ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
September 2020 • Vol. XXIII

The Changes Induced by Tartrazine in Submandibular Salivary Gland of Male Albino rats

Eman Abdel Moniem Elshorkoubally[1], RehamMagdy Amin[2] and Ahmed WaelAbou-Zeid[3]

Abstract

Tartrazine (TZ) (E 102, FD, and C Yellow) is an artificial azo dye derived from coal tar. It is orange-colored powder known as synthetic lemon yellow. It used worldwide as food additives to color several foods, drugs, and cosmetics.
In the present work 14 male albino rats were housed for 4 weeks and were divided into three groups: group I: the control group: it consisted of 7 rats and were given 1 ml distilled water using gastric intubation, group II: the Tartrazine group: it consisted of 7 rats and were given Tatrazine dose of 7.5mg/kg in 1ml distilled water using gastric intubation. The specimens were collected after the study to be prepared for light microscopic examination. This study was carried to evaluate the effects of Tartrazine on submandibular salivary gland of albino rats using Light microscope.

Results: The results revealed that Tartrazine caused severe histological; alterations in the submandibular salivary gland, the nuclei of the acini showed pleomorphism, also vacoulizations in the cytoplasm were observed and the ducts showed loss of the normal architecture and outline**.**

Conclusion: Tartrazine has severe toxic effects on submandibular salivary glands.

Keywords: Submandibular salivary gland, Tartrazine , histological.

1. Department of Oral Biology, Faculty of Dentistry, El Fayoum University.
2. Department of Oral Biology, Faculty of Dentistry, Ain Shams University.
3. Department of Oral Biology, Basic Dental Sciences, Dental Research Center, National Research Center.

Introduction:

A wide range of food additives used to preserve, dye, or enhance foods **(Toledo 1996, 1999; Hirschbruch and Torres 1998)**. The individual response to these chemicals varies according to dose, age, gender, nutritional status, and genetic factors **(Sandler 1983; Sasaki et al. 2002)**. Tartrazine (TZ) (E 102, FD, and C Yellow) is an artificial azo dye derived from coal tar. It is orange-colored powder known as synthetic lemon yellow **(Elhakim and Heraud 2007)**. It used Worldwide as food additives to color several foods, drugs, and cosmetics **(Mehedi et al. 2009)**. The azo benzene structure of tartrazine features a trisodium salt of 3-carboxy-5-hydroxy-1 (p-sulfophenyl)-4-(sulfophenyl azo) pirazolone. It is reduced in the organism to highly sensitizing aromatic amine because it is a nitrous derivative (azo class). Its main metabolite is sulfanylic acid **(Maekawa et al. 1987; Chung et al. 1992)**. Some of these amines are toxic, carcinogenic, and mutagenic **(Chung 2000; Zhang and Ma 2013)**.

The acceptable daily intake (ADI) for tartrazine is 7.5 mg/kg/day which was established by Joint FAO/WHO Expert Committee on Food Additives **(JECFA. 1964; Toledo 1996; Hirschbruch and Torres 1998; Walton et al. 1999)**. EFSA, (2016) recorded the TZ as authorized food colorant, which used as a feed additive for cats, dogs, ornamental fish, grain-eating ornamental birds, and small rodents.

These azo dyes, such asTZ, have been reported to cause adverse effects in animals, including inflammation of the lining of the stomach, increased lymphocyte, and eosinophil counts when consumed by rats for extended periods **(Moutinho et al. 2007)**. In young mice, TZ has a noticeable effect on behavior **(Tanaka 2006; Tanaka et al. 2008)**. **Tanaka (2006)** found that administration of TZ to mice in the diet had effects that lead to hepatocellular damage and biochemical and reproductive alterations in low and high doses. It also had DNA-damaging effect **(Himri et al. 2011).**

Salivary glands develop at different sites and they have very different architectures and produce different types of saliva **(Jaskoll*et al*, 2002).** The main salivary glands are 3 pairs: parotid, submandibular and sublingual glands **(Kimura *et al*, 1998)**. The salivary glands play an important role in the digestive system.

Thesecretion (saliva) is mostly serous, containing various enzymes, water, mucopolysaccharides and lubricating glycoprotein **(Adnyane*et al*, 2000).** Salivary glands of rodents are important elements regarding their adaptations to different diets, environments and taxonomic studies. To reach a delicate analysis between biology and ecology of rodents, there is need to study salivary glands histology **(YazdaniMoghaddam*et al*, 2009)**.

MATERIALS & METHODS

Experimental animals:

Fourteen adult male albino rats weighing between 150- 200 grams were used in this study. The experimental procedures were approved by ethical committee,faculty of Dentistry, Ain Shams University. The experiment was carried out under the supervision of a specialized veterinarian at the Medical Research Center, Faculty of Medicine, El-Azhar University. Rats were kept under good ventilation and were fed a standardized diet consisting of fresh vegetables, dried bread and tap water. Disposal of sacrificed bodies were done by the incinerator of El-Azhar University.

Drugs:

-Tartrazine powder*was purchased from SIGMA ALDRICH © and given daily at a dose of 7.5mg/kg in 1ml distilled water **(Himri*et al*., 2011)**.

Experimental Design:

The animals were randomly divided equally into 5groups7rats each.

1. **Group I: (Control group):**consisted of 7 rats, each rat received 1 ml distilled water each.
2. **Group II: (Tartrazine group):** consisted of 7rats,each rat

*SIGMA ALDRICH © **(Himri*et al*., 2011)**.

receivedTartrazine in 1 ml of distilled water for 4 weeks.

Sample Collection:

At the end of each group experiment period the rats were sacrificed by extra dose of anathesia. The Submandibular salivary glands were immediately dissected and processed.

Light microscopic examination:

The lobes of the submandibular salivary glands were immediately fixed in 10% neutral buffered formalin solution for 24 hours, then the fixed samples were washed, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 3–4 µm in thickness were obtained by rotatory microtome, deparaffinized, and stained with hematoxylin and eosin (H&E) stain for examination under the light microscope **(Gamble 2008)**.

Results

Histological observation of the submandibular salivary glands from the animals of **Group I (control group)**revealed normal architecture in both the secretory end pieces and the duct system the granuler convoluted tubules and the striated ducts revealed normal and intact epithelial lining (Fig 1).

The submandibular salivary glands of **Group II (TZ group)**revealed many histopathological changes. In the groupII treated with TZ thesubmandibular gland showed small vacuoles that appeared inside the cytoplasm of the acinar cells, and some nuclei showed abnormal mitosis. Furthermore, the intercalated ducts, the convoluted tubules, the striated ducts showed loss in their architecture with obvious degenerative changes; moreover, congestion of the surrounding blood vessels was also clear (Fig.2).

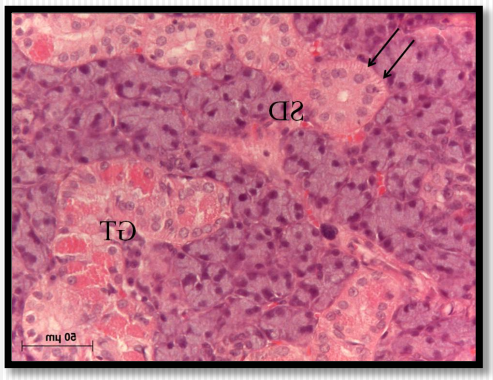

Fig. (1): A photomicrograph of the submandibular salivary gland of Group I showing normal acini, the Granular convoluted tubule (GT) with eosinophilic granules and the striated duct (SD) with the basal striations (black arrow). (H&Ex 400)

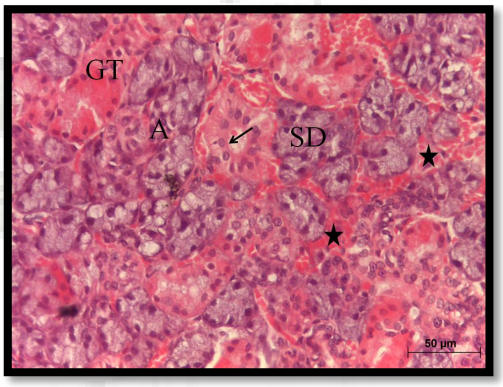

Fig. (2): A photomicrograph of the submandibular salivary gland of Group II showing ill-defined outline of the acini (A). Different sized cytoplasmic vacuoles are encountered (V). Granular convoluted tubule (GT) is showing ill-defined outline, indistinct cell boundaries and the excretory granules appear as whole eosinophilic mass. Striated duct (SD) with many of the lining cells showing loss of the supra nuclear zone (black arrow) and extravasated blood vessels inbetween the acini(asterisk). (H&Ex 400)

Discussion

Saliva and salivary glands are considered the control group revealed strong positive reaction in both strongest link between the oral and systemic health. Acini and ducts that was observed more at their basement Although the submandibular salivary gland is considered membrane. On examining the submandibular salivarythe 2nd largest salivary gland, it produces about 60% of saliva. Saliva has an important role to keep healthy oral cavity **(El-Gusbi*et al*., 2016)**.

The rat was chosen as an experimental model in this research because it can be housed, bred and handled without difficulties. Also, it has a long life span and is relatively disease-free, yet remarkable changes occur when no other diseases are apparent **(Cobo*et al*., 1992)**.In group II The histological examination of the submandibular glands of adult male albino rats exposed to TZ revealed varying changes in the glandular architecture including changes in the secretory units, blood vessels, and duct system. Abnormalities which were observed in the present study may be due to the accumulation effect of the dye since the animals received the dye for 30 consecutive days. Light microscopic figures showed TZ induced total loss of the acinar architecture, destruction of the intralobular and interlobar ducts cells, congestion of blood vessels.Common features were evident in the acinar and ductal cells polymorphisim of the cell nuclei and cytoplasmic vacuolation.

These findings were in agreement with **Himri*et al*. (2011)**, **Mehedi*et al*. (2013)** and **Saxena& Sharma (2015)** who indicated thatTZ administration alters the histological structure of livers and kidneys, also in experimental animals. **Rus*et al*. (2010)** found that TZ and carmoisine causes congestion, stasis and edema in liver and kidney of Guinea pigs leading to apoptosis in hepatocytes and atrophy of renal structures.Vacoulation of the acinar cells were previously observed by **Ghonimi and Elbaz (2015)** in the liver cells and cerebellar neurons after exposure of Westar rats to TZ.

Also same results were discovered in the study carried by **Elkhaya*et al*., (2017)**Most of hepatocytes appeared with necrotic nuclei and cytoplasmic vacuolization. Some cells had irregular shaped nuclei, while others were devoid of nuclei. Also obvious histological changes were observed in the structure of the kidney in the animal group treated with tartrazine when compared to controls. These changes includes degeneration in glomerular structure, loss of renal tubules integrity and the presence of areas of huge vacuoles.

Our results were in agreement with **Al seeni*et al*., (2018)** and **El Raby *et al*., (2019)** bothcarried out a studies on the effect of TZ on different tissues, TZ treated groups showed drastic damage of liver and kidney. Also **Abdel-Aziz *et al*., (2019)** carried out a study on the structure and function of the thyroid gland. The treated group showed deterioration of thyroid functions with various degrees of thyroid structural alterations as vacuolated cytoplasm, dark nuclei and vacuolated colloid, epithelial stratification.

El-sakhawy*et al*., (2019) carried out an investigation to study the effect of TZ administration on the cerebellum, submandibular glands, and kidneys of adult male albino rats. The results of our study were in hand to hand withthe group treated with TZ (7.5 mg/kg body weight).**El-sakhawy*et al*., (2019)** results showed that the submandibular gland showed small vacuoles that appeared inside the cytoplasm of the acinar cells, and some nuclei showed hyperchromatism and abnormal mitosis these results were in concomitant with our study.

Degenerative changes and loss of the cellular morphology which explained by **Abdin (1981)** who stated that the hydropic degeneration was a disturbance in the metabolism of the cell, resulted in morphologic abnormalities, thus the cellular architecture is lost.**Henics and Whealthy (1999)** reported that the intense vacuolations in the mammalian cells lead to cell apoptosis"shrinkage necrosis" as the cell expected to compensate by vacuolating and swelling. This agree with our findings as many nuclei in the acinar cells appeared hyperchromatic andpyknotic with abnormal

mitosis.Polymorphism indicate DNAdamage and genotoxic effect of tartarzine.

Also **El-sakhawy*et al*., (2019)** in his study the group treated with tartrazine (7.5 mg/kg body weight), the submandibular gland showed same results as our study the interlobular and the intralobular ducts showed pyknotic nuclei and loss of the striations of the striated ducts also, total loss of the architecture of the ducts with obvious degenerative changes.Our results revealed also interacinar congestion of the interacinarblood capillaries. These observations in the congestion of the blood vessels was recorded by**Mehedi*et al*. (2013)** in mice observed subcortical edema in the brain treated with 1 and 2.5%tartrazine doses in addition to congestion of blood vessels in 2.5% TZ groups. Also was observed by **Ghonimi and Elbaz (2015)** in the liver portal vessels inWestar rats exposed to tartrazine.

Elkhayat*et al*., (2017) showed that the blood sinusoids revealed dilatation and congestion as well as white blood cell. **El-sakhawy*et al*., (2019)** the submandibular gland showed congestion of the interacinar blood capillaries and also dilation of the blood vessels present in the connective tissue surrounding the interlobular ducts and engorgement with blood was evident. The dilatation and congestion of the blood vessels present in our study support the opinion of **Moubarak (2008)** who explained that it might be part of the inflammatory response to bring more blood to the areas of degeneration.TZ induces lipid peroxidation, which can damage membrane of cell organelle once this cell membrane is disturbed, it leads to various structural and functional alterations in the cell **(Gajawat et al., 2006)**.

Conclusion

The results obtained in this study revealed that tartrazine causes disruptions and distortions of the cyto-architecture of the cerebellum, submandibular glands, and kidneys. These results suggest that the functions of these organs have been adversely affected. It is recommended that caution should therefore be advocated in the use of this product.

References:

1. **Abdel-Aziz, H., Alazouny, Z., Abdelfadeel, K.andAbohashem, A.:**Effectoftartrazine on thyroid gland of male rat and ameliorating roleof Curcumin (Histological and Immunohistochemical Study). JBiochem Cell Biol. 2019, 2:1-9.
2. **Abdin, F.:** Cell and tissue damage. In: Abdin's General Pathology,4th edn.1981, p 7.
3. **Adnyane, I., Zuki, A., Noordin, M. and Agungpriyono, S.:** Histological study of the parotid and mandibularglands of Barking Deer (Muntiacusmuntjak) with special reference to the distribution of carbohydrate content. Anat. Histol. Emryol. 2010, 39: 516-520.
4. **Al Seemi, M., El Rabey, H., Al-Hamed, A. and Zamzami, M.:** Nigella sativa oil protects against tartrazine toxicity in male rats. Toxicol Rep.2018, 5:146-155.
5. **Amin, K., Abdel Hameid, H. and AbdElsttar A,:** 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 Chem.Toxicol. 2010, 48: 2994-2999.
6. **Chung K**: Mutagenicity and carcinogenicity of aromatic amines metabolically produced from azo dyes. Environ CarcinogEcotoxicol Rev.2000, 18:51–74.
7. **Chung, K., Stevens, J. and Cerniglia, C.:** The reduction of dyes by the intestinal microflora. Crit Rev Microbiol. 1992, 18:175190.
8. **Cobo, J., L.C. Hernández, M. E. Del Valle, M. Vijandeand J.A. Vega**. Immunohistochemicallocalization of epidermal growth factor and itsreceptor during odontogenesis in the rat. Eur J Orthod. 1992, 14: 333-338.
9. **El Rabey, H., Al-Seeni, M., Al-Sieni, A., Al-Hamed, A., Zamzami, M. and Almutairi, F.:** Honey attenuates the toxic effects of the low dose of tartrazine in male rats.J Food Biochem. 2019, 43; e12780.

10. **Elewa, Y., Bareedy, M., Abuel- Atta, A., Ichii, O., Otsuka, S., Kanazawa, T., Lee, S., Hashimoto, Y., Kon, Y.:** Structural characteristics of goat (Capra hircus) parotid salivary glands. Japanese. J. Vet. Res. 2010, 58: 121-135.
11. **EL-Gusbi, G., Shredah, M. and Soliman, A.:**Submandibular Glands as an Evident of the Effects of Antioxidant on Alloxan-Induced Diabetic Rats. WJMS. 2014, 11: 210-216.
12. **Elhakim, M. and Heraud, F.:** New considerations regarding the risk assessment on tartrazine: an updated toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. RegulToxicolPharmacol. 2007, 47:308–316.
13. **El-sakhawy, M., Mohamed, D. and Ahmed, Y.:**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, 26:9574–9584.
14. **Esteconodo, S., Codon, S. and Casanave, E.:** Histological study of the salivary glands in Zaedyuspichiy(Mammalia, Xenarthra, Dasypodidae). Int. J. Morphol. 2005, 23: 19-24.
15. **Gajawat, S., Sancheti, G. and Goyal, P.:** Protection against lead-induced hepatic lesions in Swiss albino mice by ascorbic acid. Pharmacologyonline. 2006, 1: 140-149.
16. **Gamble, M.:** The hematoxylins and eosine. In book: Theory and Practice of Hstology Techniques (pp.121-134).
17. **Ghonimi, W., and Elbaz, A.,:** Histological changes of selected wistar rats tissues following the ingestion of tartrazine with special emphasis on the protective effect of royal jelly and cod liver oil. J Cytol Histol.2015, 6: 1-6.
18. **Henics, T. and Whealthy, D.:** Cytoplasmic vacuolation, adaptation and cell death: a view on new perspectives and features. Biol Cell. 1999, 91: 485–498.
19. **Himri I., Bellachen S., Souna F, Belmekki F., Mohammed A., Mohamed B., Zoheir J., Berkia Z., Mekhfi H. and Saalaoui E.:** A 90-day Oral Toxicity Study of Tartrazine, a Synthetic Food Dye, in Wistar Rats, Int. J. Pharm. Pharm. Sci. 2011, 3: 159-169.
20. **Hirschbruch, M. and Torres, E.:**Toxicologia de alimentos: umadiscussão, HigAlim. 1998, 12:21–25.
21. **Jaskoll, T., Zhou, Y., Chai Y, Makarenkova, H., Collinson, J., West, J. and Carvalho, A.:**Embryonicsubmandibular gland morphogenes is; Stage- specific protein localization of FGFs, BMPs, Pax6 and Pax9in normal mice and abnormal phenotypes in FgfR2- III c(t/Delta), BMP7(-/-) and Pax6(-/-) mice. Cells. Tissues. Organs. 2002, 170: 83-90.
22. **JECFA (1964):** Specifications for identity purity and toxicological evaluation of food colours in FAO nutrition Meetings Report Series No 38B WHO Geneva.
23. **Kimura, J., Habata, I., Endo, H., Rerkamnuagchoke, W., Kurohmaru, M., Yamada, J., Nishida, T. and Tsukise, A.:**Histochemistry of comoplex carbohydrate in the major salivary glands of Hoary Bamboo rats (Rhizomyspurinosus). Anat. Histol. Embryol. 1998, 27: 147-153.
24. **Li, Y. and Schellhorn, H.:** New developments and novel therapeutic perspectives for vitamin C. J Nutr. 2007, 137:2171–2184.
25. **Maekawa, A., Matsuoka, C., Onodera, H., Tanigawa, K., Furuta, J., Kanno, J., Jang, J., Hayashi, Y. and Ogiu, T.:** Lack of carcinogenicity of tartrazine (FD and C Yellow No. 5) in the F344 rat. Food Chem Toxicol.1987, 25: 891-896.
26. **Mehedi, N., Mokrane, N., Alami, O., Ainad-Tabet, S., Zaoui, C., Kheroua., O., and Saidi, D.:** A thirteen week ad libtium administration toxicity study of

tartrazine in swiss mice. AJB. 2013, 12:4519-4529.

27. **Mehedi, N., Tabet,S., Mokrane, N., Addou, S., Zaoui, ., Kheroua, O. and Saidi, D.:** Reproductive Toxicology of Tartrazine (FD and C Yellow No. 5) in Swiss Albino Mice. Am J PharmacolToxicol. 2009, 4: 128-133.
28. **Moubarak, R.**: The effect of hypercholesterolemia on the rat parotid salivary gland (histopathological and immunohistochemical study).Cairo Dental Journal.2008. 24:19–28.
29. **Moutinho, I., Bertges, L. and Assis, R.:** Prolonged use of the food dye tartrazine (FD&C yellow nº5)and its effects on the gastric mucosa of Wistar rats. Braz. J. Biol. 2007, 67: 141-145.
30. **Rus V, Gherman C, Micl us V, Mihalca A, Nad s G.:** Comparative toxicity of food dyes on liver and kidney in guinea pigs: A histopathological study. Annals of RSCB. 2010, 15:161165.
31. **Sandler, R.:** Diet and cancer: food additives, coffee and alcohol. Nutr Cancer.1983, 4:273–279.
32. **Sasaki, Y., Kawaguchi, S., Kamaya, A., Ohshima, M., Kabasawa, K., Iwama, K., Taniguchi, K. and Tsuda, S.:** The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutat Res.2002, 519: 103-119.
33. **Saxena, B. and Sharma, S.:** Food color induced hepatotoxicity in Swiss albino rats, Rattusnorvegicus. Toxicol. Int. 2015, 22:152-157.
34. **Sharma, P., Mishra, S., Ajmera, P. and Mathur, S.:** Oxidative stress in metabolic syndrome. Indian J ClinBiochem. 2005, 20:145–9.
35. **Tanaka, T., Takahashi, O., Oishi, S. and Ogata, A.:** Effects of tartrazine on exploratory behaviour in a three-generation toxicity study in mice. ReprodToxicol. 2008 26: 156-163.
36. **Tanaka, T.:** Reproductive and neurobehavioral toxicity study of Tartrazine administered to mice in the diet. Food Chem. Toxicol. 2006, 44:179-187.
37. **Toledo, M.:**Aditivosalimentares in fundamentos de toxicología. Atheneu, Brasil. 1996, pp, 405–39.
38. **Toledo, M.F.:**RegulamentacaodeUso de CorantesNaturais. Arch. LatinoamNutr., 1999, 49: 67-70.
39. **Tsai, C., Kuo, C. and Shih, D.:** Determination of 20 synthetic dyes in chili powders and syrup-preserved fruits by liquid chromatography/tandem mass spectrometry. J Food Drug Anal.2015, 23: 453–462.
40. **Walton, K., Walker, R., Van, D., Jim, S. and Castell, J.:** The application of in vitro in the derivation of the acceptable daily intake of food additives. Food Chem. Toxicol. 1999,37: 1175-1197.
41. **YazdaniMoghaddam F, Darvish J, MahdaviShahri N, Abdulamir AS, Mousavi M, Daud SK.:** Comparative histological and histochemical inter-species investigation of mammalian submandibular salivary glands. Res. J. Appl. Sci.2009, 4: 50-56.
42. **Zhang, G., Ma, Y., Wang, S., Kong, W. and and Wang, R.:** Chiral organic contact ion pairs in metal-free catalytic enantioselective oxidative cross-dehydrogenative coupling of tertiary amines to ketones. Chemi sci. 2013, 4: 2645-2651.

ASDJ
Ain Shams Dental Journal