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ORIGINAL ARTICLE

Study of The Toxic Effect of Tartrazine Dye on Some Biochemical Parameters in Male Albino Rats

Alaa Masikh Zebalah Al-Daamy [1] and **Naseer Marza Hamza Al-Zubiady** [2]

[1,2] Biology Department, College of Education for Pure Science, University of Kerbala, Kerbala, Iraq

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Corresponding author:
Alaa Masikh Zebalah Al-Daamy
Email: alaa78@karbalaedu.org
Biology Department
College of Education for Pure Science
University of Kerbala
Kerbala
Iraq

ABSTRACT

Objectives: The present study aimed to demonstrate the toxic effects of tartrazine pigment on some biochemical parameters related to liver and kidney functions.

Methods: The experiment was conducted on male rats aged 10-15 weeks with a weight of 190-250g. The experimental animals were subjected to suitable laboratory conditions at a temperature of 20-25 degrees Celsius. The feed and water were given freely during the period of the experiment. The animals were divided into a negative control group that included nine Animals that were dosed with a physiological salt solution throughout the trial period. The positive control group included nine animals who were dosed orally with tartrazine dye at a concentration of 400 mg / kg / bw for a period of 30 days.

Results: The results of the study showed that dosing animals with tartrazine at a concentration of (400mg / kg / bw) for 30 days led to a significant increase ($P < 0.05$) in the levels of liver enzymes in the blood serum compared to the control group,This was accompanied by a marked decrease in the concentration of blood electrolytes due to the effect of accumulation of products the oxidative stress resulting from the effect of the dye on the activity of the enzyme Na + / K + ATPase, which plays an important role in regulating the concentrations of electrolytes inside and outside the cell, Noting that there has been a marked increase in the biochemical parameters, which include urea, creatinine and uric acid, which are considered among the basic criteria for determining the rate of glomerular filtration and tube excretion of the kidneys.

Conclusion: The results of the current study showed that treating male rats with tartrazine dye (400mg / kg / bw) for a period of 30 days led to significant changes in the biochemical parameters studied when compared with the control group.

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INTRODUCTION

Epstein-Barr Tartrazine dye is one of the chemically manufactured dyes, which is in the form of a powder that is added to food to give it a yellowish orange color that dissolves in water, contributing to giving color to food,

especially fruit juices, colored drinks, prepared foods, cake mixes, custard powder, soups, canned sauces, pickles, ice cream, sweets, jam, yogurt, honey products, butter and cheeses [1]. It may be found in forbidden medicines such as ecstasy pills or happiness [2].

The substance (Tartrazine) is symbolized by the symbol (E102), as the letter E denotes the permission to use the additive in all countries of the European Union and indicates its safety and added to the agreed concentration that does not cause negative effects, as the World Health Organization has determined the permissible dose from 0 to 7.5 mg per A kilogram of body weight, as for the number 102, refers to the same substance, the brand name for tartrazine is Tartrazine, and its chemical name is Tri-sodium 5-hydroxy-1 (4-sulfonato phenylazo) pyrazole-3-carbaxylate, while its chemical formula is C16H9N4NA3O9S2 Its molecular weight is 534.37 [3,4].

Numerous studies have proven that consuming this substance for long periods or using it in a dose higher than the permissible dose leads to many damages, including bronchial sensitivity, asthma, rashes, tearing of the eyes, nasal drainage, blurry vision, as well as hepatitis C, impaired liver and kidney function, and may be carcinogenic in some cases. As it has been shown to stimulate the emergence of thyroid cancer, and the toxic effect of tertrazine on the liver and kidneys is through the formation of free radicals, noting that its effect is greater on children than in adults because their hepatic enzymes are secreted in low concentrations, which causes them to accumulate in the blood and affect the rest of the organs and this is due To that these materials need to be highly efficient in liver function, especially when accumulated in large quantities [5,6].

Several studies have indicated the effect of food additives, including tartrazine, on liver and kidney function and their histological structure, as the use of high doses of colorants leads to an increase in the enzymatic activity of liver enzymes, as the level of the enzyme (AST, ALT) increased. ALP is the result of high permeability and weakness of hepatocyte membranes, as well as a high concentration of (AST) in mitochondria as a result of damage to cells in the mitochondrial membrane [7,8].

MATERIALS AND METHODS

Experimental animals: The experiment was conducted on male rats 10-15 weeks old and weighing 190-250 g. The experimental animals were subjected to suitable laboratory conditions at a temperature of 20-25 degrees Celsius. The feed and water were given freely during the experiment period. The animals were divided into the following groups:

The negative control group included nine animals who were dosed with a physiological salt solution throughout the experiment period, while the positive control group included nine animals who were dosed orally with tartrazine dye at a concentration of 400 mg / kg / bw (mg / kg / bw) at one milliliter of solution per 100 grams of body weight for a period of 30 days after the expiration of the trial period, the blood was drawn from the heart directly heart puncture, after anesthetizing the animal with chloroform using a sterile medical syringe of 5 ml capacity, then the blood was placed in plastic tubes (gel-tube) and the serum was separated from it using a quickly centrifuge (5000 RPM for a period of (5min), and then the serum was withdrawn using a micropipette and placed in an Ibendrove tube for the purpose of conducting biochemical tests in the serum. The serum was kept at -20 ° C until use.

In order to study the effect of tartrazine staining on biochemical parameters related to kidney function, the following was done:

Measuring the level of urea in the serum: The level of urea in the serum was measured according to the method used by [9]. The basic principle: It depends on the hydrolysis of urea in the presence of the enzyme Urease, according to the following equation:

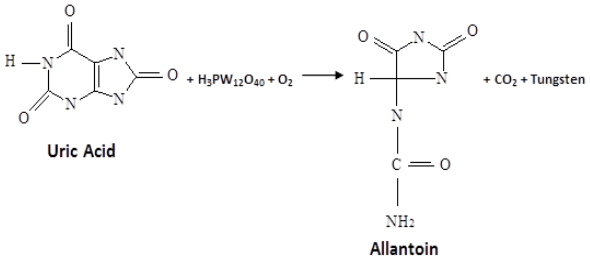
$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

Measurement of serum creatinine level: The level of serum creatinine was measured using the method [10].

The basic principle: It depends on the reaction of creatinine in a basic medium with Picric acid to be a complex red color according to the following equation :

$$\text{Creatinine} + \text{Picric acid} \xrightarrow{PH>12} \text{Red complex}$$

Determination of uric acid in serum: the basic principle - uric acid was measured using the phosphotungstic acid method. As for uric acid, it is oxidized to a colorless, soluble compound known as allantoin and CO2 [11].



Measuring the level of calcium ions in the serum: The level of calcium ions in the serum was measured using the method [10]. The basic principle: The measurement of calcium ions in the serum is based on the formation of the color complex between calcium ions and (Cresolphtalein - O) in a basic medium according to the following equation :

$$Ca^{+2} + O - \text{Cresolphthalein} \xrightarrow{OH^+} red\ complex$$

Measuring the level of potassium ions in the serum: The level of potassium ions in the serum was measured using the method [10]. The basic principle: the free potassium ion reacts in the basic medium with sodium tetraphenylboron to produce a turbid suspension of Potassium tetraphenylboron. This resulting turbidity is

used as a measure of the potassium concentration when photometric.

Measuring the level of sodium ions in the serum: The level of sodium ions in the serum was measured using the method followed by [12]. Basic principle: Sodium precipitates with Mg-uranyl acetate. The uranyl ion with Thioglycolic acid forms a yellow-brown complex.

To study the effect of tartrazine stain on biochemical parameters related to liver function:

Estimation of the effectiveness of the enzymes transporting the amine (AST and ALT): Used color method by [13], was used to estimate the activity of the enzymes transporting the amine, AST and ALT, analytical Equipment were used from the Italian company Giesse.

The effectiveness of the enzyme ALP: was estimated using an enzymatic method through ready-made packages of the type Biomerieuxsa 69280 IE toile-France based on the method used by [14], a color method based on the use of the substrate on which the base phosphatase enzyme works (Alkaline Phosphatase).

RESULTS

The results of the current study showed a clear significant effect of treatment with tartrazine pigment with a concentration of 400 mg/kg at the level of (P≤0.05) on the concentration of blood urea in the serum, as its concentration increased to 45.66 mg/dl in the toxic group after It was equal to 33.16 mg/dl in the control group (Figure 1).

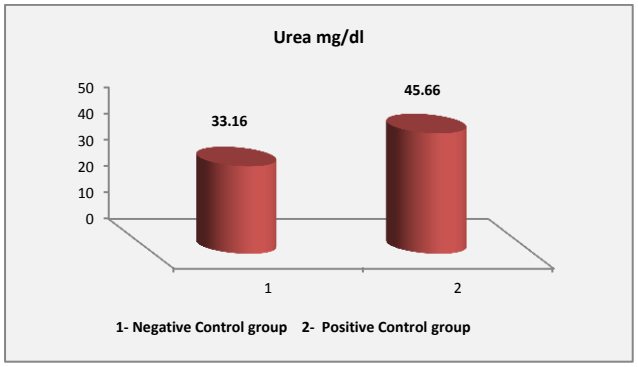


Figure 1. **The effect of toxic substance treatment (400mg/kg) on Urea concentration compared with the control group.**

The results of the present study showed a clear significant effect of treatment with tartrazine pigment with a concentration of 400 mg/kg at the level of P≤0.05 on the concentration of serum creatinine, as its concentration increased to 13.40 mg/dl in the toxic group after it was equal to 11.00 mg/dl in the control group (Figure 2).

The results of the current study showed a clear significant effect of treatment with tartrazine pigment with a concentration of 400 mg/kg at the level of P≤0.05 on the concentration of uric acid in the blood serum, as its concentration increased to 71.34 mg/dl in the toxic group after it was equal to 49.52 mg/dl in the control group (Figure 3).

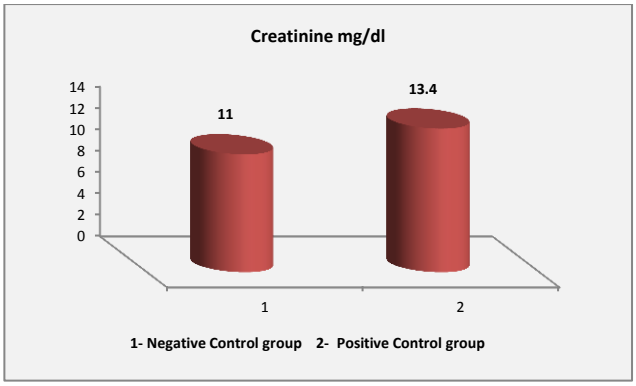


Figure 2. **Effect of toxic substance treatment (400mg/kg) on concentration Creatinine compared to control group.**

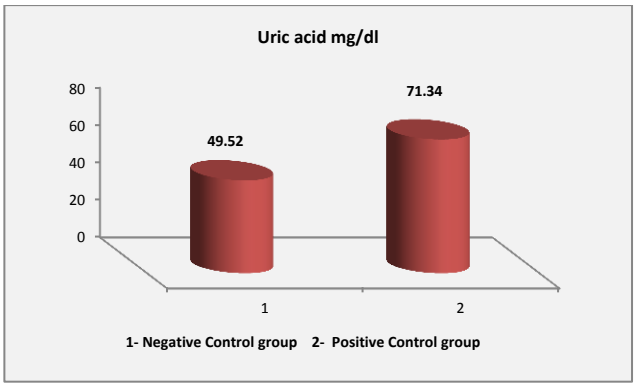


Figure 3. **The effect of toxic substance treatment (400mg/kg) on the concentration of Uric acid compared to the control group.**

The results of the current study showed a clear significant effect of treatment with tartrazine pigment at a concentration of 400 mg/kg at the level of (P≤0.05) over the concentration of Ca in blood serum, as its concentration decreased to 8.18 mg/dl in the toxic group after it was It was equal to 11.76 mg/dl in the control group (Figure 4).

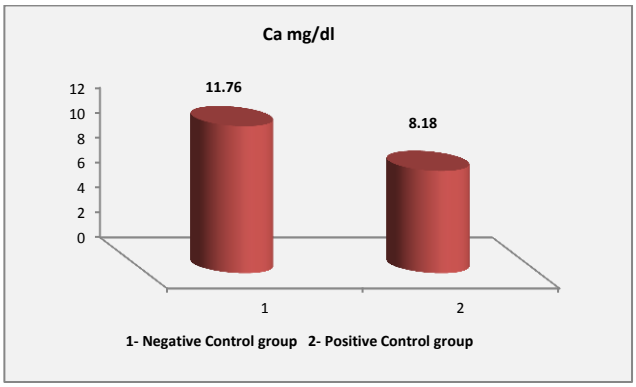


Figure 4. **Effect of toxic substance treatment 400mg/kg on Ca concentration compared with control group.**

The results of the current study showed a clear significant effect of treatment with tartrazine pigment with a concentration of 400 mg/kg at the level of (P≤0.05) on the concentration of potassium K in blood serum, as its concentration decreased to 536.20 mmol/L in the toxic group after it was equal to 634.40 mmol/L in the control group (Figure 5).

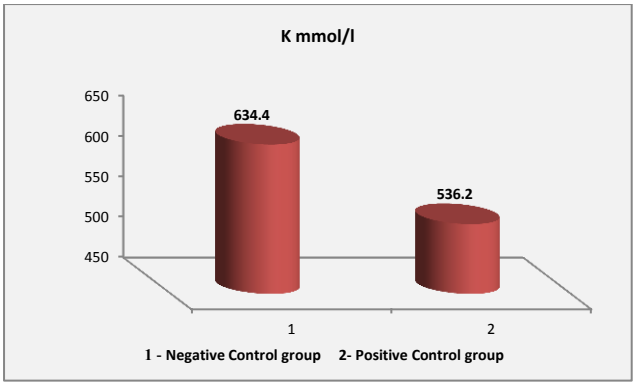


Figure 5. **Effect of toxicity treatment 400 mg/kg on concentration K compared to control group.**

The results of the current study showed a clear significant effect of treatment with tartrazine pigment at a concentration 400 mg/kg at the level of (P≤0.05) on the concentration of Na in blood serum, as its concentration decreased to 121.40 mmol/L in the toxic group after It was equal to 139.02 mmol/L in the control group (Figure 6).

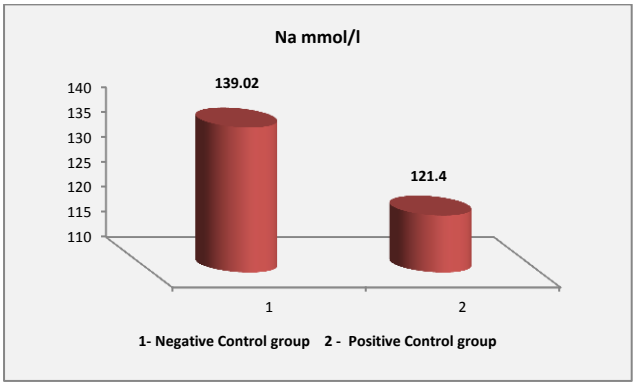


Figure 6. **Effect of toxicity treatment 400 mg/kg on concentration Na compared to control group.**

The results of the current study showed a clear significant effect of treatment with tartrazine stain with a concentration of 400 mg/kg at the level of (P≤0.05) on the concentration of the enzyme (AST) in blood plasma, as its concentration increased to 375.34 U/L in the toxic group after It was equal to 358.80 U/L in the control group (Figure 7).

The results of the current study showed a clear significant effect of treatment with tartrazine pigment with a concentration of 400 mg/kg at the level of (P≤0.05) on the concentration of the (ALT) enzyme in the blood plasma, as its concentration increased to 469.96 U/L in the toxic group after It was equal to 339.54 U/L in the control group (Figure 8).

The results of the current study showed a clear significant effect of treatment with tartrazine stain with a concentration of 400 mg/kg at the level of (P≤0.05) on the concentration of the (ALP) enzyme in the blood plasma, as its concentration increased to 712.78 U/L in the toxic group after It was equal to 633.54 U/L in the control group (Figure 9).

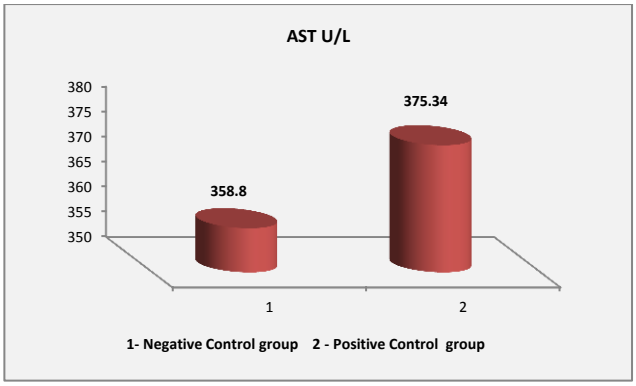


Figure 7. **Effect of toxicity treatment 400 mg/kg on enzyme concentration (ALP) compared to control group.**

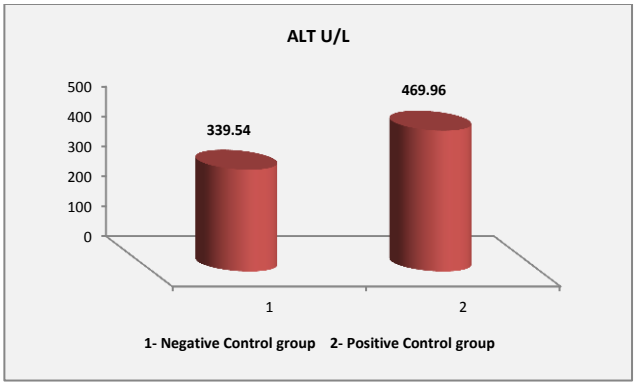


Figure 8. **Effect of toxicity treatment 400 mg/kg on enzyme concentration (ALT) compared to control group.**

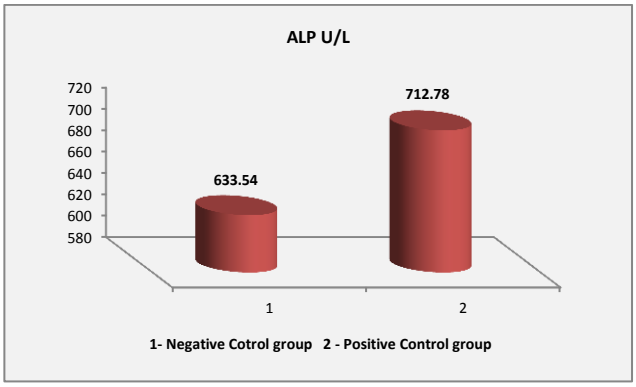


Figure 9. **Effect of toxicity treatment 400 mg/kg on enzyme concentration (ALP) compared to control group.**

DISCUSSION

The results of the study showed that the treatment of male rats with tartrazine pigment 400 mg/kg for 30 days led to a significant increase at P <0.05 in the levels of urea, creatinine and uric acid in the blood serum compared to the control group. Measuring the level of urea is an indicator to confirm the extent of the efficiency of the functional performance of both the liver and the kidneys, and the high level of creatinine in the blood leads to an evidence of acute renal failure, and the high concentration of urea and creatinine is one of the most determining factors for the rate of glomerular filtration and tubular excretion of the kidneys, and this is evidence of the harmful effect of tartrazine pigment on kidney tissues, In conformity with what he found [15] in

his study to find out the effect of some food additives, including tartrazine, on the kidney tissue of rats, this study also came in agreement with a study [15], and the reason for this increase in the concentration of urea and creatinine may be due to impaired kidney function as it affects this dye is also applied to the kidney tissues and thus weakens its ability to filter bodily fluids and nitrogenous wastes [16,17]. The researchers [18] also indicated in their study that the high concentration Creatinine in blood serum due to treatment with tartrazine dye may be due to the interference of tartrazine pigment with the metabolism of creatinine causing an increase in its concentration or through loss of renal tissue a more important part of the functional capacity of tubular excretion, and the high level of urea in the case of oxidative stress can be explained by the loss of the direct source of energy and the animal's refuge to the exploitation of proteins as an alternative source of energy, which results in the formation of large quantities of urea, [19,20] or perhaps the reason is the increase in the concentration of free radicals in the body, which raises the state of oxidative stress and leads to the oxidation of proteins and amino acids. This process results in an increase in the concentration of urea in the blood serum. As a secondary product [21], and this is what the current study showed.

As for uric acid, it is considered one of the final products of protein metabolites that increase due to the oxidative stress caused by the tartrazine pigment, as it is the final product of the purine metabolism and through it the amino groups (alpha) are excreted outside the body. Uric acid can also be considered the main catabolism product of protein as it is formed in the body as a result of the breakdown of normal cells formed in response to eating various foods, especially substances that cause oxidative stress and is usually dissolved in the blood, which facilitates its access to the kidneys, to be excreted out of the body through urine, and the level of this acid increases in the body when the body is unable to get rid of it, especially in the case of kidney failure, and this is identical to what was found in this study of its high concentration associated with kidney tissue damage due to the tartrazine pigment, which has been confirmed by many studies [17,22].

The results indicated that treatment with tartrazine dye caused a significant decrease at level P <0.05 in the levels of sodium, potassium and calcium in the blood serum of adult male rats compared to control groups. The results of the current study are in agreement with many studies that used tartrazine pigment in the study [23,24].

The disturbance in the balance of these electrolytes can be attributed to the effect of oxidative stress caused by the dye tartrazine on the activity of Na + / K + ATPase [25], which is an enzyme that plays an important role in regulating electrolyte homeostasis inside and outside the cell. Inhibition of the enzyme Na + / K + ATPase causes disturbance in the balance of these ions, which leads to a change in cellular metabolism, change in cell membrane fluidism, and thus disturbances in cell function.

The generation of active radicals can cause the severe effect of membrane-bound enzymes such as Na + / K + ATPase, Mg + 2ATPase and Ca + 2ATPase [26], and free radicals can exhibit their cytotoxic effects by causing peroxidation of phospholipids in the membrane. Cellular, that lipid peroxidation of biofilms can adversely affect many functionally important parameters such as membrane fluidity, permeability, electrical voltage and control of the transport process through the membrane, which leads to a decrease in membrane fluidity and disruption of the structural organization of cells and their ability to transport and produce energy, and this ultimately leads to a breach of stability the membrane, which in turn affects the activity of the enzyme Na + / K + ATPase, as free radicals can target this enzyme directly [27].

The decrease in the levels of sodium, potassium and calcium ions can be attributed to the effect of tartrazine pigment on the kidney function, as several studies indicated that tartrazine can disrupt the structural organization of the kidneys and thus affect the kidney function, which is reflected in the levels of electrolytes in the blood serum [28].

Liver enzymes are clinically important, as their level of effectiveness depends on the extent of cellular damage that leads to their release into peripheral fluids and then into the blood, including ALT, ALP and AST. The ALT enzyme is mainly produced in the cytoplasm of hepatocytes, so it is the most specialized in detecting liver diseases, and it is also found in other tissues, but in small quantities, such as skeletal muscles, heart, kidneys, pancreas, spleen, and lung, AST enzyme is found in the cytoplasm and mitochondria of hepatocytes. As well as the cells of other organs such as the heart, skeletal muscles, kidneys, and the brain, while the enzyme ALP is present in high concentrations in the bile ducts of the liver and in the bone and the placenta. Their concentration in the blood gives a picture of the extent of their activity in those organs, especially the liver [29].

The results of the present study showed that treatment of adult male rats with Tartrazine led to a significant increase at P <0.05 in the levels of these enzymes in the blood serum compared to the control group.

The results of the current study can be attributed to the fact that these enzymes have leaked in large quantities from liver tissue into body fluids, especially serum, and that this high leakage reflects the extent of damage to body tissues, especially the liver.

Through the results of the study, it was found that there is a significant increase in the activity of liver enzymes (ALT, AST, ALP) in the blood serum of male rats treated with tartrazine pigment 400 mg/kg. This study came in agreement with the study conducted on male rats during which pigment was dosed tartrazine at a concentration 200 mg/kg for a period of 60 days [30], and the reason for this is due to damage and necrosis of hepatocytes, as well as damage to the mitochondrial membranes as a result of the toxic effect of this dye, and

the damage may also include the kidney and heart tissues, causing an increase in the permeability of the membranes of these cells and may result in an increased concentration liver enzymes (ALT, AST) in the blood serum through an increase anabolism or decrease in the metabolism of the amine transporters, and the increase in the concentration of enzymes may occur through the production of free radicals by the tartrazine pigment, where this dye can be converted by the intestinal flora into aromatic amines, which may turn into nitrosamine, releasing free oxygen radicals (ROS) through which the side effect of tartrazine dye occurs [31,32].

As for the increase in the level of the enzyme ALP, it may be due to an increase in the activity of the lysosomes due to the influence of many factors on cell death, or perhaps due to the flow of bile to the outside or inside the hepatocytes, which leads to an increase in the concentration of the enzyme ALP in the serum [33].

REFERENCES

1. Moon J. and Shibamoto T. "Antioxidant Assays for Plant and Food Components." Journal of Agricultural and Food Chemistry. 2009; 57(5):1655-66. DOI: 10.1021/jf803537k.
2. Sharrenbroch, L., Tough A. and Matthews K.H. "Characterisation of Illicit Ecstasy and Diazepam Tablets by Colorant Identification." Analytical Methods. 2018; 10(17):2048–55.
3. Leulescu M., Rotaru A., Pălărie I. *et al.* "Tartrazine: physical, thermal and biophysical properties of the most widely employed synthetic yellow food-colouring azo dye". J Therm Anal Calorim. 134(1): 209–231 (2018). DOI: https://doi.org/10.1007/s10973-018-7663-3.
4. Who, Joint and F.A.O. Expert Consultation. "Diet, Nutrition and the Prevention of Chronic Diseases." World Health Organ Tech Rep Ser. 2003; 916(i–viii).
5. Rovina K., Siddiquee S. and Shaarani S.Md. "A Review of Extraction and Analytical Methods for the Determination of Tartrazine (E 102) in Foodstuffs." Critical Reviews in Analytical Chemistry. 2017; 47(4):309–24. DOI: https://doi.org/10.1080/10408347.2017.1287558.
6. Al-Mashhedy L.A.M. and Fijer A.N. "Acute Toxicity of Food Additives Tartrazine and Carmoisine on White Male Mice." International Journal of PharmTech Research. 2016; 9(4):364–67.
7. Amin K.A., Abdel Hameid H. and Abd Elsttar A.H. "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. 2010; 48(10):2994–99. DOI: 10.1016/j.fct.2010.07.039.
8. Rajagopal S.K., Manickam P., Periyasamy V. and Namasivayam N. "Activity of Cassia Auriculata Leaf Extract in Rats with Alcoholic Liver Injury". The Journal of Nutritional Biochemistry. 2003;14(8):452–58. DOI: https://doi.org/10.1016/S0955-2863(03)00053-6.
9. Patton, C. J. and S. R. Crouh. "Urea Colorimetric Endpoint Determination Urease-Berthelot Reaction." Annal. Chem. 1977; 49:464–69.
10. Wu, Alan H. B. "Tietz Clinical Guide to Laboratory Tests-E-Book". Elsevier Health Sciences. 2006.
11. Pileggi V.J., Giorgio J.D. and Wybenga D.K. "A One-Tube Serum Uric Acid Method Using Phosphotungstic Acid as Protein Precipitant and Color Reagent". Clinica Chimica Acta. 1972; 37:141–49. DOI: https://doi.org/10.1016/0009-8981(72)90425-1.
12. Loeb W.F. and Quimby F.W. "The Clinical Chemistry of Laboratory Animals". 1999.
13. Bergmeyer H.U. and Bernt E. "Colorimetric Assay of Reitman and Frankel." Second edition, Vol.2. Pp. 735–39 in Methods of enzymatic analysis. Elsevier. 1974. https://doi.org/10.1016/B978-0-12-091302-2.50051-7.
14. Engvall, Eva and P. Perlmann. "Enzyme-Linked Immunosorbent Assay (ELISA)." Protides of the Biological Fluids 1971; 553–56.
15. Tawfek N.S., Amin H.M., Abdalla A.A. and Fargali S.H.M. "Adverse Effects of Some Food Additives in Adult Male Albino Rats." Current Science International. 2015; 4(4): 525–37.
16. Arefin S., Hossain M.S., Neshe S.A., Rashid M.M.O., Amin M.T. and Hussain M.S. "Tartrazine Induced Changes in Physiological and Biochemical Parameters in Swiss Albino Mice, Mus Musculus." Marmara Pharmaceutical Journal. 2017; 21(3):564–69.
17. Latifa K., Essawy A., Sorour J. and Soffar A. "Tartrazine Induces Structural and Functional Aberrations and Genotoxic Effects in Vivo." Peer J. 2017; 5:e3041. DOI: 10.7717/peerj.3041.
18. Yousef M.I., El-Nassag D.E., Gasser M.H. and Ibrahim A.F.M. "Potential Protective Effects of Propolis against Hepatotoxicity and Nephrotoxicity Induced by Monosodium Glutamate in Rabbits." Alexandria Science Exchange Journal. 2019; 40(1):30–42. DOI: 10.21608/ASEJAIQJSAE.2019.26639.
19. Habib Y., Farrokhi F.R., Rezaee R. and Sahebkar A. "Oxidative Stress Induces Renal Failure: A Review of Possible Molecular Pathways." Journal of Cellular Biochemistry. 2018; 119(4):2990–98. DOI: 10.1002/jcb.26450.
20. Oliva L., Alemany M., Remesar X. and Fernández-López J.A. "The Food Energy/Protein Ratio Regulates the Rat Urea Cycle but Not Total Nitrogen Losses." Nutrients. 2019; 11(2):316. DOI: 10.3390/nu11020316.
21. Amiri M. "Oxidative Stress and Free Radicals in Liver and Kidney Diseases; an Updated Short-Review." Journal of Nephropathology. 2018; 7(3): 127-131. DOI: 10.15171/jnp.2018.30.
22. El-Desoky, G.E., Abdel-Ghaffar A., Al-Othman Z.A., Habila M.A., Al-Sheikh Y.A., Ghneim H.K., Giesy J.P. and Aboul-Soud M.A.M. "Curcumin Protects against Tartrazine-Mediated Oxidative Stress and Hepatotoxicity in Male Rats." European Review for Medical and Pharmacological Sciences. 2017; 21(3): 635–45.
23. El Rabey H.A., Al-Seeni M.N., Al-Sieni A.I., Al-Hamed A.M., Zamzami M.A. and Almutairi F.M. "Honey Attenuates the Toxic Effects of the Low Dose of Tartrazine in Male Rats." Journal of Food Biochemistry. 2019; 43(4): e12780. DOI: https://doi.org/10.1111/jfbc.12780.
24. Abd-Elhakim Y.M., Moustafa G.G., Hashem M.M., Ali H.A., Abo-EL-Sooud K. and El-Metwally A.E. "Influence of the Long-Term Exposure to Tartrazine and Chlorophyll on the Fibrogenic Signalling Pathway in Liver and Kidney of Rats: The Expression Patterns of Collagen 1-A, TGFβ-1, Fibronectin, and Caspase-3 Genes." Environmental Science and Pollution Research. 2019; 26(12):12368–78. DOI: 10.1007/s11356-019-04734-w.
25. Choudhary, A.K. and Rathinasamy S.D. "Aspartame Induces Alteration in Electrolytes Homeostasis of Immune Organs in Wistar Albino Rats." Biomedicine & Preventive Nutrition. 2014; 4(2):181–87. DOI: https://doi.org/10.1016/j.bionut.2013.12.006.

26. Sabour A.N. and Ibrahim I.R. “Effect of Sodium Benzoate on Corticosterone Hormone Level, Oxidative Stress Indicators and Electrolytes in Immature Male Rats.” Sci. J. Med. Res. 2019; 3(11): 101–6. DOI: 10.37623/SJMR.2019.31104.
27. Krata N., Zagożdżon R., Foroncewicz B. and Mucha K. “Oxidative Stress in Kidney Diseases: The Cause or the Consequence?” Archivum Immunologiaeet Therapiae Experimentalis. 2018; 66(3):211–20. DOI: 10.1007/s00005-017-0496-0.
28. Al-Seeni M.N., El Rabey H.A., Al-Hamed A.M. and Zamazami M.A. “Nigella Sativa Oil Protects against Tartrazine Toxicity in Male Rats.” Toxicology Reports. 2018; 5:146–55. DOI: https://doi.org/10.1016/j.toxrep.2017.12.022.
29. Mazahreh T.S., Aleshawi A.J., Al-Zoubi N.A., Altabari M. and Aljarrah Q. “Comparison of Postoperative Liver Function between Different Dissection Techniques during Laparoscopic Cholecystectomy.” Future Science OA. 2020; 6(4):FSO462. DOI: https://doi.org/10.2144/fsoa-2019-0160.
30. Abdelgayed,S.S., Ali A.F., Osama S. Eltawil O.S. and Bakeer A.M. “Toxicological and Histopathological Studies on the Effect of Tartrazine in Male Albino Rats". 2016; 10(8):.527-532. DOI: 10.13140/RG.2.2.31938.58568.
31. Pavithra, K.G., kumar P.S., Jikumar V. and Rajan P.S. “Removal of Colorants from Wastewater: A Review on Sources and Treatment Strategies.” Journal of Industrial and Engineering Chemistry. 2019; 75:1–19. DOI: https://doi.org/10.1016/j.jiec.2019.02.011.
32. Balta I., Sevastre B., Mireşan V., Taulescu M., Raducu C., Longodor A.L., Marchiş Z., Mariş C.S. and Coroian A. “Protective Effect of Blackthorn Fruits (Prunus Spinosa) against Tartrazine Toxicity Development in Albino Wistar Rats.” BMC Chemistry. 2019; 13(1):1–11. DOI: 10.1186/s13065-019-0610-y.
33. Hamad Z.M. “Protective Effect Ethanol Extract of Nigella Sativa. L on Hepatic Damage Induced by Naphthalene in Male Rats.” Journal of Al-Qadisiyah for Pure Science (Quarterly) . 2012; 12(2):1–8.