

Histological and Immunohistochemical studies of the effect of vitamin C on the parotid salivary glands of male albino rats after chromium exposure.

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Abstract: 30 male albino rats (200-220 gm body weight) were utilized in the current study. They were divided into three groups, 10 animals each. The first group was considered control group and received drinking tap water for 4 months. The second group was the experimental group and received drinking tap water containing 300 ug/L of Cr (VI) as chromium trioxide for 4 months. The third group, received drinking tap water containing 300 ug/L of Cr (VI) as group II. In addition the animals of group III were injected intramuscularly by 10 mg/kg body weight of vitamin C, twice weekly for the whole duration of the experiment. Samples from the parotid salivary glands were fixed in 10% buffered neutral formalin and prepared routinely for paraffin sectioning and staining for histological and immunohistochemical investigation of proliferating cell nuclear antigen (PCNA). Group II animals revealed histopathological degenerative changes in parotid salivary glands. The nuclei of the acinar cells showed signs of atypism, hyperchromatism and abnormal mitosis. The connective tissue stroma revealed increase in thickness and showed hyalinization. There was prominent inter-acinar edema. The blood vessels were dilated and engorged with blood. The immunoexpression of PCNA in the nuclei of the acinar cells was intense after 4 months daily administration of drinking tap water containing 300 ug/L of Cr (VI). Group III received vitamin C in addition to Cr (VI), revealed some recovery to the normal structure of the parotid salivary glands, which might revealed the protective effect of vitamin C as an antioxidant against cytotoxic effect of hexavalent chromium.

[El-Sakhawy, M.A and Shredah, M. **Histological and Immunohistochemical studies of the effect of vitamin C on the parotid salivary glands of male albino rats after chromium exposure.** *Life Sci J* 2014;11(10):822-826]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 130

Key words: Parotid, Chromium, PCNA.

1. Introduction:

Chromium is a chemical element which has the symbol Cr.

A major development was the discovery that steel could be made highly resistant to corrosion and discoloration by adding metallic chromium to form stainless steel.

This application, along with chrome plating (electroplating with chromium) currently comprise 85% of the commercial use for the element. Trivalent chromium (Cr (III)) ion is possibly required in trace amount for sugar and lipid metabolism, although the issue remains in debate. In larger amounts and in different forms, chromium can be toxic and carcinogenic. The most prominent example of toxic chromium is hexavalent chromium (Cr (VI)) (**Cronin, 2004**). Chromium compounds are found in the environment, due to erosion of chromium containing rocks and can be distributed by volcanic eruption. The concentration range in soil is between 1 and 300 mg/Kg, in sea water 5 to 800 ug/L and in rivers and lakes 26 ug/L to 5.2 mg/L (**Kotas and Stasicka, 2000**). The acute oral toxicity for chromium (VI) range between 50 and 150 ug/L.

In the body, chromium (VI) is reduced by several mechanisms to chromium (III) already in the

blood before it enters the cells. The chromium (III) is excreted from the body, whereas the chromate ion is transferred into the cell by a transport mechanism. The acute toxicity of chromium (VI) is due to its strong oxidational properties. After it reaches the blood stream, it damages the kidneys, the liver and blood cells through oxidation reactions. Hemolysis, renal and liver failure are the results of these damages. Aggressive dialysis can improve the situation (**Dayan and Paine, 2001**). Chromium salts (chromates) are also cause of allergic reactions in some people. Chromates are often used to manufacture, amongst other things, leather products, paints, cement, mortar and anti-corrosives. Contact with products containing chromate can lead to allergic contact dermatitis and irritant dermatitis, resulting in ulceration of the skin, sometimes referred to as "chrome ulcers". This condition is often found in workers that have been exposed to strong chromate solutions in electroplating, tanning and chrome-producing manufacturer (**Basketter et al., 2000**).

Proliferating cell nuclear antigen (PCNA) was originally described in proliferating mammalian cells as a nuclear protein. The highly homologous nature of PCNA suggests that protein plays an essential role in DNA replication (**Nakane et al.,**

1989). Recently, it is found to be necessary for proliferation with no relation to cyclin protein. Proliferating cell nuclear antigen (PCNA) was involved in the cellular cycle (Itall *et al.*, 1990), and could be identified in replicating cells of both benign and malignant lesions. Higher expression of this marker had been shown in aggregative tumors (Tsuji *et al.*, 1995; Cardoso *et al.*, 2000; and Lazzarbolet *et al.*, 2000). Vitamin C as an antioxidant and immune enhancer is one of the important water soluble vitamins and essential for collagen synthesis (Naidu, 2003).

No available studies were done according to our knowledge to investigate the effect of hexavalent chromium on the parotid salivary glands of male albino rats and to study the effect of vitamin C as an antioxidant and immune enhancer on the effect of Cr (VI) on the parotid salivary glands. This promoted the present research.

2. Material and methods

2.1 Experimental animals:

The study was carried out on thirty (30) adult male albino rats weighing about 200-220 gm; they were caged in the animal room in the Faculty of Veterinary medicine Cairo University throughout the experimental period (4 months). The animals were maintained on stock diet and kept under fixed appropriate conditions of housing and handling. Animals in each group were caged in separate cages. The animals were classified into 3 groups.

2.2 Grouping of the experiment:

Group I (control group): received drinking tap water for 4 months.

Group II (Experimental group): received drinking tap water containing 300 ug/L of hexavalent chromium Cr (VI) as chromium trioxide (ADWIC, Laboratory chemicals, Egypt) for 4 months (The permissible concentration of Cr (VI) is 50-100 ug/L-Current drinking water standards. Washington: Environmental protection agency, 2000)

Group III (Experimental group): received drinking tap water containing 300 ug/L of hexavalent chromium Cr (VI) as group II. In addition, the animals were injected by intramuscular injection of 10 mg/kg body weight of vitamin C (Cevaryl 1000 mg/5 ml ampoule (Memphis Co. for Pharm. & Chemical Ind. Cairo, Egypt) two times per week for the whole duration of the experiment.

2.3 Obtaining of specimens and tissue preparation:

Samples from parotid salivary glands were obtained after 4 months of the experimental period. Rats were sacrificed by cervical decapitation then the parotid salivary glands were carefully dissected out, fixed in 10% neutral buffered formalin and prepared for histological and immunohistochemical study.

2.4 Histological examination:

Specimen from parotid salivary glands were washed, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of 5-6 um in thickness were cut out, deparaffinized and stained with Haematoxylin and Eosin (H&E) for examination under the light microscope (Bancroft *et al.*, 1994).

2.5 Immunohistochemical examination for detection of PCNA:

Immunohistochemistry was performed on paraffin sections, and mounted on coated glass slides. Antigen was retrieved in citrate buffer (pH 6.0) microwave digestion (2 cycles of 12 minute each). Endogenous peroxidase was blocked with 0.05% hydrogen peroxide for 30 min. After incubation with a 1:20 dilution of normal horse serum, the slides were incubated over night at 4 °C with primary antibodies (Dako, 1:50). Secondary antibodies associated with a streptavidin-biotin-peroxidase method were applied (Dako A/S). Diaminobenzidine was used as chromogen. All sections were counter-stained with haematoxylin. The sections were washed with phosphate buffered saline after each step. Negative controls were used using non-immune serum instead of the primary or secondary antibodies. The method used was outlined according to (Ramos-Vara 2005).

3. Results:

Examination of control group sections of parotid salivary glands revealed that, the gland consisted of secretory acini and ducts. These serous acini appeared round and had a narrow lumen. The acini were lined by pyramidal cells with apical acidophilic cytoplasm. Their nuclei were prominent, deeply stained, spherical in shape and basally situated (Figure 1). The duct system presented intercalated, striated and excretory ducts. The intercalated ducts were hardly identified, as they were compressed between the acini. The striated ducts were lined by a single layer of columnar cells which showed well-defined outlines and central, rounded, darkly stained nuclei. The cytoplasm appeared eosinophilic and showed basal striation. Thick fibrous connective tissue was present between the lobes and lobules of the parotid glands. Both acini and ducts revealed negative immune reaction to PCNA (Figure 2).

Examination of sections of the parotid glands contributed that the daily administration of Cr (VI) caused multiple histopathological and immunohistochemical changes in the parotid salivary glands. There was hydropic degeneration of the cytoplasm. There were ill-distinct cell boundaries and vacuolization in the acini. The intercalated, striated and interlobular ducts were dilated and their cytoplasm showed complete signs of degeneration (Fig. 3). The nuclei of the acinar cells showed signs of

atypism, hyperchromatism and abnormal mitosis (**Fig.4**).The connective tissue stroma, both intra and interlobular revealed increase in collagen fiber

thickness and showed hyalinization (**Fig.5**).All blood vessels were dilated and engorged with blood (**Fig.6**).

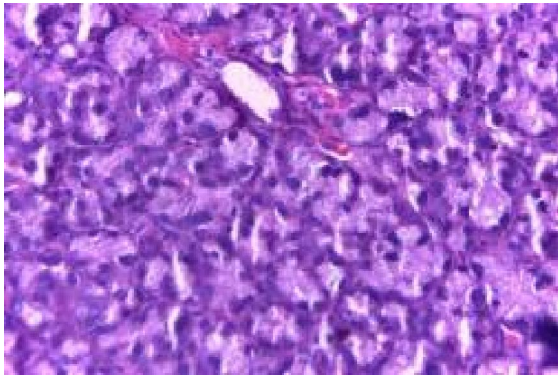


Figure 1: Photomicrograph of parotid salivary glands of albino rats of control group showing normal structure (H&E x400).

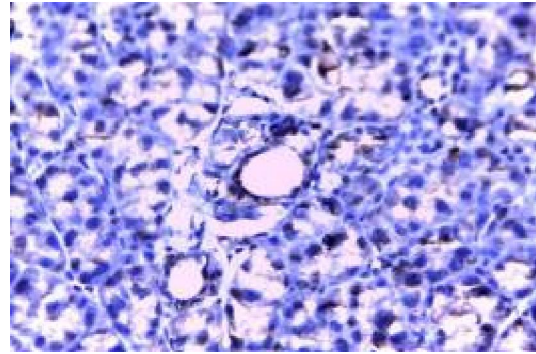


Figure 2: Photomicrograph of parotid salivary glands of albino rats of control group showing negative immune reaction (PCNA x400).

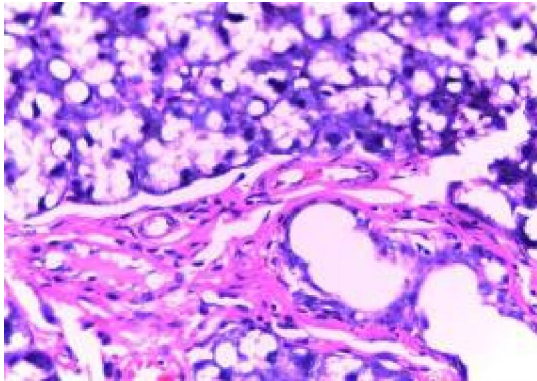


Figure 3: Photomicrograph of parotid salivary glands of group II showing degenerative changes and vacuolization in the acini and epithelial lining the excretory duct (H&E x400).

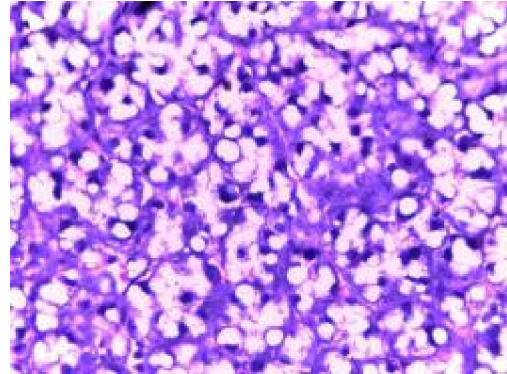


Figure 4: Photomicrograph of parotid salivary glands of group II showing different forms of hyperchromatic nuclei, abnormal mitosis and atypism (H&E x400).

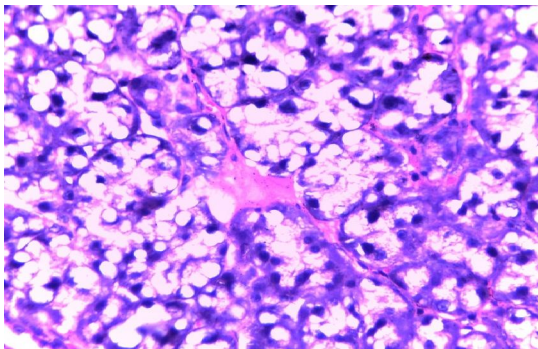


Figure 5: Photomicrograph of parotid salivary glands of group II showing increase in thickness and hyalinization of connective tissue septa (H&E x400).

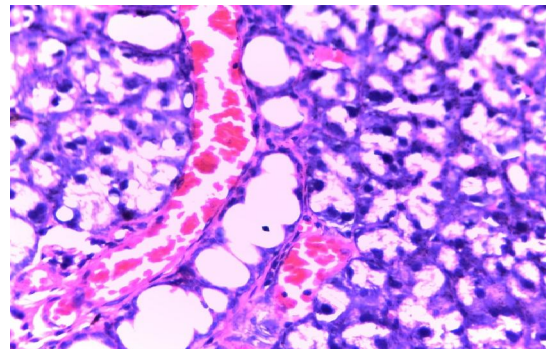


Figure 6: Photomicrograph of parotid salivary glands of group II showing dilatation and congestion of the blood vessels (H&E x400).

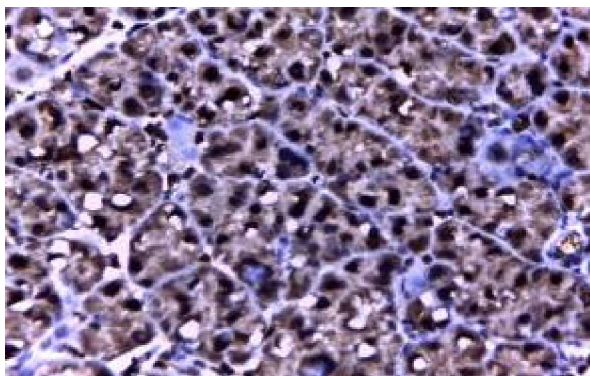


Figure 7: Photomicrograph of parotid salivary glands of group II showing intense immunoreexpression in the nuclei of acinar cells (PCNA x400).

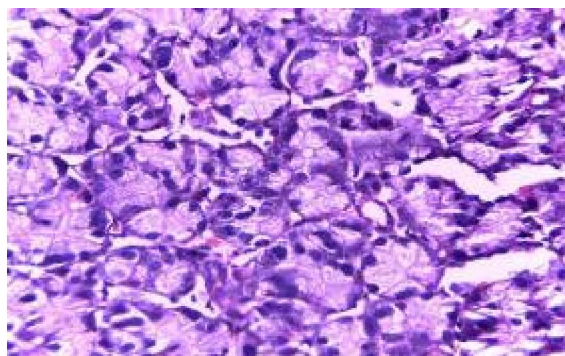


Figure 8: Photomicrograph of parotid salivary glands of albino rats of of group III showing normal structure of the acini (H&E x400).

Some acini were completely ruptured so that the nuclei were expelled out of the acini. There was a prominent interacinar edema. The nuclei of the acinar cells of this experimental group showed intense immunoreactivity for PCNA (**Fig.7**). The cytoplasm of the acinar cells revealed weak positive immune reaction. The nuclei of the epithelium lining the ducts revealed intense immunoreexpression to PCNA. Examination of the parotid salivary glands of group III showed some recovery to the normal structure. The acini were arranged in circular fashion. Some acinar cells exhibited normal structure showing many eosinophilic zymogen granules (**Fig.8**). Some acinar cells were distended and their cytoplasm showed signs of degeneration. The nuclei showed some mitotic figures. The excretory ducts were dilated and lined with normal epithelium. The blood vessels were still dilated and engorged with blood. The nuclei of some acinar cells revealed moderate positive immunoreexpression to PCNA.

4. Discussion:

In the present work, the effect of chromium administration on the parotid salivary glands of male albino rats was studied. The results of the present study showed that chromium administration to rats resulted in many histopathological changes in the parotid salivary glands. The serous cells and ductal epithelial cells revealed degeneration and vacuolization. The connective tissue septa showed increased in thickness and hyalinization. The blood vessels were enlarged and congested. Interacinar edema was evident. **Osman et al. (2006)** studied the effect of chromium on the tongue of rats. They reported atrophy of the lingual papillae. They attributed this atrophy to the mechanism of epithelial reproduction through the cytotoxic effect of chromium. **Manygoats et al. (2002)** stated that the cellular damage, morphological changes, chromatin

condensation and DNA fragmentation might be due to the toxic effect of chromium. **Thomson et al. (2012)** on their studies of the effect of hexavalent chromium on the alimentary canal of F344 rats and B6C3F1 mice reported that, the histopathological findings in the rat small intestine were generally similar to those reported for mice. In mice, cytoplasmic vacuolization was observed. Also villous atrophy and crypt cell hyperplasia were noticed. Vacuolization can be a sign of injury and thus suggests that damage to the villous epithelium resulted in crypt epithelial hyperplasia in mice. There are many potential causes of vacuolization including altered lipid metabolism, sequestration of absorbed material, autophagy, endoplasmic reticulum stress, and proteasome dysfunction (**Mimnaugh et al., 2006., Franco and Cidlowski.,2009**).

The proliferative activity of the parotid salivary glands was detected in the present work using an immunohistochemical staining of proliferating cell nuclear antigen (PCNA). The present study revealed that intense expression of PCNA was recorded in the nuclei of the acinar cells of the parotid salivary glands after 4 months of Cr (VI) administration. This could be an indication for increasing proliferation rate, as an attempt to repair and renew the damaged cells. **Pusztai et al. (1993)** stated that, the accelerated proliferation might indicate an increased mutagenic risk on cells. **Itall et al. (1990)** concluded that PCNA which were involved in the cellular cycle could be identified in replicating cells of both benign and malignant lesions.

Reduction of Cr (VI) to the less permeable and bioavailable Cr (III) is thought to occur primarily in the stomach, as a mechanism of detoxification. Gastric reduction has been hypothesized to be efficient, such that oral exposure to Cr (VI) would not result in toxicity or carcinogenicity, except perhaps in

the stomach (**De Flora et al.,1997; Proctor et al.,2002**).

Cr (VI) is genotoxic in a number of in vitro and in vivo test system (**De Flora et al., 1990**); however, the mechanisms of genotoxicity and carcinogenicity are not fully understood. Because Cr (VI) as chromate structurally resembles sulfate and phosphate, it can be taken up by all cells and organs throughout the body through non-specific anion transporters (**Costa, 1997**).

Once inside the cell, indirect DNA damage may occur through the generation of oxygen radicals during intracellular reduction of Cr (VI) through the more reactive pentavalent and tetravalent chromium to Cr (III) (**O'Brien et al., 2003**). Cr (III), the final product of intracellular reduction of Cr (VI), has been shown to interact directly with DNA and other macromolecules to induce chromosomal alterations and mutational changes (**O'Brien et al., 2003**) (**Reynolds et al. et al., 2007**).

The present investigation proved that the degenerative changes induced by hexavalent chromium in the parotid salivary glands of male albino rats decreased in the group given vitamin C. The antioxidant effect of vitamin C was evidently to partly prevent the toxicity of Cr (VI).

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