

*Evaluating the Antioxidant Effects of Onion (*Allium cepa*) on Blood Biochemical Factors and Antioxidants after Consuming Tartrazine in Rats*

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ABSTRACT

Colors have been added to food naturally and artificially for centuries to make them look more appetizing. According to the Food and Drug Organization, eight artificial colors were registered for the nutrition application, one of them, is tartrazine, which is widely used in foods and cosmetics. Food colors significantly decrease weight and increase proteins, liver enzymes, thyroid hormones, total cholesterol, and triglyceride. Onion, as an antioxidant, can reduce the harmful effects of artificial food colors on weight gain, antioxidant activity, and blood biochemical factors. The present study aimed to evaluate the effects of the onion's antioxidant properties on serum biochemical factors and antioxidants in Wistar rats after consuming tartrazine. Eighty Rats were divided into four groups, 20 mice in each. The first group received water without additives and was considered the control, the second group received tartrazine, the third group received tartrazine with onion juice, and the fourth group received only onion juice through gastric gavage. This experiment was performed for 60 days, and then the antioxidant activities of superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), and the biochemical parameters of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were measured. Tartrazine decreased the antioxidant activities of SOD, CAT, GPx, and the biochemical parameters of HDL and LDL. The results showed that the consumption of tartrazine causes the production of free radicals, which is the reason for the significant reduction of antioxidant activities and serum biochemical factors. Onion, as an antioxidant in this study, reduces the effects of tartrazine on antioxidant activities and serum biochemical factors.

Keywords: *Allium cepa*, Antioxidant, Blood factors, Rat, Tartrazine

1. Introduction

Food colors that are naturally or artificially added to food can be one of the criteria for choosing food due to the attractiveness they create. However, 95% of the used colors are artificial. The reason for the wide use of these materials is their easy production, low price, attractiveness, and the existence of various artificial colors. According to the law of the Food and Drug Organization in 1954, eight artificial colors were registered for nutrition applications, one of which is tartrazine, which is widely used in foods and cosmetics. This substance is known as lemon color and is widely used in ice cream, juices, non-alcoholic drinks, snacks, sauces, etc. Studies show that artificial colors cause different reactions. According to previous studies, it has been observed in the laboratory that this substance is genotoxic and carcinogenic. During this study performed on male albino mice, tartrazine caused an increase in body weight in the experimental group; however, a significant decrease in the weight of the testicles and epididymis and in the motility and amount of sperm were observed. In terms of morphology, significant changes have been observed in high doses (1).

According to previous studies, food colors cause a significant decrease in weight and an increase in proteins and liver enzymes, thyroid hormones, total cholesterol, triglyceride, and transaminase. Although bleeding areas were seen in the liver and kidneys, no adverse effects were observed in the stomach tissue (1). Sulfanilic acid is the main metabolite of tartrazine. In some countries, including Sweden, Switzerland, and Norway, this substance is no longer used (2). Meanwhile, antioxidants play a significant role in cell and tissue protection of testicles and sperms. An antioxidant is a molecule that can slow down or prevent the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing substance. These reactions produce free radicals, which initiate a series of cell-damaging reactions. There are two antioxidant systems in the body; the preventive

antioxidant system with proteins such as albumin, ceruloplasmin, etc., which are bound to metals and prevent the formation of reactive oxygen species; thus, inhibiting the start of a chain reaction. This is while the scavenger antioxidant system, such as vitamins A, C, and glutathione peroxidase enzyme, removes the generated reactive oxygen species (3). A two-year onion plant is with hollow and cylindrical leaves, and stems and its height reaches up to one meter. Onion flowers are white or purple in the form of umbrellas at the end of the stem. There are different types of onions, and they are currently cultivated in all regions of the world. Onions are an important source of phenolic and flavonoid compounds. Research has shown that onions contain exogenous and endogenous antioxidants such as selenium, glutathione, vitamins, and flavonoids (e.g., quercetin and isorhamnetin). Therefore, it is concluded that onion reduces the harmful effects of tartrazine on biochemical factors, blood antioxidants, and testicular tissue changes. According to the research performed by Khaki et al., onion, as a medicinal plant, has been effective in spermatogenesis (4).

Considering the high consumption of artificial colors in food and cosmetics and the possibility of reducing their harmful effects on the biochemical and antioxidant factors of blood using natural antioxidants, the consumption of onion together with tartrazine can be important in terms of innovation. Therefore, the present experiment aimed to evaluate onion effects on blood biochemical and antioxidant factors after tartrazine consumption in Wistar rats.

2. Materials and Methods

2.1. Animals

To conduct this research, 80 Wistar rats were purchased from the Pasteur Center in Iran. The rats were about 8 weeks old and weighted around 250 ± 10 . During the research period, these animals were placed in light for 12 h and in darkness for 12 h (9 am to 9 pm). The temperature of the storage room was 25.3 to 23.9 degrees Celsius, and the room humidity

was 55-60%. All the animals in this research were euthanized according to animal protection by the Animal Ethics Committee of the Islamic Azad University, Rasht, Iran. A total of 80 rats were divided into three groups of study and one group of control. The first group was the control group with drinking water without additives. In the second group, 2 ccs of tartrazine 1% was given daily by gavage. In the third group, 2 units of tartrazine 1% along with 3 cc of onion juice were gavaged to the mice. In the fourth group, only 3 ccs of onion juice was gavaged daily. Onion juice was prepared from 20 g of onion with a juicer. After 60 days after the start of the experiment, blood samples were taken from the eye area of the experimental mice to check the chemical factors and blood antioxidants. After 10 min of centrifugation at 3,000 rpm, serum and plasma were separated from the blood samples and kept at -20°C until the experiments. To measure high-density lipoprotein (HDL) and low-density lipoprotein (LDL), the quantitative detection kits of Pars Azmoun Company and spectrophotometer model Ce1010 of England were used. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes in plasma were also measured using a Ransel and Randox commercial kit at wavelengths of 505 and 340 nm, respectively, by a spectrophotometer. Catalase (CAT) enzyme was measured based on the amount of H_2O_2 decomposition at 240 nm wavelength and 20°C .

At the end of the study, the animals were euthanized by CO_2 gas for 2 h (9-11 am) according to the Animal Protection Act.

2.2. Statistical Analysis

The collected data were statistically analyzed in a completely randomized design using the general linear model (GLM) with the statistical software SAS (2004). The statistical model applied was $Y(i) = \mu + T_i + \epsilon_{ij}$, where $Y(i)$ is the value of each observation, μ is the overall mean of the trait, T_i is the effect of experimental diets, and ϵ_{ij} is the residual error. Mean separation was performed using Duncan's multiple range test at a 5% probability level.

3. Results

3.1. The Results of the Effect of Tartrazine and Onion Juice on Blood High-density Lipoprotein (HDL) Levels

The mean value of HDL was 19.9 ± 0.39 in the control group, 10.5 ± 0.358 in the tartrazine group, 11.2 ± 0.497 in the tartrazine and onion juice group, and 24.9 ± 0.789 in the onion juice group. In the statistical analysis, the P -value was less than 0.05, showing that the amount of HDL in all four groups has a significant difference between the groups. Therefore, it can be concluded that the consumption of tartrazine and onion juice has a significant effect on the level of HDL (Figures 1 and 2).

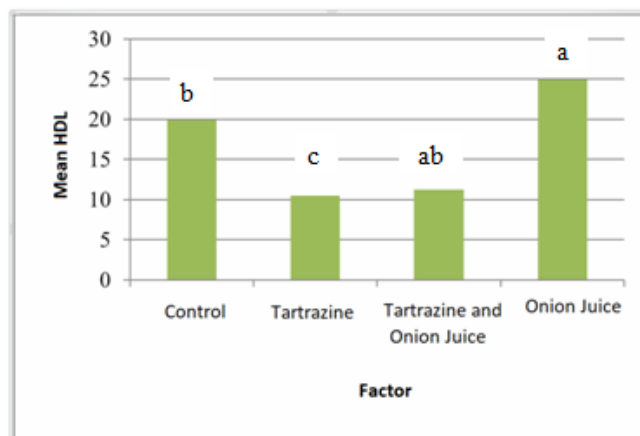


Figure 1. Mean high-density lipoprotein in the four groups (mg/dl)

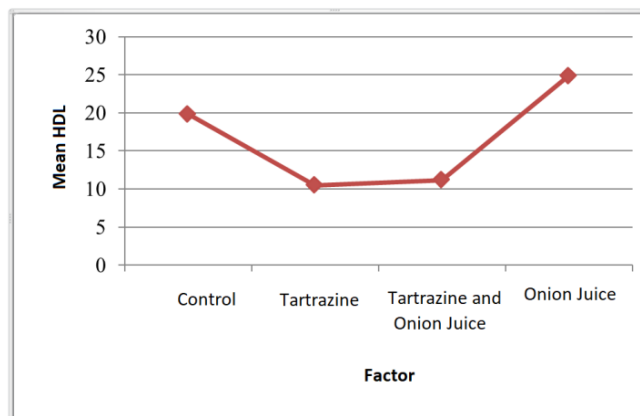


Figure 2. Mean high-density lipoprotein in the four groups (mg/dl)

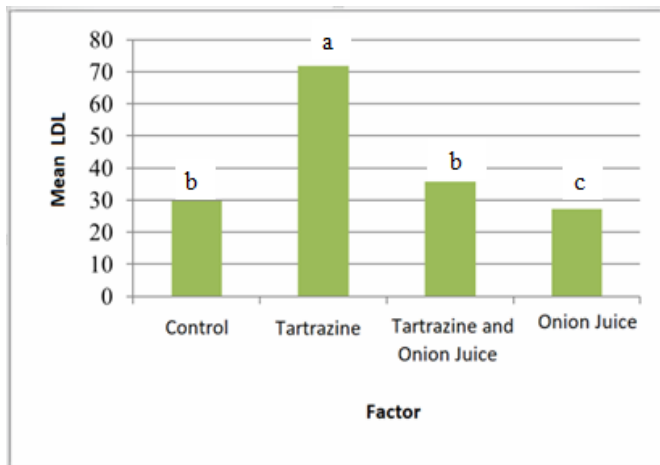


Figure 3. Mean low-density lipoprotein in the four groups (mg/dl)

3.2. The Results of the Effect of Tartrazine and Onion Juice on Blood Low-density Lipoprotein (LDL) Levels

The mean value of LDL was 29.8 ± 0.498 in the control group, 71.8 ± 0.298 in the tartrazine group, 35.7 ± 0.581 in the tartrazine and onion juice group, and 27.1 ± 0.596 in the onion juice group. In the statistical analysis, the *P*-value was less than 0.05, showing that the amount of LDL in all four groups has a significant difference between groups. Therefore, it can be concluded that the consumption of tartrazine and onion juice significantly affect the level of LDL (Figures 3 and 4).

3.3. The Results of the Effect of Tartrazine and Onion Juice on the Amount of Superoxide Dismutase (SOD)

The average value of SOD was 1.87 ± 0.1985 in the

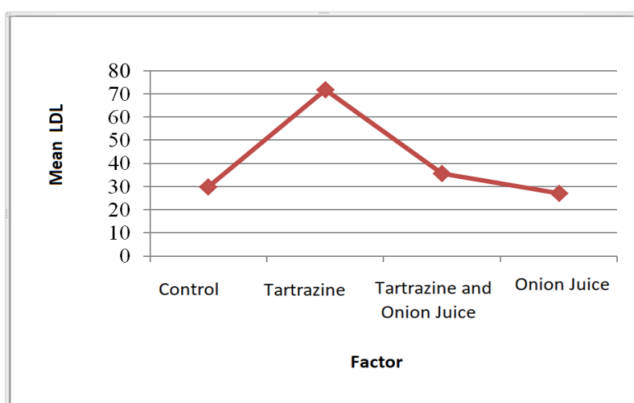


Figure 4. Mean low-density lipoprotein in the four groups (mg/dl)

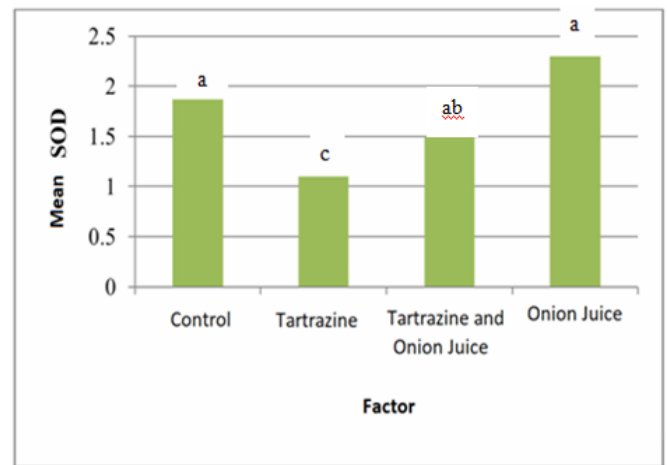


Figure 5. Mean value of superoxide dismutase in the four groups (proteinV/mg)

control, 1.1 ± 0.1421 in the tartrazine group, 1.5 ± 0.1281 in the tartrazine and onion juice group, and 2.3 ± 0.1594 in the onion juice group. In the statistical analysis, the *P*-value for the difference between the control group and the mixture of tartrazine and onion juice was more than 0.05 and in the rest less than 0.05, and this shows that the difference in the mean SOD in the control group and the mixture of tartrazine and onion juice is not significant. The amount of SOD in the remaining groups had significant differences from each other. Therefore, it can be concluded that the consumption of tartrazine and onion juice does not affect the level of SOD. The reason can be the inverse effect of tartrazine and onion juice since onion juice has an increasing effect and tartrazine has a decreasing effect (Figures 5 and 6).

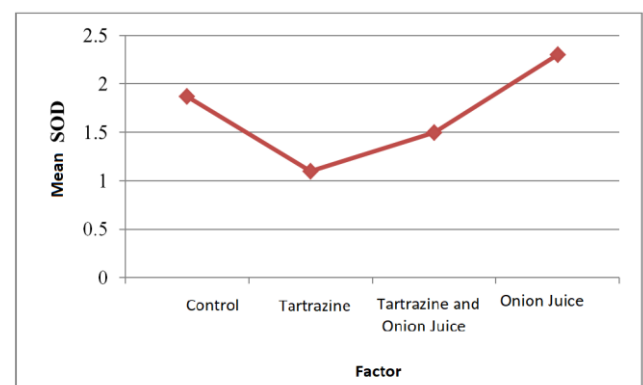


Figure 6. Mean value of superoxide dismutase in the four groups (V/mg protein)

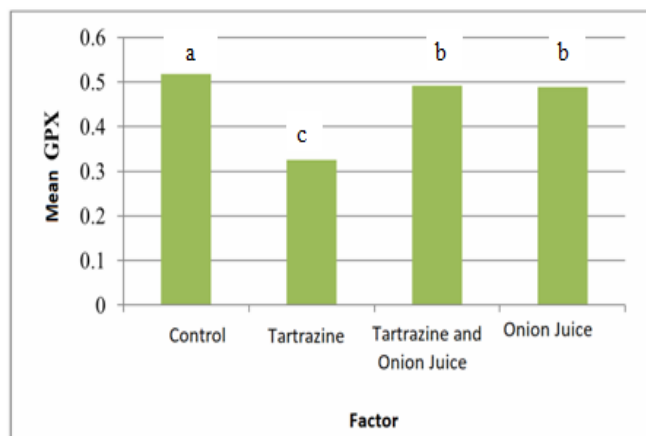


Figure 7. Mean glutathione peroxidase in the four groups (proteinV/mg)

3.4. The Results of the Effect of Tartrazine and Onion Juice on the Amount of Glutathione Peroxidase GPx

The mean value of GPx was 0.518 ± 0.0017 in the control group, 0.326 ± 0.0618 in the tartrazine group, 0.491 ± 0.0096 in the tartrazine and onion juice group, and 0.489 ± 0.0026 in the onion juice group. In the statistical analysis, the *P*-value in all groups had a significant difference at the 0.05 level with the control. The results showed that consuming tartrazine and onion juice together removes the effect of tartrazine on GPx; on the other hand, tartrazine significantly reduces GPx. Moreover, onion juice also reduces the amount of GPx (Figures 7 and 8).

3.5. The Results of the Effect of Tartrazine and Onion Juice on the Amount of Catalase (CAT)

The mean value of CAT was 67.9 ± 0.481 in the

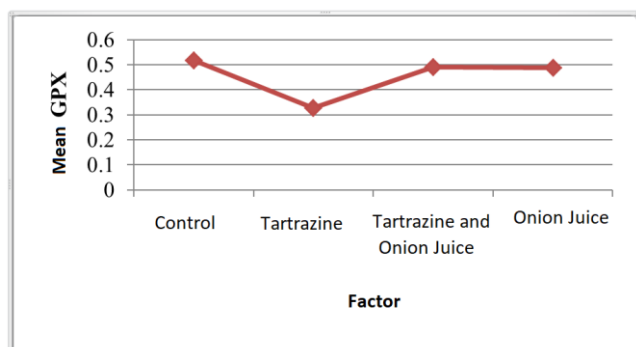


Figure 8. Mean glutathione peroxidase in the four groups (V/mg protein)

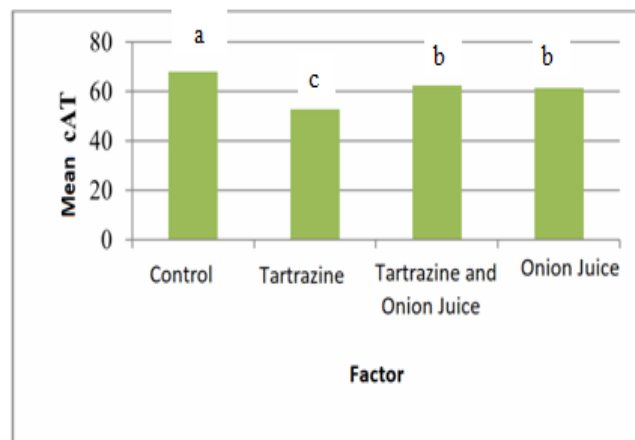


Figure 9. Mean Catalase in the four groups (proteinV/mg)

control, 52.6 ± 2.1468 in the tartrazine group, 62.4 ± 0.318 in the tartrazine and onion juice group, and 61.2 ± 0.318 in the onion juice group. In the statistical analysis, the *P*-value of all groups had a significant difference at the 0.05 level with the control. Therefore, it can be concluded that the consumption of tartrazine and onion juice together leads to the disappearance of the effect of tartrazine on CAT. On the other hand, tartrazine has a significant reducing effect on the amount of CAT. Onion juice also reduces the amount of CAT (Figures 9 and 10).

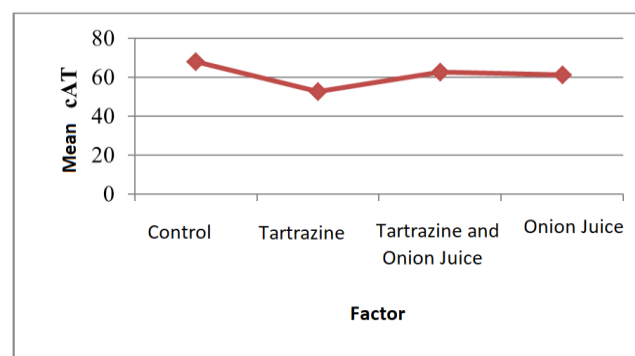


Figure 10. Mean Catalase in the four groups (V/mg protein)

4. Discussion

In recent decades, scientists have conducted numerous studies on free radicals. Their extensive effect on biological systems led to many research works being done on this subject. These mechanisms may be important in the pathogenesis of diseases and

aging. Many reports confirm that the use of antioxidant supplements is effective in reducing the level of oxidative stress and slowing down or preventing the development of the side effects caused by diseases. Many synthetic antioxidant compounds have toxic or mutagenic effects, and this has attracted attention to natural antioxidants. Many plants are active in scavenging free radicals. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (5).

The flavonoids in onions are present in the form of glycosides of quercetin and kaempferol, and they show antioxidant activity (6). These compounds give high medicinal value to onion. High antioxidant activity for onions is reported in research (7).

One of the chemicals that induce free radicals, and unfortunately, its consumption has increased among people, is tartrazine food color. Tartrazine is a synthetic food coloring additive and is soluble in water. Children are often attracted to colored foods, especially sweets, and drinks, and they are among the main consumers of colored foods. In a developing country like India, the high use of food colors resulted in serious health risks among its people (8).

Some behavioral changes in children from using tartrazine, such as irritability, restlessness, and sleeping disorder, have been reported. Tartrazine causes changes in kidney and liver biochemical profiles. Tartrazine and other synthetic food colors have been reported to cause asthma and hives in sensitive people (6).

One of our goals in this research was to investigate the effects of tartrazine on changes in biochemical factors and blood antioxidants. After the experiment, we concluded that the consumption of tartrazine decreases the amount of HDL, blood antioxidants, SOD, CAT, and GPx. Tartrazine also increases LDL (9).

Quercetin is a bioactive compound of plant origin that has antioxidant properties found in onions, tea, and other plants with its polyphenolic chemical structure (10). The researchers reported that quercetin showed cell and tissue protective properties against

oxidative stress and reactive oxygen radicals in diabetic rats (11). As an important flavonoid and a strong antioxidant, this compound removes free radicals and reduces oxidative stress (12). Another research has shown that quercetin, as a polyphenolic antioxidant, increases the activity of antioxidant enzymes, including catalase enzyme (13). They stated that quercetin found in plants has a protective effect on cells under oxidative stress, and by giving electrons to free radicals, they can reduce the destruction of cellular DNA.

In a study, it was shown that tartrazine reduces antioxidant enzyme activity and stimulates the production of free radicals (14). In another study, it was shown that the consumption of onion extract reduces oxidative stress, prevents the action of carcinogens, and supports the body's antioxidant defense system (15).

In another study, it was shown that the amount of serum antioxidants increased, and the toxic effects of tartrazine decreased (16).

In a study, it was revealed that tartrazine dye causes a decrease in blood parameters such as hemoglobin, hematocrit percentage, Total erythrocyte count (TEC) and total leukocyte count (TLC), blood glucose level, and a significant increase in the number of lymphocytes, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and triglyceride, alkaline phosphatase and total cholesterol levels (17).

One of the objectives of this research was to evaluate the effect of onion juice on blood biochemical factors and blood antioxidants after consuming tartrazine. Based on the findings of the present research, the combined use of tartrazine and onion juice does not affect the amount of SOD. Consuming tartrazine and onion juice together causes the loss of the adverse effect of tartrazine on GPx and TAC levels.

The obtained results showed that the consumption of tartrazine with the production of free radicals significantly decreases the activity of antioxidant enzymes and blood biochemical factors. Onion, which

is used as an antioxidant in this study, reduces the harmful effects of tartrazine on the activity of antioxidant enzymes and blood biochemical factors.

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Authors' Contribution

Study concept and design: A. Z. and A. S.

Analysis and interpretation of data: M. P.

Revision of the manuscript: A. Z., A. S. and M. P.

Ethics

All the animals in this research were euthanized according to animal protection by the Animal Ethic Committee of the Islamic Azad University, Rasht, Iran.

Conflict of Interest

The authors declare no conflict of interest.

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