Histological and ultrastructural study of the effect of potassium dichromate with evaluation of potential protective role of vitamin C on submandibular salivary gland of rats

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Abstract: Potassium dichromate is a heavy metal found in rocks, plants and animals that commonly used in paints, stainless steel manufacturing and food additives. Contamination of water with dichromate results in serious damage in body. Vitamin C is a potent hydrophilic antioxidant able to scavenge a variety of free radicals and oxidative molecules. The aim of this study is to evaluate the effect of potassium dichromate on submandibular salivary glands (SMGs) of rats with evaluation of the protective role of vitamin C. The present work was carried on healthy 30 adult male albino rats which were randomly divided into three groups. Group (I) act as control group, group (II) which received potassium dichromate and group (III) which received the same dose of potassium dichromate but with vitamin C. At the end of the experiment, the rats were sacrificed and the submandibular salivary glands were collected. The specimens then underwent light and electron microscopical study. Light microscopical examination of SMG specimens in group (II) revealed fatty degenerative changes with loss of normal architecture. Interestingly, these damaging effects of potassium dichromate on SMGs were decreased in group (III) after treatment by vitamin C. Ultrastructural study of specimens of potassium dichromate group showed degenerative changes represented by cytoplasmic accumulation of lipid droplets that substitute SMG structure together with wide perinuclear membrane, distended rough endoplasmic reticulum cisternae (RER). Vitamin C-treated glands showed mild ultrastructural changes with almost normal RER, mitochondria, nuclei, perinuclear membrane and secretory granules. So, we can conclude that the exposure to chromium caused damaging effects on salivary glands and these damaging effects may be decreased by using vitamin C as a protective agent.

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Key Words: potassium dichromate, vitamin C, submandibular salivary gland, rats

1. Introduction:

Potassium dichromate, known by the formula (K2CrO7), is an inorganic soluble hexavalent chromium compound [(1)]. Chromium (Cr) is a heavy metal found in rocks, volcanic dust, plants and animals [(2)]. These compounds are commonly used in paints, stainless steel manufacturing, cement dust, wood treatment and food additives [(3)].

Chromium is present in two different stable forms, hexavalent Cr(VI) that can readily penetrate anionic channels in cell membranes and trivalent Cr(III) which doesn't cross cell membrane rapidly [(4)]. Cr(III) occurs naturally in the environment and is an important nutrient and dietary supplement, whereas Cr (VI) is produced by industrial processes [(5)].

Exposure to chromium is found among industrial workers and contamination of water with Cr(VI) is a worldwide problem resulting in serious damage in the body [(6, 7, 8)]. Moreover, Cr(VI) was found to interact with oxygen resulting in the formation of oxidizing agent that is known to induce allergic dermatitis and carcinogenesis in animals and humans [(9)].

On the other hand, Vitamin C (Ascorbic acid) is an essential micronutrient that performs important metabolic function in humans [(10)]. It is a potent hydrophilic antioxidant able to scavenge a variety of free radicals and oxidative molecules [(11)].

In addition, Vitamin C is transported to the cells in oxidized form as dehydro-ascorbic acid [(10)]. The highest concentration of vitamin C has been found in glandular tissue and the lowest in muscles and adipose tissues [(12)]. Moreover, it has been reported that vitamin C works as a major reducer of Cr(VI) in animals and cell culture models [(13)].

To the best of our knowledge, this study is the first to evaluate the histopathological and ultrastractural effect of Potassium dichromate on submandibular salivary gland of rats and to illustrate the potential protective role of vitamin C.

2. Material and methods:

Chemical:

Potassium dichromate (K2cr207) was supplied by El-Nasr pharmaceutical chemicals.

Animals:

The present work was carried out on healthy 30 adult male albino rats weighing between 200 to 300 g that were collected from Physiology Department, Medicine Faculty, Tanta University. All animals were conditioned at room temperature at natural photoperiod for 1 week before the start of the experiment. A commercial balanced diet and tap water ad libitum were provided. All animals received human care in compliance with the guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publication Number 85-23, Rev. 1985).

Experimental Design:

Animals were randomly divided into three groups 10 rats each. Group (I) act as control group (A positive control), which received intra-peritoneal injection of one ml distilled water; the solvent of the material used in this work. The second group (II) was the experimental group, which received potassium dichromate in a dose of 0.4mg/kg intra peritoneal/day for 15 days. The third group (III) was the protective group, which received the same dose of potassium dichromate but concomitant with vitamin C in dose of 25 mg/day orally for the same period.

At the end of the experiment, the rats were scarified and the submandibular salivary gland (SMG) was collected. The specimens then divided into two parts; one processed for light microscopical study and the second part was processed for electron microscopical study.

Specimen's collection and preparation:

At day 15 after all rats were sacrificed by decapitation. The SMGs of left side were dissected and immediately fixed in 10 % in buffered formalin for 24 h and embedded in paraffin. 5-µm thick cross-sections were taken from paraffin-embedded tissues and were examined under a light microscope after H&E staining, for histological evaluation.

While, the SMGs of the right side of each animal were cut into small parts of one cubic mm. that were fixed in glutraldehyde to be prepared for ultrastructural examination by the transmission electron microscope (TEM). Specimens were washed in three changes of phosphate buffer at pH 7.4. Secondary fixation was achieved in 1% osmium tetra-oxide at 40C, for 1.5 hours followed by rinsing in phosphate buffer. Specimens were then dehydrated in ascending grades of ethyl alcohol, the cleared in propylene oxide and embedded in epoxy resin. Semi-thin sections of 1-2 microns were cut and stained with 1% toluidine blue and examined by light microscopy for detection of the site to be studied by TEM. Ultra-thin sections were then cut using the ultra-microtome, mounted on copper grids and stained with uranyl acetate and lead citrate. The grids were examined by Joel TEM 100 CX trans-mission-electron microscopes at Electron Microscope Unit (EM), Faculty of Medicine, Tanta University, Tanta, Egypt.

3. Results:

Histopathological study of SMGs in group (I) revealed normal acinar and ductal cells together with the secretory terminal portions predominantly of the serous type and were composed of pyramidal cells with a foamy basophilic cytoplasm. These cells surrounding a narrow lumen together with a duct system consisted of intercalated and striated ducts. Also, it showed granular convoluted tubules that characterized by their columnar cells containing excretory granules. Whereas, a higher magnification showed striated ducts which having a wider lumen compared to intercalated ducts (Fig.1 A&B). H&E stained sections of group II showed severe degenerative changes represented by cytoplasmic accumulation of lipid droplets that substitute SMG structure. In addition, loss of normal architecture and degenerated acini were also detected (Fig. 2 & 3). After vitamin C treatment, the morphological appearance of SMGs was much closer to that of the controls (Fig. 4 A) with rare accumulation of few fat droplets (Fig. 4 B).

Under TEM, The acini of Group I showed normal pyramidal cells with basal round nuclei and accumulated secretory granules (Fig.5). Also, it showed normal ductal cells (striated ducts) with abundant mitochondria and normal nuclei (Fig. 6). TEM of Group II showed obvious changes as wide perinuclear membrane, distended rough endoplasmic reticulum cisternae (RER), irregular nuclei with, clumping and margination of heterochromatin, degenerated mitochondria and ill-defined hazy secretory granules (Fig. 7 &8). Mild ultrastructural alterations were noted in vitamin C-treated glands. They showed improved salivary gland structure with almost normal RER, mitochondria, nuclei, perinuclear membrane and secretory granules (Fig. 9 A&B).

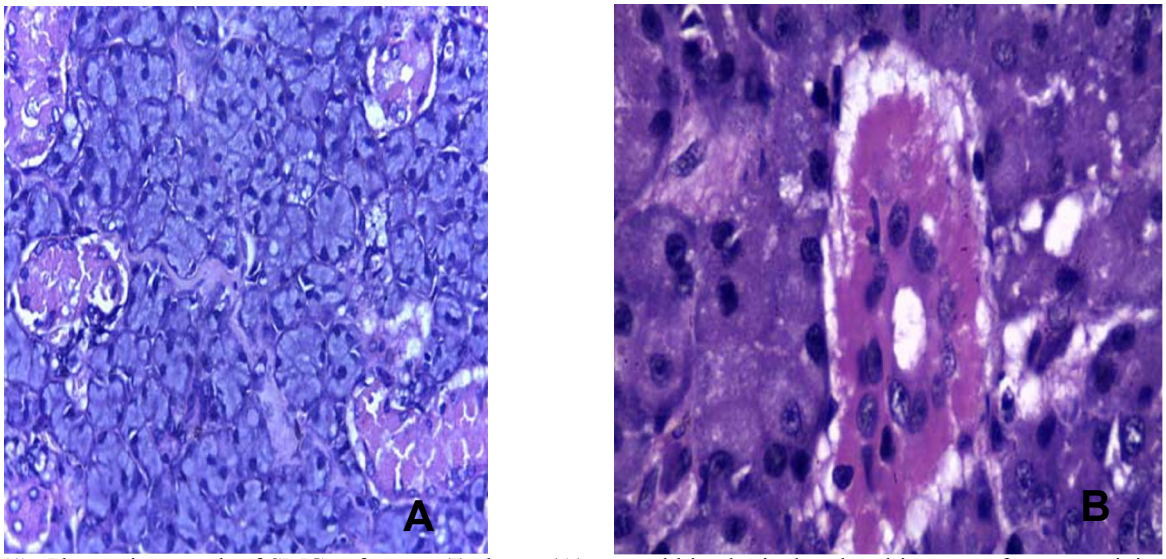

Fig. (1): Photomicrograph of SMGs of group (I) shows (A) normal histological and architecture of serous acini with duct system composed of intercalated ducts, striated ducts together with granular convoluted tubules and (B) represents higher magnification of the pervious photomicrograph showing striated duct with its lumen (H&E Ax100 & Bx400).

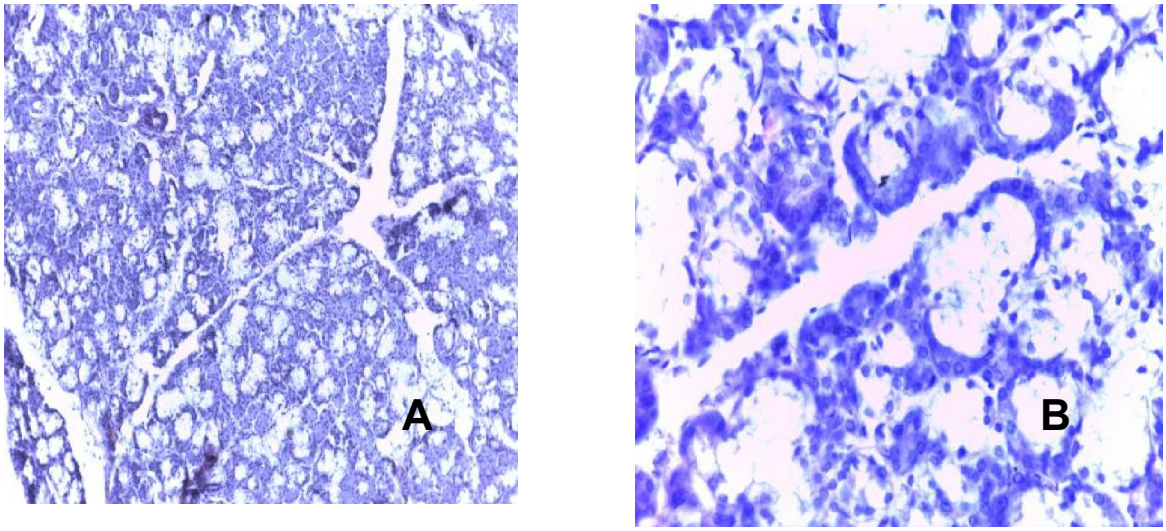

Fig. (2): Photomicrograph of SMGs of group (II) shows: (A) serous acini with loss of acinar structures and (B) is the higher magnification of the same section showing disturbed lobular architecture, fatty degenerative changes because of cytoplasmic lipid deposition (H&E Ax100 & Bx200).

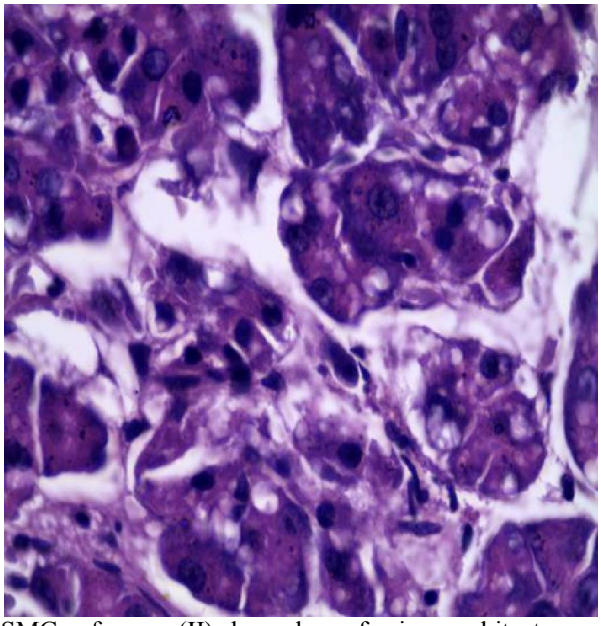

Fig. (3): Photomicrograph of SMGs of group (II) shows loss of acinar architecture with fatty degenerative changes associated with cytoplasmic lipid deposition (H&E x400).

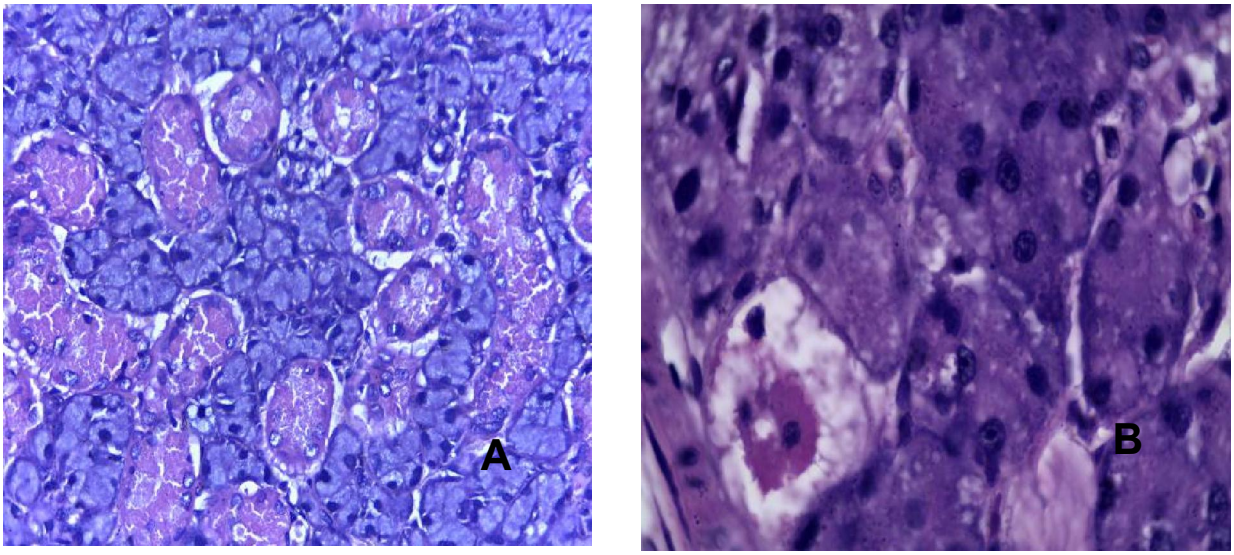

Fig. (4): Photomicrograph of SMGs of group (III) shows: (A) almost normal histological and architecture of serous acini together with nearly normal distribution of convoluted tubules. (B); A higher magnification that shows nearly normal lobular architecture distortion (H&E Ax100 & Bx400

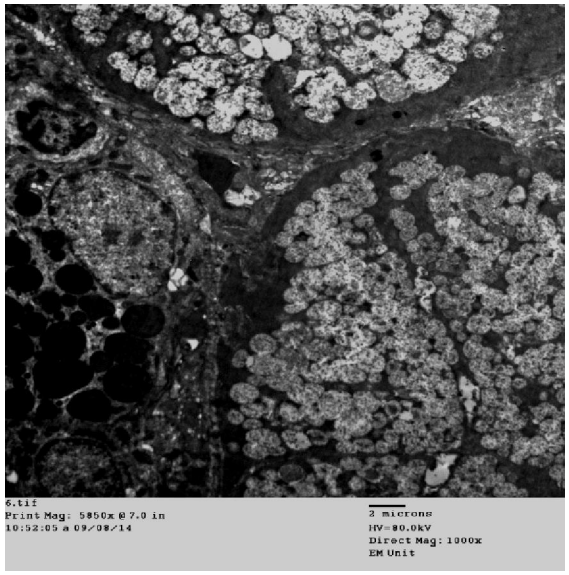

Fig (5): TEM of group (I) reveals cells of serous acini with intact cell boundaries together with electron dense basal nucleus that shows well intact and defined nuclear envelop with secretory granules of varying size and density. Also, it shows normal intercellular canaliculi (uranyl acetate and lead citrate x1000).

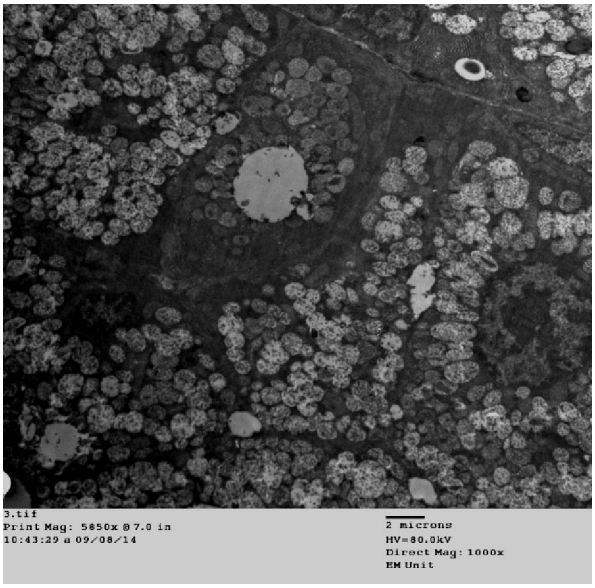

Fig (6): TEM of group (I) reveals striated duct with intact cell boundaries together with its will defined lumen (uranyl acetate and lead citrate x1000).

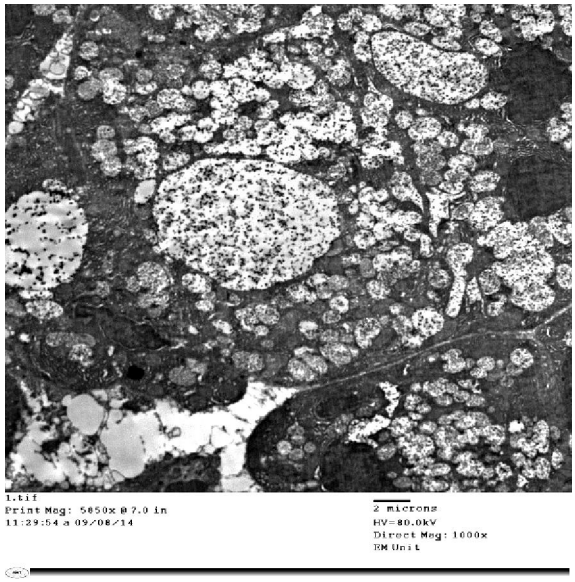

Fig (7): TEM of SMGs acini of group (II) reveals electron dense nucleus with ill definite outline. Also, a fusion of several secretory granules that associated with dilated intracellular canaliculi could be observed. (uranyl acetate and lead citrate x10000)

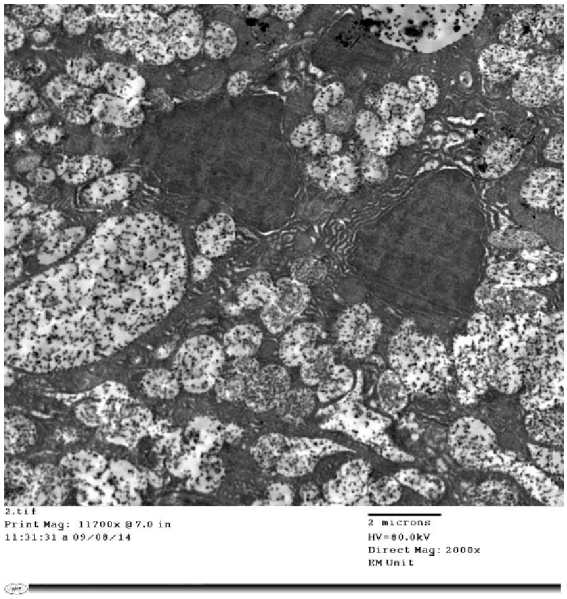

Fig (8): Higher magnification TEM of the previous SMGs acini of group (II) reveals ill definite outline of bifid nucleus and dilated RER (uranyl acetate and lead citrate x20000).

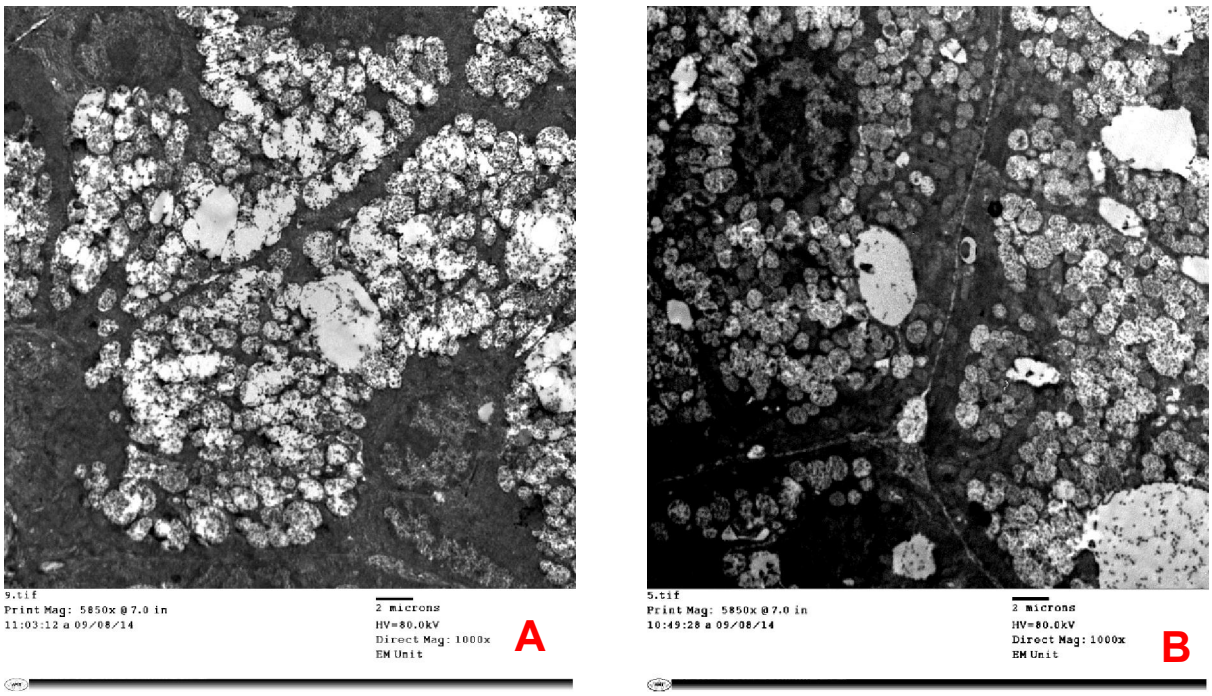

Fig. (9): A & B; TEM of SMGs acini of group III reveals electron dense nucleus having a regular outline, occasional confluence of secretory granules, few intracytoplasmic vacuoles and mild dilatation of intracellular canaliculi (uranyl acetate and lead citrate x1000)

4. Discussion

Potassium dichromate is known to act as oxidative compound on different body organs when individual is exposed to it on considerable amounts [9]. According to our knowledge, this study is the first to illustrate the effect of potassium dichromate on salivary glands.

In the present study, light microscopic examination of H&E stained sections of potassium dichromate group; salivary glands revealed abnormal acinar architecture, and variable-sized nuclei, in addition to ductal and vascular dilatation. Furthermore, the electron microscopic findings showed evidence of cell injury such as DNA fragmentation, dilated rough endoplasmic reticulum (RER) and increased mitochondria. In previous study, it was reported that Cr (VI) caused thyroid toxicity via enhancing cellular oxidative stress and decreasing activity of antioxidants[14]. Moreover, it was demonstrated that Cr(VI) often generates free radicals, which subsequently activate O_2 and produce reactive oxygen species (ROS), including hydroxyl radicals, singlet oxygen, superoxide and hydrogen peroxide[15], and consequently lead to DNA damage [16].

In addition chromium accumulated in the pituitary gland, considerably reduced cell activity, induced apoptosis and affected the pituitary hormone synthesis in developing rats [17]. In previous studies, gonads exposed to chromate were shown with significant decrease in serum sex hormones level associated with suppressed reproductive functions, further exposure to chromate agents have been reported to cause reproductive dysfunctions [18].

Furthermore, our results are consistent with that obtained in thyroid gland in which multiple damaging effects of chromium compound on the thyroid gland were revealed on the form of irregularity in size and shape of the follicles, others undergoing necrosis leaving empty spaces [19]. Moreover, these changes somehow similar to the effect on salivary glands that are exposed to other oxidative agents such as gamma irradiation [20].

These changes were attenuated on administration of vitamin C which is well-known as anti-oxidant. The group received the drug showed a milder degree of the effects as shown in both light and electron microscopical sections. These results may be explained by the ability of vitamin C to remove free radicals and oxidative molecules [11]. Moreover, vitamin C is a redundant of Cr(VI) that was demonstrated in animals and culture models [13].

In conclusion, exposure to chromium caused damaging effects on salivary glands. These damaging may be decreased by using of vitamin C which indicates a protective role for vitamin C.

Recommendation

Based on our data, vitamin C (ascorbic acid) is recommended for persons working in conditions where they are exposed to potassium dichromate as a

protective means. In addition, further investigations are required to explain the exact mechanism of the effect of potassium dichromate on salivary glands.

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