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Research Article

MITIGATING TARTRAZINE-INDUCED PATHOLOGICAL CHANGES IN THE PAROTID GLAND: A HISTOLOGICAL AND ULTRASTRUCTURAL INVESTIGATION WITH OMEGA-3 FATTY ACID INTERVENTION

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ABSTRACT

There is a wide variety of food additives that contain tartrazine. Different body organs can be adversely affected by it. The antioxidant, anti-apoptotic, and anti-inflammatory properties of omega-3 fatty acids are well documented. To determine whether omega-3-fatty acids protect the parotid gland against changes induced by tartrazine. A total of 21 albino rats were divided into three groups in this study. The control group received tartrazine at 320 mg/kg daily, the tartrazine-treated group received tartrazine at 320 mg/kg body weight daily, while the tartrazine and omega-3 treatment group received omega-3 daily at 300 mg/kg body weight, as well as tartrazine. Four weeks were spent treating the patient. A variety of light and electron microscopy techniques were used on parotid gland specimens, including hematoxylin and eosin, Sirius red stains, and caspase-3 immunofluorescence. There was an increase in darkly stained nuclei, as well as cytoplasmic vacuolations of parotid acini in the tartrazine-treated group. There is a significant difference in % between the control group and the group immunostained for caspase-3 and collagen fibers. Tartrazine-treated acinar cells showed irregular nuclei, dominant electron-lucent granules, and dilated rough endoplasmic reticulum, as well as degenerative changes in ductal epithelium, according to ultrastructural analysis. Studying the structure of the parotid gland histologically and morphometrically revealed that omega-3 improved its structure. Tarrazine's structural effects on the parotid gland were ameliorated by omega-3 treatment..

Keywords: - Corneal, Sensitivity, Mechanical, Chemical, Thermal.

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INTRODUCTION

Parotid, sublingual, and submandibular glands make up the major salivary glands in humans. Roa & Del Sol, 2019 [1] state that the parotid gland is also serous in rodents. Located below and behind the ear, the parotid gland is caudally adjacent to the submandibular gland in rodents. Parathyroid glands are composed of two parts: parenchyma (glandular secretory tissue) and stroma (the connective tissue that supports the gland). In the parenchyma, the main duct consists of collecting ducts, intercalated ducts and striated ducts that must be joined together to form the main duct [2].

Various aromatic amines can be formed when the azo dye molecule is cut [3]. On application, tartrazine provides a yellow to orange color to foods. It is a synthetic azo dye that is primarily used as a food coloring. Also sometimes called E102 or Yellow 5 or C.I. 19140, it is licensed by the FDA to be used as a colorant in drugs, food, and cosmetics. Zinc dioxide is a synthetic

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food dye commonly found in drinks, fruit juices, chocolates, cookies, chewing gum, jam, candy, ice cream, sauces, and mustards

A significant amount of attention has been paid to the application of active antioxidants due to the oxidative damage that tartrazine does to various body organs, along with their widespread use in food coloring. Omega-3 fatty acids have not previously been studied in their role as protective agents against tartrazine-induced oxidative stress. Thus, in order to investigate whether omega-3 fatty acids can protect albino rats against the detrimental effects of tartrazine on their parotid glands, the present study was conducted.

MATERIALS AND METHODS

Design and treatment of animals in experiments:

The study involved 21 adult male albino rats weighing between (220 and 250 g), aged three months. Cages were kept at 23°C with 22% humidity, 12 hours of light and 12 hours of darkness with rat cage temperature maintained at 23°C. Food and water were provided to them at no cost. It took them 15 days to become acclimated to the laboratory conditions. We randomly divided the rats into three groups of seven rats each. None of the medications were administered to Group I (control group). A four-week tartrazine treatment was administered via gastric tube to rats in Group II (the tartrazine-treated group) using tartrazine dissolved in distilled water 320 mg/kg body weight once a day. A four-week tartrazine and omega-3 treatment regimen was implemented for group III (tartrazine and omega-3 treated group) that included omega-3 administration at a dose of 300 mg/kg body weight once a day by a gastric tube. Anaesthesia was administered through inhalation of ether and perfusion of saline solution into the heart after the experiment. Parotid salivary glands were carefully dissected off the body of the sacrificed animal and removed intact after they were located below the ear on the lateral side of the submandibular salivary glands. An immunohistochemistry and histological study was conducted on the right glands. Transmission electron microscopy was performed on the left parotid salivary glands.

Analyses of histology:

A study of light microscopy:

A 10% neutral formalin solution was applied to the specimens immediately and they were rinsed in distilled water for 48 hours. Their dehydration in successive grades of ethyl alcohol was proceeded by embedding them in paraffin. After cutting and processing the sections, the following were done:

Histological staining for eosin and hematoxylin to explore parotid gland cytoarchitecture [4]

The presence of Sirius red to identify collagen fibers and observe fibrosis [5].

Staining with immunohistochemistry:

Positively charged slides were used to mount 4 m thick sections. Deparaffinization was then performed on the sections. For 30 minutes, H2O2 in PBS was used to block endogenous peroxidase activity. A 0.01 M sodium citrate buffer (pH 6.0) was used for microwaving sections for antigen retrieval. Once the sections had been rinsed in PBS and blocked with normal goat serum, rabbit anti-cleaved caspase 3 monoclonal antibody was incubated overnight with them. A secondary biotinylated goat anti-mouse antibody was then added to the slides and incubated for three hours at room temperature with diluted 1:150 in PBS; Strepavidin-labeled peroxidase complex was incubated for three hours in PBS (diluted 1:150; Zymed) at room temperature. DAB (Sigma-Aldrich) was used as a dissolving agent for visualizing the reaction. Mayer's hematoxylin was used to counterstain the sections. After dehydration, mounting, and examination, they were photographed [6].

Transmission Electron Microscopic Study:

Specimens of the parotid gland were cut into small pieces (1 mm3) fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4). They then were fixed for 1h in 1% osmium tetroxide in same buffer. Then, dehydration and embedding in epoxy resin was carried out. Semithin sections (0.5 µm) were cut, stained by toluidine blue stain, examined, and photographed. Ultrathin sections (80-90 nm) were cut using the ultramicrotome and mounted on copper grids and stained with uranyl acetate and lead citrate.

Morphometric Study:

A digital camera with a 0.5x photo adaptor was mounted on an Olympus microscope with an objective lens x 40 to photograph Sirius red stained sections and caspase-3 immunostained sections with an Olympus digital camera (DP27). We used ImageJ software (Java 8. 1.6.0. 2017. NIH, USA) to analyze the resultant images for morphometric analysis. In order to convert pixels to micrometers, the software converted pixels to micrometers. Across each group, five different sections of each animal were randomly selected at a magnification of x400. Measuring parameters included the following:

Amount of staining (%) on collagen fibers (%):

To evaluate red stained collagen fibers within a standard measuring frame [7], the RGB (Red, Green, Blue) threshold was used to detect red stained areas (fortea, green, blue).

In the area immunostained by Caspase-3, the percentages are:

An area percentage was calculated for caspase-3 immunostaining. Image J software was used to quantify the positive staining area and the percentage of positive staining to a standard measuring frame was expressed as a percentage. No matter the intensity of the brown color, the areas displaying brown color were studied.

Statistical analysis

Statistical analysis was conducted on the data obtained. In the table, the mean values are represented along with their standard error means (SEM). Statistics were analyzed using SPSS 19.0 software (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA). Tukey's multiple comparison test and one-way analysis of variance (ANOVA) were used to determine whether there were any differences between the groups. In this study, P 0.05 was considered significant.

RESULTS

Results of histopathology

Microscopy results under light:

Ink staining with hematoxylin and eosin:

Normal histological structures were observed in the control group. There are thin delicate connective tissue septa dividing the parotid gland into lobes and lobules. Its parenchyma contains acinus and ducts. The excretory (interlobular) duct was lined by pseudostratified columnar epithelium. Saccules with rounded nuclei at the base and eosinophilic cytoplasm towards their apex had acini lined by pseudostratified columnar epithelium. There are low cuboidal cells with central nuclei lining the intercalated duct, located between acini and compressed among them. There were spherical vesicular nuclei at the centers of the striated ducts as well as basal striations in the epithelial cells lining the ducts.

Among the tartrazine-treated groups (Group II), thickened connective tissue septa and homogeneous acidophilic material were observed between the lobules. The cellular lining of the interlobular ducts appears disorganized. A pyknotic nucleus and vacuolated cytoplasm were present in the interlobular duct lining. Periductal vessels were found to be congested. Nuclei and cytoplasm of the acinar cells showed deep staining. Between the acini, red blood cells were found to be extravasated . Flattened, disorganized cellular linings were observed in the intercalated ducts. As a result of shedding of the epithelial lining of the striated duct, the lining cells in the duct showed loss of their striations, vacuolation, and pyknotic nuclei.

There were thin connective tissue septa separating the parotid gland parenchyma among the tartrazine and omega-3 treated groups. There was a pyramidal cellular lining surrounding the rounded nuclei of the parotid gland acini. Experimental and control groups had similar intercalated, striated, and interlobular ducts.

Stained red by Sirius

A Sirius-red stain of samples from the control group indicated fine collagen fibers between lobules, around ducts, and within acini. Connective tissue septa and blood vessels within interlobular ducts, vessels, and septa of tartrazine-treated animals contained dense collagen fibers. Collagen fibers were observed around blood vessels and ducts in the tartrazine and omega-3 treatment group.

Toluidine Blue Staining

On examination of semithin slices of parotid glands in the control group, pyramidal cells with basal rounded vesicular nuclei with prominent nucleoli were found to line close-packed serous acini within the parenchyma. Granules of varying densities were packed into the cytoplasm. There were low cuboidal cuboidal cellular linings surrounding oval vesicular nuclei in the intercalated ducts between the acini. Columnar strutted duct lining cells had striated cytoplasm and round nuclei with rounded condensations in the center. A myoepithelial cell enveloped them. It was surrounded by myoepithelial cells and had a pseudostratified lining.

In the tartrazine-treated group the parotid gland showed that serous acini lining cells appeared with darkly stained irregular nuclei. Several vacuolations were observed in the cytoplasm and there was an accumulation of lightly stained granules instead of densely stained ones. It was possible to observe disorganized acini. Generally, the cytoplasm of many intercalated ducts was vacuolated, with discontinuities in the epithelial lining. A vacuolated cytoplasm as well as pyknotic nuclei were found in the living cells of the interlobular ducts, as well as exfoliated cells in its lumen. In this study, blood vessels were found to be congested. Intercellular spaces appeared dilated in some acini.

Generally, the pyramidal cells appeared to be normal in the tartrazine and omega-3 treated acini. There are granules of different densities in the cytoplasm as well as rounded basal nuclei. There was a near normal lining in the intercalated ducts, the striated ducts, and the interlobular ducts.

Staining with immunohistochemistry:

In the control group, acinar cells and duct cells stained mildly with caspase-3 antibodies. Tartrazine, however, induced strong nuclear and cytoplasmic reactions in the group that was exposed to antioxidants. It shows that tartrazine plus omega-3 induced moderate

immune reactions against caspase-3 in acinar cells and duct cells of rats.

Analyses conducted using electron microscopy:

In the control group, acinar cells had well-defined nucleoli, rounded euchromatic nuclei and abundant rough endoplasmic reticulum aligned in parallel cisternae around the nucleus. The junctional complex among cells and the canaliculi between cells were observed. A great number of spherical secretory granules of different electron- density and well-defined membrane in the supranuclear region of cytoplasm were observed with some granules are of high electron-dense content, some were of moderate homogeneous electron- dense content and other granules were of a low density. The striated duct's lining cells were observed to have regular nuclear membrane and euchromatic nuclei with rounded centrifugal nuclei. Small secretory granules were visible in the apical cytoplasm. Cell walls were dotted with mitochondria. Junctional complexes were observed near the lumen in addition to apical microvilli.

Those with tartrazine treatment showed irregular heterochromatic nuclei in their acinar cells. Secretory granules with low electron density or electron lucency showed electron lucency above the nucleus. A large number of vacuoles were also visible in the cytoplasm. A dilated intercellular canaliculus without junctional complexes can be observed. A nuclear membrane indentation was observed in some cells. We observed outlines that were fused of many granules. The rough endoplasmic reticulum cisternae were dilated perinuclearly, and their cystic dilation was irregular. In the cells lining the striated ducts, there are condensed nuclei and mitochondria with deconstructed cristae, while microvilli are absent throughout the cells.

In both tartrazine-treated and omega-3-treated acinar cells, the basal euchromatic nucleus was rounded, the surface electron density granules were high; the surface electron density secretory granules were moderate, and the rough endoplasmic reticulum was normal. Lining cells of striated ducts had a rounded nucleus at the apex, small secretory granules at the apex, and mitochondria in both the middle and lower parts.

Findings from Morphometrics

Tarrazine treated groups showed a significant increase in collagen fiber staining compared to control groups in the parotid gland. Compared to tartrazine treatment alone, however, collagen fiber staining significantly decreased in the omega-3 and tartrazine treated groups (Table 1).

Comparing the tartrazine treated group to the control group, the area percentage of caspase-3 immunstaining was significantly higher in the parotid gland. Compared to the tartrazine treated group, the omega-3 and tartrazine treated groups immunostain significantly less for caspase-3. (Table 1).

Table 1: In each experimental group, the mean and standard deviation are indicated by the percentage of collagen fibers stained and caspase-3 immunostained areas:.

	Group of controls	**The group that was treated with tartrazine**	**An Omega 3 fatty acid and tartrazine treatment group**
Collage fiber staining area percentage	13.87± 0.50	25.11 ±0.73*	15.22±0.21#
Caspases-3 immunostaining percentage in the area	9.31±0.47	28.64± 0.51*	12.88± 0.29#

* The difference between the control group and the experimental group is significant (p 0.05).

DISCUSSION

Tartrazine is one of the widely used food additives all over the world. Scientist have found that azo dyes can cause health defects, exert a risk to the nervous system and gastrointestinal tract [8]. Omega-3 fatty acids were found to be effective in improving tartrazine-induced parotid gland pathology.

A tartrazine oral administration altered the histological structure of the parotid gland in albino rats in the present study. The nuclei of acinar cells exhibited abnormal dark staining and there were numerous vacuoles within cells. According to El-Sakhawy et al., [9], these results are similar to those obtained after tartrazine is administered to adult male albino rats to study their submandibular glands. The nuclei of the acinar cells showed hyperchromatism and abnormal mitosis, and small vacuoles appeared inside the cytoplasm. Tarrazine administration also altered the histological structure of liver and kidney as reported by Saxena & Sharma [10]. Accordingly, Ghonimi and Elbaz [11] observed vacuolations in Westar rats' cerebellar neurons after exposure to tartrazine, as well as widespread degeneration in the liver.

By converting semiquinone radicals and aromatic amines into azo dyes, peroxidases and azo reductases catalyze the reactions. Among the oxidative

stress-related disorders that are caused by semiquinone radicals are hydroxyl radicals, superoxide radicals, and hydrogen peroxide [12]. Considering that tartrazine induces malondialdehyde as the end product of lipid peroxidation with a reduction in glutathione levels, it has been demonstrated that tartrazine causes oxidative stress in rats [13]. Administration of tartrazine as well, decreased the activity of antioxidant enzymes (catalase, superoxide dismutase, and glutathione reductase)

Tartrazine-treated rats with congested blood vessels and collagen fibers deposition around the acini, ducts, and constrictions in the parotid tissue showed a higher degree of collagen deposition than their control counterparts based on statistical analysis. In tartrazine treated rats, mucosal collagen fibers were detected in an increased amount in the jejunal mucosa, according to Kandeel & Sharaf Eldin, [14]. Acini can be disorganized due to the presence of oxidation products, such as lipid peroxidation products during damage to tissue, inflammatory signals are released, causing fibroblast differentiation and consequently fibrosis [15].

In the parotid tissue that had been treated with tartrazine, cells were observed interstitially and blood vessels were congested. A tartrazine-induced oxidative-inflammatory stress is thought to be responsible for these findings [8].

Acinar cells have low density secretory granules, which can be confirmed by ultrastructural examination of toluidine blue stained semithin sections. The presence of granules that are electron-lucent is a sign of a degenerating parotid gland, according to Selim [16].

Ultrastructural examination confirmed that semithin sections exhibited dilated intercellular canaliculi. A shift in the integrity of plasma membranes and disruption of intercellular junctions may be attributed to reactive oxygen species[17]. It has been suggested that myoepithelial dysfunction in the parotid gland acini may explain the observed dilation of intercellular canaliculi. Degenerating mitochondria and pyknotic nuclei were observed in the present study. Hepatocytes and renal tubule epithelium were also affected by tartrazine treatment.

In this study, omega-3 fatty acids combined with tartrazine were found to provide a protective effect against the degenerative changes in the parotid gland of the tartrazine-treated group. There was a minimal cytoplasmic vacuolation in the ducts and serous acini of the parotid gland in the tartrazine and omega-3 groups.

CONCLUSION:

The effects of tartrazine on the acini of the parotid gland and the duct system of rats are degenerative histologically, ultrastructurally, and immunohistochemically. Providing omega-3 fatty acids concomitantly reduces the severity of these effects and improves the histological picture. As much as possible, tartrazine should not be used excessively as a food additive.

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