ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
March 2022 • Vol.25

Histopathological evaluation of the effect of vitamin E on reversing changes induced by Tartrazine in parotid glands of albino rats

Iman Mostafa Abelmeguid [1], Radwa T Elsharkawy[2], Raneem F. Obeid[2]

Aim: Tartrazine (TZ) is an artificial azo dye made from coal tar. It is used all over the world as food coloring in a variety of foods, medicines, and cosmetics.
was to assess the harmful effects of Tartrazine on the parotid gland and also study the reparative effect of Vitamin E on affected tissues.
Materials and methods: Rats were grouped into Control group and experimental groups; tartrazine treated groups and Vitamin E and tartrazine treated groups. The control group was administered orally with water alone. Each subgroup consisted of 5 rats. All doses were given daily by oral intubation using a gastro pharyngeal tube. Sacrifice was carried out on two intervals: after14 days and after28 days The experimental group was administered orally with tartrazine (7.5 mg/kg, b.wt.). The specimens were collected after the study to be prepared for light microscopic examination.
Results: The findings demonstrated that tartrazine caused severe histological changes in the parotid glands, including acini nuclei, changes in the cytoplasm, and loss of the normal architecture and ducts. Vitamin E treated group was nearly similar to control group and it obviously reversed the damage caused by Tartrazine.
Conclusion: According to our study findings, it's interesting to note that vitamin E lowered the toxicity of Tartrazine and reversed the degeneration produced in the parotid gland tissues.

Keywords: Tartrazine, Parotid gland, Vitamin E

1. Oral Pathology department, Future University in Egypt, Cairo, Egypt.
2. Oral Biology department, Faculty of Oral and Dental Medicine, Future University in Egypt, Cairo, Egypt.
Corresponding author: Iman Mostafa Abelmeguid, email: iman.mostafa@fue.edu.eg

Introduction

The parotid gland is the largest of the major salivary glands, including the submandibular and sublingual glands.[1] It plays an essential function in the oral cavity because it secrets saliva which facilitates speaking, chewing, swallowing, and digesting.[2]

Reactive oxygen species (ROS) are physiological byproducts of cellular biochemical reactions essentials for cellular metabolism. The term describes numerous molecules derived from oxygen which have accepted extra electrons and can oxidize other molecules which by consequence can lead to variable effects on cellular physiology.[3] Oxidative stress is defined as the imbalance between the oxidants and antioxidants, in favor of the oxidants leading to cellular damage. Oxidative damage results when the generation of ROS produced exceeds the cellular capacity to destroy them to protect or repair it. ROS led to alterations in the structure of the membrane protein and changes in enzymatic activity. Early investigations showed that ROS are capable of damaging proteins, lipids, and DNA.[4]

Vitamin E is the major lipid-soluble component in the cellular defense system and is exclusively obtained from the diet. It has numerous important roles within the body because of its antioxidant activity. Oxidation has been linked to numerous possible conditions and diseases, including cancer, ageing, arthritis, cataracts; vitamin E has been shown to be effective against all of these.[5] Vitamin E is found in various foods and oils including nuts, seeds, and vegetable. It is a potent chain-breaking antioxidant that inhibits the production of ROS molecules when fat undergoes oxidation and during the propagation of free radical reactions. It is in the cell and organelle membranes where it can exert its maximum protective effect. It acts as the first line of defense against lipid peroxidation, protecting the cell membranes from free radical attack.[6] Vitamin E also possesses anti-cancer properties. This is probably because of its ability to stimulate the p53 tumor suppressor gene; the downregulation of mutant p53 proteins and his anti-angiogenic effect mediated by the blockage of transforming growth factor alpha.[7]

Dyes in food, drugs, cosmetics are used to maintain color. They are classified as artificial or natural.[8] Customers became aware of the harmful effects of artificial dyes; thus they prefer natural colorants and consequently synthetic dyes have become undesirable.[9]

Tartrazine (E102, TZ) is a synthetic organic azo dye and considered to be a low-cost alternative for curcumin and saffron. It is one of the most widely used artificial food colorant. It is also used pharmaceuticals and cosmetics fields and it is derived from coal tar.[10] Coal tar colors are metabolized by azoreductase and peroxidase to produce superoxide, hydroxyl radicals and hydrogen peroxide.[11] It is a nitrous derivative and is known to cause allergic reactions such as asthma and urticaria. It has been the focus of many studies on mutagenesis, carcinogenesis, and cell viability.[12]

TZ has cytotoxic, genotoxic, and clastogenic effects by binding directly to DNA.[13] This toxicity is achieved by the reductive biotransformation of the azo linkage either directly or indirectly.[14] It can be reduced metabolically in the animal's intestine by the intestinal microbiota, producing these metabolites sulfanilic acid and aminopyrazolone.[15] which consequently can cause oxidative stress and ROS.[16]

The TZ dye was tested in a large number of in vivo studies at various doses.[17] The present study intends to assess TZ's harmful effect on the parotid glands of rats because the literature's evidence on its toxicity is conflicting.

The aim of the present study is to evaluate the effect of TZ's and co-administration of vitamin E on the structure of parotid gland by microscopic examination of hematoxylin and eosin (H&E) stained sections.

Material and Methods

Forty adult male albino rats were used in this study. The research was held at the Medical Research Center, Faculty of Medicine, Ain Shams University, under the supervision of a specialized veterinarian. The rats were housed in mesh wire separate cages. Each cage contained five rats, under precise temperature, humidity, and dark/light cycle. Rats stayed under good ventilation and fed a normalized diet consisting of fresh vegetables, dried bread, and tap water. Sacrificed bodies were discarded by the incinerator of Ain Shams University. The Research Ethics Committee of Ain Shams University accepted and authorized the experimental procedure.

A) Materials:

i) Animals: forty adult male albino rats weighing between 200- 250 grams were used in this experiment.

i) Drugs:

- Tartrazine powder was purchased from SIGMA ALDRICH © and given daily at a dose of 7.5mg/kg in 1ml distilled water.[16]
- Oral supplementation of vitamin E capsules was given daily at a dose of 400IU/kg vitamin dissolved in 1 ml olive oil.[18] Vitamin E capsules were cut open and carefully emptied into a clean container and olive oil added to it.[19]

B) Methods:

i) Experimental Design:

Albino Rats had one week for acclimatization. Then rats were randomly divided into two main groups (table 1):

- Control (group 1) (distilled water/olive oil)
- Experimental (group 2) (TZ +/- vitamin E).

Each group consisted of 20 rats. All doses were given daily by oral intubation using a gastro pharyngeal tube. Sacrifice was carried out on two intervals: after14 days and after 28 days.[20]

Table 1: Study groups

A) Group 1: Control Group	B) Group 2: Experimental Group
a) Group 1 A: Given 1ml distilled water then killed after 14 days.	**a) Group 2 A (T14):** Given TZin1ml distilled water and killed after 14 days.
b) Group 1 B: Given 1ml distilled water then killed after 28 days.	**b) Group 2 B (T28):** Given TZ in1ml distilled water and killed after 28 days.
c) Group 1 C: Given 1ml distilled water plus 1ml olive oil then killed after 14 days.	**c) Group 2 C (TE 14):** Given TZ in 1ml distilled water plus vitamin E in 1 ml olive oil then killed after 14 days.
d) Group 1 D: Given 1ml distilled water plus 1ml olive oil then killed after 28 days.	**d) Group 2 D (TE 28):** Given TZ in 1ml distilled water plus vitamin E in 1 ml olive oil then killed after 28 days.

b) Sample Collection

By the end of the experimental period, the rats were sacrificed by giving them an overdose of anesthesia (chloroform, HPLC Sigma Aldrich), parotid glands were immediately removed, labeled, and fixed in 10% formaldehyde and prepared for staining.

c) Histological Preparation and Evaluation

T he fixed specimens were dissected and fixed in 10% formalin solution then washed properly under running water then dehydrated through ascending concentrations of alcohol 50%, 60%, 80%, 90%, 96% and absolute alcohol at the end. The specimens were transferred to xylol to be cleared from alcohol. Glands were infiltrated in paraffin wax and embedded in the center of paraffin

wax blocks. The embedded specimens were sectioned by the microtome (4-6 microns thick) and deparaffinized in descending concentrations of alcohol 96%, 70% then in distilled water. After all, the sections were stained by H&E stain (Bancroft and Stevens, 1996) and examined by light microscope (Trinocular microscope Olympus, BX46) to detect any structural changes in the parotid tissues.

Results

Histological Evaluation Results:

Group 1A, 1B, 1C and 1D:

The histological examination of the parotid gland after 14 and 28 days showed normal architecture of the gland where lobes and lobules, separated by connective tissue septa, consisted of secretory acini and ducts. The striated ducts were lined by a single layer of columnar cells which showed well-defined outlines and central, rounded darkly stained nuclei. The excretory ducts appeared large with wide lumens and lined by pseudostratified columnar cells. The serous acini form the terminal secretory units of the parotid gland. The acini were nearly spherical with pyramidal cells that made up each acinus.

Group 2A (T14):

The histological examination of group 2A showed distorted cells with varying sizes of cytoplasmic vacuoles and numerous degenerative changes. The cells lining the acini did not show the typical pyramidal outline.

Group 2B (T28):

The histological examination of group 2B (T28) showed striated duct with shorter cells almost flattened in some areas. Nuclei are pushed basally. Cytoplasm appears scant with no basal striations. More degenerative changes in acini and dilated blood capillaries with numerous RBCS are seen (fig.1)

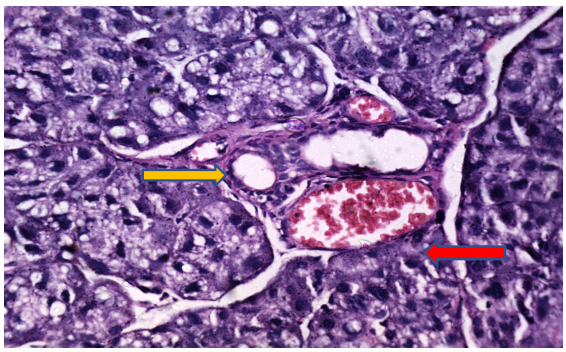

Fig.1. Photomicrograph of parotid gland of group 2B T28 showing striated duct with shorter cells almost flattened in some areas (yellow arrow). More degenerative changes in acini and dilated blood capillaries with numerous RBCS are seen (red arrow) (original magnification x400)

Group 2C (TE14):

The histological examination of the parotid gland after adding Tartrazine in 1ml distilled water and adding Vitamin E in 1 ml olive oil, and sacrificing animals after 14 days, showed almost normal architecture of parotid acini except for periductal fibrosis (fig.2).

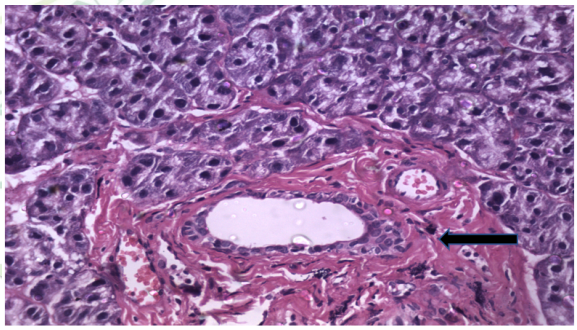

Fig.2: Photomicrograph of excretory duct of parotid gland of group TE14 showing relatively normal lining. Increase in the fibrous C.T surrounding the duct was also observed and almost normal acini (original magnification X400)

Group 2D (TE28):

The histological examination of the parotid gland after adding Tartrazine in 1ml distilled water and adding Vitamin E in 1 ml olive oil then sacrificing animals after 28 days, showed nearly normal architecture of parotid gland, striated ducts regained their basal

striations and excretory with pseudostratifications of its epithelium (Fig.3)

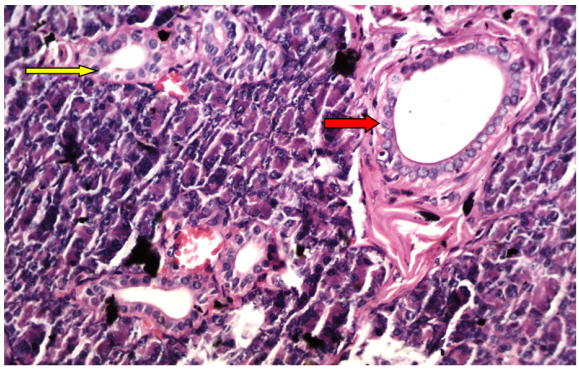

Fig.3: Photomicrograph of group TE28 showing almost normal gland architecture. Striated duct (yellow arrow) regained the basal striations, while the excretory duct (red arrow) with pseudostratified columnar epithelium (original magnification x 400)

Discussion

Previous studies have shown that the widespread use of TZ in food causes cellular damage as well as increased ROS generation.[21] TZ poisoning has been linked to various tissues, including the liver, kidneys, and brain.[22] Salivary glands and lingual mucosa have both been related to oral tissue damage after TZ.[23]

In the current study, the histological examination of the control group showed a normal glandular architecture.
Groups exposed to TZ for 14 days then sacrificed showed distorted cells with varying sizes of cytoplasmic vacuoles, also striated duct showed shortened cells, indistinct cell boundaries and loss of basal striations. Sacrificing rats after 28 days revealed more degenerative changes; cells of striated duct became shorter and almost flattened in some areas with loss of pseudo stratification in excretory duct. These changes could be due to the cumulative effect of TZ.

Our results were hand in hand with Shorkoubally et al. (2020)[24] who studied the changes induced by TZ in submandibular salivary gland of albino rats. Moreover, Mehedie et al. (2013)[25], and Saxena & Sharma (2015)[26] found that TZ changed the histological structures of the kidney and liver in experimental animals.

El khayate et al (2017)[15] found similar results in their study. They noticed that most of the hepatocytes had necrotic nuclei and cytoplasmic vacuolization. Some cells had no nuclei, while others had nuclei in a variety of shapes. Furthermore, the kidney's structure was clearly aberrant when comparing the TZ treated animal group to the controls. Large vacuoles, regions where the integrity of the renal tubules has been compromised, glomerular structural degradation, and other changes were also found. Our findings concurred with those of Al Seeni et al. (2018)[27] and El Raby et al. (2019)[28], who also investigated the effects of TZ on numerous organs. El-Sakhawy et al. (2019)[29] found that groups receiving TZ showed extensive liver and renal damage.

Previous studies found vacuolation, inflammation and liver cell damage due to TZ food colorant in male albino rats. It was also found that TZ increased catalase and Glutathione-S Transferase (GST) activities in spleen and kidneys, but decreased them in the liver of treated rats, it may elicit catalase and GST responses dependent on tissue-specific oxidative-stress status.[30]

Golli et al. (2016)[31] detected that the kidney is the main organ affected by TZ exposure. Rus et al., (2010)[17] reported altered histopathological changes in kidneys of guinea pigs given 1-3% TZ in water for 3 months.

The results of El-Sakhawy et al. (2019)[29] were in line with those of our study and showed that the submandibular gland had nuclei that showed hyperchromatism and abnormal mitosis as well as tiny vacuoles that formed inside the cytoplasm of the acinar cells. According to Abdin (1981)[31], morphologic abnormalities and the destruction of cellular architecture were the

results of hydropic degeneration, which was brought on by a disruption in the metabolism of the cell. According to Henics and Whealthy (1999)[32], the severe vacuolations in mammalian cells lead to cell death, known as "shrinkage necrosis," because the cell anticipates compensating by vacuolating and expanding. This supports our observations, which showed many alterations in acinar cells of the parotid gland. DNA damage and genotoxic effects of TZ are indicated by pleomorphism. The interlobular and intralobular ducts in the submandibular gland functioned similarly to our study's findings in the group given TZ (7.5 mg/kg body weight), according to El-Sakhawy et al. (2019)[29]study, pyknotic nuclei, loss of the striations in the striated ducts, and complete destruction of the duct architecture, together with clear signs of degeneration. According to Elkhayate et al. (2017)[15], white blood cells, as well as dilatation and congestion, were visible in the blood sinusoids. Agreeing with Elsakhawy et al. (2019)[29], the submandibular gland had engorgement with blood as well as congestion of the interacinar blood capillaries and dilated blood vessels present in the connective tissue surrounding the interlobular ducts. Our study's findings on blood vessel dilation confirm Moubarak's (2008)[33] assertion that it might be a result of the inflammatory response to increase the flow of blood to the areas of degeneration. Lipid peroxidation, which TZ induces, can harm the membranes of cell organelle. The cell's structural and functional features alter as a result.[34]

As we just discussed, an imbalance between ROS and antioxidants indicates oxidative stress. It occurs when the capacity of the antioxidants is exceeded by the overall quantity of ROS. Accordingly, oxidative stress may result from an increase in ROS generation, a decrease in antioxidant systems, or both.[35] This damage to proteins, DNA, and cellular organelles eventually results in cell death. Also, when TZ reaches the colon, it can be significantly reduced by the intestinal bacteria and converted by the liver's mammalian azo reductase to free aromatic amines. The interaction of these active amino groups with meals high in nitrate allows these amines to create ROS as part of their metabolism.[12]

Moreover, an earlier study demonstrated that oxidative stress increases nitrous oxide (NO) synthesis in epithelial cells, causing enhanced NO release, increased nitrite generation, and decreased cell viability.[36] The histological alterations in the parotid glands in our work could be explained by any of these mentioned hypotheses.

On the other hand, supplementing with vitamin E for 14 and then 28 days dramatically enhanced the structure of the salivary glands. With no or little cytoplasmic vacuolization observed in the glandular components, both groups treated with vitamin E displayed symptoms of healing that were nearly too close to normal texture. The basal striations of striated ducts returned, and the excretory ducts had cell lining with a pseudostratified columnar epithelium.

The fact that vitamin E is a potent antioxidant that prevents the synthesis of ROS molecules may help to explain these results. Additionally, it has been discovered that vitamin E neutralizes the already-present free radicals while also having the power to prevent the creation of new free radicals.

These earlier findings may help to explain how vitamin E assisted in reducing the harmful effects of TZ, which were mostly brought on by a higher amount of ROS.[37]

Another explanation for vitamin E's function is that it greatly stabilizes the cell by raising the lipid content of cell membranes, which contributes to a more stable membrane. As a result, vitamin E promotes membrane repair by limiting the oxidation of phospholipids,

which could obstruct membrane fusion processes.[38]

Conclusion

According to our study findings, it's interesting to note that vitamin E lowered the toxicity of Tartrazine and reversed the degeneration produced in the parotid gland tissues.

References

1. Chason HM, Downs BW. Anatomy, head and neck, parotid gland. In: StatPearls. StatPearls Publishing; 2021.
2. Ghannam MG, Singh P. Anatomy, head and neck, salivary glands. 2019;
3. Dumanović J, Nepovimova E, Natić M, Kuča K, Jaćević V. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Front Plant Sci. 2021;11:552969.
4. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;2017.
5. Mansour-Gueddes S Ben, Saidana-Naija D. Vitamin E: Natural Antioxidant in the Mediterranean Diet. In: Vitamin E in Health and Disease-Interactions, Diseases and Health Aspects. IntechOpen; 2021.
6. Ebhohimen IE, Okanlawon TS, Osagie AO, Izevbigie ON. Vitamin E in Human Health and Oxidative Stress Related Diseases. In: Vitamin E in Health and Disease-Interactions, Diseases and Health Aspects. IntechOpen; 2021.
7. Alqahtani S, Kaddoumi A. Vitamin E transporters in cancer therapy. AAPS J. 2015;17(2):313–22.
8. Authority EFS. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA J. 2022;20(3).
9. Shakoor S, Ali F, Ismail A, Rahman ZU, Sabran MR, Mohtarrudin N. Toxicity of tartrazine, curcumin and other food colorants; possible mechanism of adverse effects. Online J Vet Res. 2019;23(6):466–508.
10. Silva MM, Reboredo FH, Lidon FC. Food colour additives: A synoptical overview on their chemical properties, applications in food products, and health side effects. Foods. 2022;11(3):379.
11. Cemek M, Büyükokuroğlu ME, Sertkaya F, Alpdağtaş S, Hazini A, Önül A, et al. Effects of food color additives on antioxidant functions and bioelement contents of liver, kidney and brain tissues in rats. J Food Nutr Res. 2014;2(10):686–91.
12. Moutinho ILD, Bertges LC, Assis RVC. Prolonged use of the food dye tartrazine (FD&C yellow n° 5) and its effects on the gastric mucosa of Wistar rats. Brazilian J Biol. 2007;67:141–5.
13. Atlı Şekeroğlu Z, Güneş B, Kontaş Yedier S, Şekeroğlu V, Aydın B. Effects of tartrazine on proliferation and genetic damage in human lymphocytes. Toxicol Mech Methods. 2017;27(5):370–5.
14. Chequer FMD, Dorta DJ, de Oliveira DP. Azo dyes and their metabolites: does the discharge of the azo dye into water bodies represent human and ecological risks. Adv Treat Text Effl. 2011;48:28–48.
15. Khayyat L, Essawy A, Sorour J, Soffar A. Tartrazine induces structural and functional aberrations and genotoxic effects in vivo. PeerJ. 2017;5:e3041.
16. Himri I, Bellahcen S, Souna F, Belmekki F, Aziz M, Bnouham M, et al. A 90-day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats. Group. 2011;300(00).
17. Rus V, Gherman C, Miclăuş V, Mihalca A, Nadăş GC. Comparative toxicity of food dyes on liver and kidney in guinea pigs: A histopathological study. Ann Rom Soc Cell Biol. 2010;15(1):161–5.
18. Noaparast Z, Hosseinimehr SJ. Radioprotective agents for the prevention of side effects induced by radioiodine-131 therapy. Futur Oncol. 2013;9(8):1145–59.
19. Abd El Fadil H, M Mahmoud N, Mohamed Abd El-Mottleb D. Ameliorative Effect Of Vitamin E Aganist Some Adverse Effects Of Levofolxacin In Male Rats. Assiut Vet Med J. 2020;66(165):55–66.
20. Elsharkawy RFOHSART. Histological evaluation of the Antioxidant effect of Vitamin E on reversing the negative impact of tartrazine on extraction socket healing. (Randomized controlled trial). Egypt Dent J [Internet]. 2020 Jan 1;66(1):285–92. Available from: http://dx.doi.org/10.21608/edj.2020.77544
21. Liu Z, Ren Z, Zhang J, Chuang C-C, Kandaswamy E, Zhou T, et al. Role of ROS and Nutritional Antioxidants in Human Diseases. Front Physiol. 2018;9:477.
22. Hosieny NA, Eldemerdash M, Ahmed SM, Zayed M. Toxic effects of food azo dye tartrazine on the brain of young male albino rats: Role of oxidative stress. Zagazig J Forensic Med. 2021;19(1):60–73.
23. Zaki NT, Al Ankily M, Amin RM, Halawa AM. The possible protective role of vitamin e on the induced silver nanoparticles toxicity on filiform and circumvallate tongue papillae of albino rats

histological and immunohistochemical study. 2020;
24.Elshorkoubally EAM, Amin RM, WaelAbou-Zeid A. The Changes Induced by Tartrazine in Submandibular Salivary Gland of Male Albino rats. Ain Shams Dent J. 2020;23.
25. Mehedi N, Mokrane N, Alami O, Ainad-Tabet S, Zaoui C, Kheroua O, et al. A thirteen week ad libitum administration toxicity study of tartrazine in Swiss mice. African J Biotechnol. 2013;12(28).
26.Saxena B, Sharma S. Food color induced hepatotoxicity in Swiss albino rats, Rattus norvegicus. Toxicol Int. 2015;22(1):152.
27. Al-Seeni MN, El Rabey HA, Al-Hamed AM, Zamazami MA. Nigella sativa oil protects against tartrazine toxicity in male rats. Toxicol reports. 2018;5:146–55.
28. El Rabey HA, Al-Seeni MN, Al-Sieni AI, Al-Hamed AM, Zamzami MA, Almutairi FM. Honey attenuates the toxic effects of the low dose of tartrazine in male rats. J Food Biochem. 2019;43(4):e12780.
29. El-Sakhawy MA, Mohamed DW, Ahmed YH. Histological and immunohistochemical evaluation of the effect of tartrazine on the cerebellum, submandibular glands, and kidneys of adult male albino rats. Environ Sci Pollut Res. 2019;26(10):9574–84.
30. El Golli N. Toxicity induced after subchronic administration of the synthetic food dye tartrazine in adult rats, role of oxidative stress. Recent Adv Biol Med. 2016;2(2016):652.
31. Abdin F. Cell and tissue damage. Abdin's Gen Pathol 4th edn p. 1981;7.
32. Henics T, Wheatley DN. Cytoplasmic vacuolation, adaptation and cell death: a view on new perspectives and features. Biol Cell. 1999;91(7):485–98.
33. Moubarak R. The effect of hypercholesterolemia on the rat parotid salivary gland (histopathological and immunohistochemical study). Cairo Dent J. 2008;24(1):19–28.
34. Gajawat S, Sancheti G, Goyal PK. Protection against lead-induced hepatic lesions in Swiss albino mice by ascorbic acid. Pharmacologyonline. 2006;1:140–9.
35. Frances DEA, Ingaramo PI, Ronco MT, Carnovale CE. Diabetes, an inflammatory process: oxidative stress and TNF-alpha involved in hepatic complication. 2013;
36. Pittalà V, Fidilio A, Lazzara F, Platania CBM, Salerno L, Foresti R, et al. Effects of novel nitric oxide-releasing molecules against oxidative stress on retinal pigmented epithelial cells. Oxid Med Cell Longev. 2017;2017.
37. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of vitamin E in human health and some diseases. Sultan Qaboos Univ Med J. 2014;14(2):e157.
38. Howard AC, McNeil AK, McNeil PL. Promotion of plasma membrane repair by vitamin E. Nat Commun. 2011;2(1):1–8.

.