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

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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 Tartrazine dose of 7.5mg/kg in 1 ml 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 vacouliations 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.
**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 (azu 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 as TZ, 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 (Jaskollet 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.

The secretion (saliva) is mostly serous, containing various enzymes, water, mucopolysaccharides and lubricating glycoprotein (Adnyanee 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 5 groups 7 rats 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 7 rats, each rat
received Tartrazine 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 anesthesia. 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 granular 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 group II treated with TZ the submandibular 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 in between 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 salivary the 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 polymorphism of the cell nuclei and cytoplasmic vacuolation.

These findings were in agreement with Himriet et al. (2011), Mehdiet al. (2013) and Saxena& Sharma (2015) who indicated that TZ administration alters the histological structure of livers and kidneys, also in experimental animals. Ruset 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. Vacuolation 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 Elkhayat 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 seeniet et al., (2018) and El Raby et al., (2019) both carried 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-sakhawyet 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 with the group treated with TZ (7.5 mg/kg body weight). El-sakhawyet 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 Whealthey (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 and pyknotic with abnormal
mitosis. Polymorphism indicate DNA damage and genotoxic effect of tartrazine.

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 interacinar blood capillaries. These observations in the congestion of the blood vessels was recorded by Mehediet 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 in Westar rats exposed to tartrazine.

Elkhayet 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.

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