recent studies have determined its daily use at specific concentrations due to its toxic effects and the fact that it causes genetic mutations and cancerous diseases for users (Sharma *et al*., 2024).

Tartazine dye is one of the most important AZO dyes and is orange-yellow in color. It is a chemically manufactured dye in the form of a powder that is added to foods and dissolves in water. It contributes to giving color to foods such as fruit juices, colored drinks, ready-made foods, cakes, soups, pickles, ice cream, jam, yogurt, honey products, butter, and cheeses. Tartazine dye can be used in some cosmetics and personal care products. It is also widely used as a coloring agent in many medicines, and is added to antacids, cough syrup, lotions, and vitamins. It is also used in many other materials such as stamp inks, glue, and colored chalk (Muhammad *et al*., 2024).

Tartrazine is symbolized by the symbol (E102). Its trade name is Tartrazine, This is a frequently utilized synthetic yellow azo color. Certain individuals may exhibit adverse reactions to Tartrazine. These reactions encompass urticaria, pruritus, edema, asthma, and, in rare instances, allergies. Consequently, eliminating tartrazine from food products is essential for persons who are susceptible or sensitive for it (Alshammari *et al*., 2024). Many studies have indicated the effect of tartazine dye on kidneys, liver lipid and antioxidants parameters in different types of animals. A study was conducted on a group of rats of both sexes, where different concentrations of tartazine dye (10 mg/kg bw 500, 7.5, 5.75) were administered orally for 90 days. An increasing in the activity of enzymatic for each of (ALT, AST, ALP) was observed. As for the kidneys, a disturbance in kidney functions occurred, such as an increase in the level of urea and creatinine in male rats when they were administered (500 mg/kg bw) of tartazine for three month, in comparison to the negative control group. In another study, three doses of tartazine dye (50, 25, 75 mg/kg bw) were employed to illustrate its impact on several biochemical parameters in male white rats. It was noted that albumin, ALP, MDA, and total protein levels in the serum of the animals receiving treatment rose with increasing concentration, whereas antioxidants levels (SOD, GSH, CAT) in the serum of treated animals declined with increasing concentration. This is due to necrosis of liver cells, infiltration, rupture, and significant alterations in the defense mechanism of antioxidants (Amchova *et al*., 2024).

The aim of the research: Assessment of the adverse impacts of tetrazzin on various physiological and biochemical markers within the body.

Materials and Methods

This study utilized 12 males laboratory white rats of the species Rattus norvegicus, with weights ranging from 190 to 230 grams and ages between 11 and 13 weeks. The specimens were acquired from the animal facility of the College of Pharmacy at the University of Karbala. This experiment occurred during September 1 to November 1 of the academic year 2024. The animals were housed in plastic cages with metal lids used for the rearing of rats. The cage floors were covered with sawdust, necessitating periodic replacement to ensure cleanliness. The animals were provided with water and feed ad libitum, under appropriate ventilation, at a temperature of 25°C and with natural light, and were allowed a two-week acclimatization period to the experimental settings.

Experiment design

Upon completion of the acclimatization phase for the animals, they were segregated into two groups, each including six animals. as follows:

1-The initial group (G1): The animals of this group were considered negative control and were given water and fodder freely for 30 days.

2- The second group (G2): The animals of this group were considered positive control and were given daily aqueous solution of tartrazine for 30 days.

blood sample collection: After the end of the experiment, the animals were anesthetized using the closed method, which includes placing the animals in a tightly closed container, noting the placement of a cotton ball containing chloroform. After a few minutes, the animal was anesthetized. Then, blood was extracted directly from the heart via puncture utilizing a sterile 5 ml medical syringes to acquire the maximum volume of blood. Samples of blood were directly deposited into sterile test tubes devoid of anticoagulants (Gel tubes), thereafter transferred to a centrifuge, and spun at a velocity of 3000 rpm for 15 minutes for isolate the serum, which was transferred to small plastic tubes (Eppendorf tube) that were clean, dry and marked. The serum was kept in the refrigerator until the biochemical tests were performed.

Results and discussion

1- The effect of tertrazine therapy on liver enzyme levels (AST, ALP, ALT).

The findings of the present investigation, as illustrated in Table No. (1), indicated a substantial elevation ($P \leq 0.05$) in the levels of liver enzymes (ALT, AST, ALP) in the tartazine-treated group (G2) compared to the negative control group (G1). The findings of the present study align with those of Sudarta *et al.* (2022), who observed a notable elevation in liver enzyme levels in groups administered with tartrazine dye in their diet compared to the control group without the dye, and they explained this increase in enzyme concentrations as damage to some liver tissue cells, which led to the leakage of these enzymes into the bloodstream. A study conducted by Amchova *et al*. (2024) supports this notion. indicated an increase in liver enzymes in groups of animals that were treated with high concentrations of tartrazine in food, and these enzymes decreased when the dye concentrations in food were reduced. It seems that reducing the dye concentration lowers the limits, and they indicated that high concentrations of tartrazine in food cause the release of many reactive free radicals into the blood, which contribute in one way or another to oxidative damage to the body's cells, which is associated with damage to liver and kidney cells alike. On the other hand, Desoky *et al*.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 1, 2026, Pages 6205-6207

(2022) indicated that dosing experimental animals with tartrazine was associated with a notable elevation in the levels of liver enzymes, including AST, ALP, and ALT. Tartrazine caused necrosis in liver cells, accompanied by inflammatory symptoms that led to the liver cells releasing these enzymes into the bloodstream, which was accompanied by a clear increase in their concentrations.

2- The effect of treatment with tartrazine on kidney concentration (urea and creatinine):

On the other hand, The findings of the present investigation indicated a notable elevation in the levels of urea and creatinine in the tartazine-dosed group of animals (G2) compared to the undosed group (G1). The rise was statistically significance at the level of (P≤0.05). The findings of the present investigation corroborate those of Desoky *et al*. (2022), indicating that tartazine administration resulted in a substantial elevation (P≤0.05) in urea and creatinine concentrations in the G2 group relative to G1. This also resulted in an elevation of uric acid and total proteins levels, with a clear decrease in the weight of some body organs, especially the liver and kidney. The study suggested that taking different doses of tartazine causes various kidney diseases, which are accompanied by an increase in blood creatinine as a result of the destruction of kidney cells, which reduces the kidney's ability to filter body fluids and nitrogenous waste. In the same direction, researchers (Tuli) and others (2015) indicated that the increase in creatinine concentration is due to the interference of the dye with creatinine metabolism, causing it to rise, or through the loss of kidney tissues, part or all of the ability to excrete renal tubules. On the other hand, researchers (Verduzco) and others (2019)) that the increase in urea concentration in groups that took tartrazine came from oxidative stress to which the animal was exposed, this led to the depletion of the primary energy source, prompting the animal to utilize proteins as an alternate energy source, resulting in the production of substantial quantities of urea and an increase in its concentration in blood plasma . Or the increase in urea may be due to an increase in the concentration of free radicals in the body, which in turn leads to the oxidation of proteins and amino acids, which results in an increase in the concentration of urea, as it is produced secondary in the blood (Amiri, 2018).

Table (1):The data illustrates the concentration of hepatic enzymes and kidneys parameters in the examined groups.

Means ± S.E					group	N
creatinine	urea	ALT	AST	ALP		
0.24± 0.004 A	0.49±32.66 C	0.44±82.00 B	1.40± 134.66 C	4.60±316.33 C	G1/ Normal	1
0.26± 0.006 A	2.20±38.33 B	5.96± 129.16 A	2.96±170.00 A	3.94± 874.00 A	G2 /toxicity	2

* Averages with the same or similar letters are not significantly different

3- The effect of treatment with trazapine on the concentration of fats (HDL, LDL, TC, TG)

Findings of the present investigation, as illustrated in Table No. (2), revealed a statistically significance difference at the level of (P≤0.05) in the concentrations of fats (HDL, LDL, TC, TG) between the negative control group (G1) and the toxic control group (G2). Fat is the primary constituent of several cellular membranes. Certain studies have demonstrated varying impacts of food colorants on fat levels. The most significant compound is tartazine, as the findings of the current study align with those of Usman & Muhammad *et al.* (2024), who observed an elevation in the concentrations of fats (HDL, LDL, TC, TG) in animals administered tartazine. Conversely, the current study contradicts the results of Imafidon et al. (2015), who reported a notable reduction in body weight and blood sugar levels in animals treated with varying doses of tartazine, with no impact on the lipid profile.. Animals were used as a basis for interpreting the situation in humans. The results of the study by researcher Saxena in (2015) The findings of the current study align with previous research on animals treated with tartrazine, which indicated an elevation in the concentrations of lipids (LDL, TC, TG) and a notable increase in triglycerides, total cholesterol, and low-density lipoprotein within liver tissue. Despite the elevation in all serum lipid indicators except HDL, the findings of the study conducted by Amin *et al.* in 2023 showed that when using tartazine and giving it to animals, the results showed consistency with the results of the current study in the increase in fat concentrations, including (LDL, TC, TG) and their disagreement in the increase in HDL. The reason is likely to be due to the difference in tartazine concentrations or environmental conditions or the health status of the animal, as the results of the study by researcher El Golli in (2016) do not agree with the findings of the current study in the fat file in treating animals with tartazine, which caused an increase in HDL (and a decrease in TC.LDL). The reason is likely to be due to the difference in concentrations or environmental living conditions of the animals.

Table (2) shows the concentration of fats (HDL, LDL, TC, TG) in the studied groups

Means± S.E				group	N
TG	TC	LDL	HDL		
1.06±42.00 BC	1.17±64.50 A	0.42±32.50 B	1.19±24.83 B	G1/ Normal	1
5.64±66.00 A	1.20±66.33 A	0.76± 34.66 A	0.42± 17.50 C	G2 /toxicity	2

* Averages that have common or similar letters do not differ significantly

4-The effect of treatment with tartrazine on the concentration of oxidation enzymes in the studied groups.

Findings of the present investigation, as illustrated in Table No. (3), indicated a statistically significance difference at the level of (P≤0.05) in the concentration of oxidative enzymes (MDA, CAT, SOD, GSH) when comparing the toxic control group (G2) to the negative control group (G1). Tartrazine is a pigment frequently utilized in the food and dye sectors. The over utilization beyond acceptable limits jeopardizes human health and the aquatic ecosystem. While previous studies reported harmful effects such as mutations, cancer, reproductive toxicity, allergies and asthma. The current study showed an increase in oxidation enzymes, which is consistent with the results of the study by researchers Desoky *et al*. (2022). The findings of the present study align with those of Desoky et al. (2017), which demonstrated that dietary exposure to tartrazine resulted in a statistical significance increasing ($p<0.05$) in lipid concentrations, liver enzymes, kidney function parameters, and the oxidative stress index MDA. The activities of various antioxidant enzymes (i.e., CAT, SOD, GSH) and GSH concentration, which indicates non-enzymatic capacity for antioxidants, were considerably reduced ($p<0.05$) compared to control rats not exposed to tartrazine. Tartazine induced alterations in several parameters, enzymes, and concentrations that heightened oxidation in the organisms of animals.. The findings of the present study contradict those of researchers Abd El-Rahman *et al*. (2024), who observed an elevation in MDA levels and a reduction in antioxidant enzymes (CAT, SOD, GSH) when administering tartazine dye to animals. The findings of the present investigation concurred with those of Ibrahim *et al.* (2024), indicating that the administration of tartrazine to animals resulted in an elevation of oxidative enzymes. The findings of the present investigation contradict those of Erdemli *et al.* (2024), who observed a reduction in malondialdehyde (MDA), superoxide dismutase (SOD), and total oxidation state (TOS) levels., and an increase in the oxidative stress index (OSI), glutathione (GSH), glutathione peroxidase (GSH-Px), and catalase (CAT). In addition, scientists and researchers Haridevamuthu and others indicated (2024) They elucidated that tartrazine at an ecologically suitable dosage (50 mg/L) markedly produces oxidative stress, modifies antioxidant responses (SOD, CAT, and GSH), causes cellular damage (MDA and LDH), triggers apoptosis, and disrupts mitochondrial function.

Table (3) demonstrates the quantity of oxidative enzymes in the examined groups

Means ± S.E				group	N
SOD	GSH	MDA	CAT		
0.34±36.50 B	0.22± 50.50 A	0.56 ±51.50 C	0.30±41.16 B	G1/ Normal	1
0.87± 30.83 C	1.49±23.66 C	0.55±57.33 A	2.23± 38.66 C	G2 /toxicity	2

* Averages with the same or similar letters are not significantly different

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ISSN: 1053-7899
Vol. 36 Issue 1, 2026, Pages 6205-6207

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