Histological changes of rat tongue papillae due to chromium toxicity and the protective role of vitamin E

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Chromium is an essential trace element whose physiological role is related primarily to the maintenance of normal glucose tolerance, because it serves as a cofactor for the peripheral action of insulin. It is present in the environment in several different forms including chromium (0), chromium (III), and chromium (VI). Hexavalent chromium (Cr VI) is thought to be the most toxic of the released metal ions. This study was done to evaluate such toxic effect on rat lingual papillae and the curative effect of vitamin E on the induced structural changes using light and electron microscopy. The results showed that chromium induced degenerative changes with the lingual papillae especially the filiform, which can be countered by vitamin E supplementation having an antitoxic effect which advocate re-epithelization and regeneration of the connective tissues.

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Introduction
Chromium is a naturally occurring element found in rocks, animals, plants, soil, and in volcanic dust and gases. It is present in the environment in several different forms. The most common forms are chromium (0), chromium (III), and chromium (VI). No taste or odor is associated with chromium compounds. Chromium (III) occurs naturally in the environment and is an essential nutrient, while chromium (VI) and chromium (0) are generally produced by industrial processes. The metal chromium, which is the chromium (0) form, is used for making steel. Chromium (VI) and chromium (III) are used for chrome plating, dyes and pigments, leather tanning, and wood preserving (1).

Chromium is highly resistant to oxidation, even at high temperatures. It is the sixth most abundant element in the earth’s crust, where it is combined with iron and oxygen in the form of chromate ore. Chromium is used in three basic industries: metallurgical, chemical, and refractory (heat-resistant applications), and these industries are the second largest source of ambient chromium (2).

Chromium exists in a series of oxidation states from -2 to +6...
valence; the most important stable states are 0 (elemental metal), +3 (trivalent), and +6 (hexavalent). The health effects of chromium are at least partially related to the valence state of the metal at the time of exposure. Trivalent Cr (III) and hexavalent Cr (VI) compounds are thought to be the most biologically significant. Cr (III) is an essential dietary mineral in low doses. Certain compounds of Cr (VI) appear to be carcinogenic, but insufficient evidence exists to determine whether Cr (III) or chromium metal can be human carcinogens. Cr (VI) is generally considered 1,000 times more toxic than Cr (III) \(^{(3)}\).

The toxicity, mutagenicity, and carcinogenicity of chromium compounds is a well-established phenomenon with supporting epidemiological evidence from all forms of life. Of the two environmentally available forms of chromium, hexavalent and trivalent, the hexavalent form has been demonstrated to be associated with the toxic parameters and classified as human carcinogen and mutagen \(^{(4)}\). Several studies have shown that the cellular uptake of chromate is several fold greater than that of the trivalent ion, because trivalent chromium is predominantly octahedral and diffuses slowly \(^{(5)}\). The tetrahedral hexavalent ion has been shown to enter the cell through general anion channels and bind to cellular components, causing disruptions in biochemical pathways. Reductive metabolism of chromium within the cell leads to the formation of various intermediate forms, Cr (V), Cr (IV), and Cr(III) \(^{(6)}\).

Chromium (III) negatively affects some enzyme activity, oxygen consumption, and intracellular ATP levels and is mutagenic. While there is overwhelming evidence to show that Cr(VI) complexes are mutagenic in bacterial and mammalian cells, most of the Cr (III) complexes are shown to be nonmutagenic. Emerging reports on the biotoxicity of chromium have emphasized the effects of the coordinated legend on metal activity \(^{(7)}\).

Chromium is a mineral that is necessary for impaired glucose tolerance, elevated blood cholesterol and triglyceride levels, promotion of weight loss, acne, stimulates enzymes in metabolism of energy, healthy blood circulatory system, synthesis of fatty acids, cholesterol and protein \(^{(8)}\). The most common toxic effects of hexavalent chromium are dermatitis, allergic reactions, skin and mucous membrane ulcerations, gastro-enteritis and hepatocellular deficiency.

After oral or dermal absorption of Cr (IV), kidney is the main
target organ for chromium accumulation, which might result in acute tubular necrosis in humans \(^{(9,10)}\). The dorsal surface of the tongue is heavily coated by the lingual papillae, especially the filiform papillae. They are sensitive to changes in the body, where they response to a number of systemic and local factors. Depapillation of the tongue has been described as a side effect of a number of medications usually antibiotics, cancer chemotherapeutic agents and metal toxicity \(^{(11)}\).

Vitamin E is an essential nutritional element which has a biologic antioxidant role for a variety of nutrients, metabolites, hormones and enzymes and it maintains the function of intracellular organelles and cellular membrane integrity \(^{(12)}\). No available studies were done according to our knowledge, to investigate the effect of chromium toxicity on dorsal surface of the tongue as well as the role of vitamin E against structural changes of the tongue.

**The purpose of this study** was to evaluate the histological changes of the epithelium and connective tissues of the tongue papillae due to chromium toxicity and the effect of vitamin E against such changes.

**Material and methods**

Twenty-four adult male albino rats (180-240g.) were used in this study and were kept under the same environmental condition. They were divided into two groups: **Group I**: Control group (6 rats) received tap water containing permissible concentration of hexavalent chromium Cr (VI) 50-100ug/L (50-100 ppm) \(^{(13)}\) for three months.

**Group II**: Study group (18 rats) was subdivided into:

- **Group A** (9 rats) received drinking water containing 450 ug/L of hexavalent chromium Cr(VI) as chromium trioxide* supplied in the form of brownish-red granules, ad libitum daily for three months.

- **Group B** (9 rats): received the same drinking water as in group A, but for four months and given oral dose of vitamin E in oil (2.5 mg) daily along the fourth month. The drinking water containing hexavalent chromium was prepared by weighting the toxic dose, and the measured amount were added and dissolved in 1 liter of tap water. After three months, the rats in group I and group II A were sacrificed. While rats in group II B were sacrificed after four months. Tongues from all groups were dissected and cut longitudinally into two halves, one half of each specimen was prepared for light microscopic examination using H & E stain and the other half for scanning electron microscopic examination.

* Winlab laboratory chemicals, leicestershore, LE6, 9E, Uk
Results
I. Light microscopic observations:
Group I (control group)
The dorsal surface of the tongue revealed evenly distributed prominent filiform papillae, regular in size, shape and orientation with normal covering keratinized epithelium formed of four layers. Well formed connective tissue, muscles and nerve fibers. The fungiform papillae were also revealed normal mushroom shaped and normal covering epithelium (Fig. 1)

Group II (study group)

Group II A:
The dorsal surface of the rat tongue in chromium toxicity revealed an overall different irregular appearance of the filiform papillae. They showed acanthosis and hyperkeratosis of the covering epithelium and hyaline degeneration of the basal and parabasal layers in some areas (Fig.2), while in other areas prominent secondary papillae, deeply stained nuclei and focal hyperkeratinization overlying the atrophied papillae (Fig.3). Decreased collagenous density and muscle fibers were observed (Fig.4). The fungiform papillae exhibited atrophic appearance and degeneration of the epithelial layers. The taste buds were absent and they were ill differentiated if present and covered with a thick layer keratinized epithelium (Fig.5)

Group II B:
The dorsal surface of the rat tongue in chromium toxicity treated with vitamin E showed re-epithelization of all epithelial layers, formation of well formed keratin layer all over the surface, well shaped filiform and fungiform papillae. Regeneration of the connective tissues and muscle fibers were seen (Fig 6, 7)

II. Scanning electron microscopic observations
Group I (control group)
The dorsal surface of the tongue exhibited evenly distributed filiform papillae with pointed tips. The papillae were regular in distribution, height, shape and orientation (Fig.8). Well formed fungiform papillae were observed in between the filiform papillae with well patent taste pore (Fig. 9)

Group II (study group)

Group II A:
The appearance of the filiform papillae ranged from blunting of the papillae tip, loss of height and thickness and randomly distributed (Fig. 10). The fungiform papillae revealed ill defined taste pore and increased desquamation of the superficial cells (Fig.11)
Group II B: With vitamin E supplementation for 30 days, the filiform papillae showed signs of regeneration through restoration of their normal thickness, height and orientation (Fig. 12). The fungiform papillae restored their integrity, distribution and taste function (Fig.13).

Fig1. Control group (I), dorsal surface of rat tongue showing numerous regular orientations of the lingual papillae covered by a keratinized epithelium formed of four layers. The connective tissue is heavily formed of dense fibers and muscles. H&E X 100.

Fig.2 Study group (IIA) chromium toxicity, dorsal surface of the tongue revealed ill formed filiform papillae with hyperkeratosis, acanthosis and hyaline degeneration of the basal and parabasal epithelial layers H&E X 400.
Fig.3 Study group (IIA), Atrophied filiform papillae with focal hyperkeratosis and deeply stained nuclei  H&E  X 400.

Fig.4 Study group (IIA), overall ill formed filiform papillae in shape, size and orientation with atrophy of the connective tissue fibers and tongue musculatures  H&E  X 100.

Fig.5 Study group (IIA), altered fungiform papilla exhibiting multiple areas of hyaline degeneration, ill differentiated taste bud and thick layer of keratinized epithelium. The connective tissue shows inflammatory cells infiltration  H&E  X 400.
Fig. 6 Study group (IIB), after vitamin E supplementation, the dorsal surface of the tongue shows signs of tissue regeneration. Normal thickness of keratinization is covering the whole epithelial surface. Well-formed filiform papillae, while some degenerative changes still present. H&E X 400.

Fig. 7 Study group (IIB), well formed fungiform and filiform papillae. Normal density of the connective tissue and muscle fibers. H&E X 100.

Fig. 8 SEM of the dorsal surface of the tongue in control group (I). The filiform papillae are regular in size, shape and orientation. X 200.
Fig.9 SEM. Higher magnification of the previous figure showing well formed fungiform papilla with distinct taste pore on the surface X 500.

Fig.10 SEM of the study group (IIA) chromium toxicity. The filiform papillae are rounded, short, thin and irregularly distributed X 200.

Fig.11 SEM of the study group (IIA). Desquamated fungiform papilla with absence of the taste pore X 500.
Fig. 12 SEM of the study group (IIB) after vitamin E administration showing partial restoration of the normal appearance of the dorsal surface of the tongue. The filiform papillae appears regular in shape, size and prominent with a pointed tip X 200.

Fig. 13 SEM of the study group (IIB) showing regenerated fungiform papilla with taste pore X 500.
Discussion

Significant quantities of toxic metallic elements such as cadmium, chromium, lead, mercury and nickel are being introduced into our environment from natural man-made sources. Chromium at the physiological levels is involved in the regulation of normal carbohydrate metabolism in mammals and so it is considered as an essential trace element\(^{(14)}\). The trace elements have been shown to influence a number of biochemical and physiological processes. The two major recognized functions are to act as cofactors for metal-ion-activated enzymes or to form such a tight complex with the protein that the two are isolated together as a unit called metallocenzyme. Trace elements play a part in the synthesis and structural stabilization of both proteins and nucleic acids. They are also constituents of proteins and hormones. In addition, they are involved in the function of sub cellular systems such as mitochondria, as well as in membrane transport, nerve conduction, and muscle contraction. Some of them (Cu, Zn, Mn, and Cr) act as antioxidants\(^{(14)}\).

The entry routes of chromium into the human body are inhalation, ingestion, and dermal absorption. Occupational exposure generally occurs through inhalation and dermal contact, whereas the general population is exposed most often by ingestion through chromium content in soil, food, and water. Rates of chromium uptake from the gastrointestinal tract are relatively low and depend on a number of factors, including valence state with Cr (VI) more readily absorbed than Cr [III]), the chemical form (with organic chromium more readily absorbed than inorganic chromium), the water solubility of the compound, and gastrointestinal transit time\(^{(15)}\). It was found that, after entering the body from an exogenous source, Cr (III) does not readily cross cell membranes, but binds directly to transferrin, an iron-transporting protein in the plasma. In contrast, Cr (VI) is rapidly taken up by erythrocytes after absorption and reduced to Cr (III) inside the cell. Regardless of the source, Cr (III) is widely distributed in the body and accounts for most of the chromium in plasma or tissues. The greatest uptake of Cr (III) as a protein complex is via bone marrow, lungs, lymph nodes, spleen, kidney, and liver\(^{(16)}\).

Ingestion of Cr (VI) (250,500 or 750 ppm as potassium dichromate,K\(_2\) Cr\(_2\) O\(_7\)) through drinking water by female rats for 3 months prior gestation was toxic to embryo and fetus. There was a significant reduction in
number of implantations and number of fetuses and an increase in number of resorption and pre-implantation and post-implantation losses. Skeletal abnormality was also observed in fetuses (16). Apoptosis appear to be the mode of cell death in the presence of both Cr (V) and Cr (VI) (17).

The filiform papillae are widely distributed on the dorsal surface of the tongue and they undergo lengthening, loss and atrophic changes faster and earlier than other papillae. It was stated that the filiform papillae are of high metabolic activity, so any enzymatic disturbance, vascular insufficiency or nutritional deficiency result in atrophy of these papillae. The filiform papillae were seen as a mirror that reflects the general health status (18). The atrophy of the lingual papillae in this study may be due to the mechanism of inhibition of epithelial reproduction through the cytotoxic effects of the Cr(III) despite its lowered cellular permeability compared with Cr(VI), the charge and ionic character of Cr(III) complexes have been found to play a prime role in mediating cellular entry of Cr(III). The observed cellular damage in terms of morphological changes, chromatin condensation, formation of apoptotic bodies, and DNA fragmentation suggests that Cr(III) can be cytotoxic at the nuclear level (19). Not only can cellular uptake of chromium be toxic, but also the accumulation of trivalent ions at the cellular membrane surface is thought to inactivate or denature nucleoside permeases (20).

In view of the effect of chromium on man and marine life, it is found that the El- Max Bay in Alexandria represents one of the highest contaminated area with chromium where it receives various sources of pollutants. Doses of chromium used in this study were not usually found in the environment, but it may be encountered in the workplace in the vicinity of industrial establishments as mentioned by Kanojia et al (15). The dose given to the study group corresponded to the chromium concentration in El-Max Bay (21). The estimated safe and adequate daily dietary intake for trivalent chromium between 50-200 ug/day (22), however, food processing such as meat grinding and homogenization using stainless-steel equipment may increase chromium contend of food (23).

As fibroblasts are believed to be an important source of reactive oxygen metabolism (24), so the oxidative effect of chromium on carbohydrates leads to disturbance in fibroblasts function and consequently
decreasing collagen density and muscle formation

Vitamin E is antioxidant which prevents the oxidation action of chromium, it protects the biological function of cell membrane against the deleterious effects of molecular oxygen. In this respect it maintains the function of intracellular organelles such as mitochondria and Lysosomes, and ensures the stability and integrity of cell membrane \(^{(25,26)}\). This could be the cause of re-epithelization and formation of new cells and taste buds in group IIB treated with vitamin E. Degeneration of the lining connective tissue and tongue musculatures as an effect of chromium toxicity followed by regeneration depends on the role of vitamin E as a cofactor in some enzymes \(^{(27)}\).

**In conclusion,**
Vitamin E is an essential nutrient element could be given to help in restoring the integrity of the damaged oral epithelial tissue and taste sensation after degenerative effect of chromium toxicity.

**References**


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الملخص العربي

دراسة هستولوجية على التغيرات في الحلمات اللسانية عند الفئران الناتجة عن التسمم بالكروم ومدى التأثير العلاجي للفيتامين (E) عليها

الكروم هو أحد العناصر الأساسية الضئيلة المقدرة والتي تلعب دوراً فسيولوجيًا مبديئاً في الحفاظ على النسبة الطبيعية للجلوكوز في الجسم وقد نتجت نتائجه استخدامه على تفاعل الأنسولين الجانبي. ويوجد الكروم في الطبيعة على هيئة عديدة ومختلفة مثل كروم ثلاثي الكافو وكروم سداسي الكافو والذي يعتبر من أكثر العناصر سمية.

أجرت هذه الدراسة على أربعة وعشرين فأرا لمعارفة مدى التأثير التسميمي للكروم على حلمات اللسان وأيضاً دراسة مدى التأثير العلاجي للفيتامين (E) على التغيرات الناتجة من التأثير التسميمي وذلك باستخدام المجهر الضوئي والمجهر الإلكتروني المناسب.

وقد أظهرت النتائج أن جميع حلمات اللسان الخيطية والقطارية قد اختلفت شكلها، كما لوحظ اضطراب في تكوين الخلايا المكونة للغشاء الخاص المغطي للحلمات اللسان وبراعم التذوق ومع زيادة كثافة طبقة الكيراتين مع حلول في النسيج الضام.

وقد لوحظ عند إعطاء فيتامين (E) في ماء الشرب، تأثير إيجابي، حيث وجد أنه مضاد للتسمم ولد تأثير فعال في إعادة بناء الخلايا المدمرة وتكوين النسيج الخشفي والضام بشكل طبيعي وإرجاع الحلمات اللسانية إلى شكلها الطبيعي.

وعلى ذلك فإن زيادة عنصر الكروم تؤدي إلى تأثير تسميمي على أنسجة الجسم وصحة الفم واستخدام فيتامين (E) له دور أساسي فعال في مقاومة أعراض التسمم.