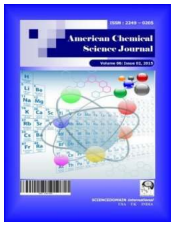

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Modulatory Role of Zinc Chloride on Cadmium Chloride-induced Histological Changes in Tongue Papillae of Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SEA and AAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors BAJ and AAA managed the analyses of the study. Author SEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cadmium (Cd) is recognized as one of the most toxic heavy metals with very strong accumulation. In dentistry, Cd may be released from dentures, intraoral alloys. Some strategies have been postulated to neutralize heavy metal intoxication such as zinc supplementation. The aim of this research project was to investigate the effect of Cd on tongue papillae of rats, and the possible modulatory role of zinc.
Materials and Methods: *In vivo* study was carried out on forty adult male *Albino* rats. All the animals were maintained on standard laboratory diet, rats were divided into four equal groups:

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GI: Rats were drinking purified water for 6 months. **GII:** Zinc was given in aqueous solutions of zinc chloride for the same period as group I. **GIII**: Rats were receiving Cd also in the form of aqueous solutions of cadmium chloride for the same period as the other groups. **GIV**: Rats were receiving similar cadmium treatment as group III along with zinc treatment as group II. After 6 months, rats were sacrificed and tongue was studied under light microscope using routine H&E stain.

Results: Results of this study demonstrated that 70%, 70%, 50%, 30%, 70%, and 70% of the total cases of group III revealed abnormal shape of filiform papillae, hyperkeratosis, acanthosis, epithelial dysplasia and inflammatory cell infiltration with dilation of blood vessels respectively, while all cases (100%) in group I and II showed normal histological features. These Differences were statistically significant ($p<0.005$). Cases in group IV showed abnormal shape of filiform papillae (70%) without any changes at the cell level in the epithelium and the connective tissue.

Conclusion: Cadmium chloride may cause histopathological changes on the tongue mucosa of rats. While zinc chloride may prevent histopathological cell changes.

Keywords: Cadmium; toxic heavy metals; Zinc; tongue papilla.

1. INTRODUCTION

Cadmium (Cd) is recognized as one of the most toxic heavy metals with very strong accumulation, and a half-life of 26 years in human body [1]. So it has enough time to accumulate and cause tissue damage in several organs [2]. Cd is used in nickel-cadmium batteries, plastic stabilizers, and paint pigments [3]. It can be found in soils because insecticides, fungicides, sludge, and commercial fertilizers that contain cadmium are used in agriculture. Cd may be found in reservoirs containing shellfish. Cigarettes also contain Cd, smoking is the main source of human exposure to environmental Cd [4]. Smoking 20 cigarettes one may absorb 1–2 µg of Cd into the blood [1]. So most human Cd exposure comes from food, water, air, or absorption through the skin [5].

Moreover, in dentistry, Cd may be released from intraoral alloys [6], as the intermetallic compound dental amalgam may contain approximately 4.5 µg/g Cd in the metal–matrix alloy [7]. Another potential source of Cd is a metal dental bridge in which a Cd-containing alloy has been used for soldering [8]. Even in dental acrylic-based resin, Cd might be used as coloring agent in denture base materials [9]. Cd elution in the mouth is increased with the raise in the acidity of the saliva [10].

Cadmium is a multi-target toxicant for a number of cell types and tissues. The etiology and pathology of cadmium toxicity depends on and varies highly at different concentrations and tissue types [11,12]. It has been reported that Cd caused hepatic toxicity [12], histopathological changes in the renal tissue [13], focal testicular necrosis [14], osteomalacia, osteoporosis, hypertension, anaemia, arteriosclerosis and cancer [15-17]. Cd has also been shown to inhibit the activities of various pancreatic proteases [18]. Regarding the intraoral tissues, exposure to cadmium induces ultrastructural changes in the mucous and serous cells of the submandibular salivary gland, which are mainly observed in the cell nucleus, Golgi Apparatus [19], higher odds of periodontal disease [5] and grouping of numerous bacteria which are attached the queratinized squamous epithelial cells of the tongue mucosa [20] Cd-induced peroxidation caused the release of free oxygen radicals [21]. These free radicals cause the stimulation and destruction of sensitive macromolecules and tissues [22].

Many strategies have been postulated to neutralize heavy metal intoxication such as zinc supplementation [23], vitamin C and selenium [24,25]. They cause the inhibition of peroxidation, mopping up of free oxygen radicals and disorganization and breakage of peroxidation chain reactions [26] via inhibition of gluthathione peroxide, Protein Kinase C (PKC) and calcium metabolism [24,25] leading to blockage of the oxidative mechanisms [26].

Little information is available in the literature about the effects of Cd toxicity on the histology tongue mucosa, which is the largest organ in the oral cavity and heavily coated by the lingual papillae, especially the filliform papillae. They are sensitive to changes in the body, where they response to a number of systemic and local factors, some medications usually antibiotics, cancer chemotherapeutic agents and metal toxicity [27].

Therefore, the aim of this research was to study the modulatory role of zinc chloride on cadmium chloride-induced histological changes in tongue papillae of rats

1.1 Objectives

The objectives of this study are:

- First, to examine the tongue papillae of adult male albino rats after cadmium administration at the light microscopic levels.
- Second, to study the histological changes of the tongue papillae after simultaneous supplementation of zinc along with cadmium.

2. MATERIALS AND METHODS

This study was carried out on forty adult male albino rats weighting between 200-225 gms. All animals were maintained on standard laboratory diet, rats were divided into four equal groups as follow:

Group I (Control group): Rats were drinking Nestle water (Nestle water, Springs water factory. Dammam Saudi Arabia) for 6 months.

Group II (Zinc group): Zinc (Loba Chemie Laboratory Reagents & Fine chemicals, India) was given in aqueous solutions of zinc chloride for the same period as group I.

Group III (Cadmium group): Rats were receiving cadmium (Sigma-Aldrich, Fluka' Analytical, India) also in the form of aqueous solutions of cadmium chloride (at a concentration of 5 mg/dm^3) for the same period as the other groups**.**

Group IV (Cadmium and Zinc group): That was a combination group, the rats were receiving similar percentage of cadmium as group III along with zinc treatment as group II.

Cadmium was obtained as cadmium chloride ($CdCl_2$), it was dissolved in drinking water from Nestle (Nestle water, Springs water factory, Dammam Saudi Arabia)at a concentration of 5 mg Cd/dm^3 (equivalent to environmental exposure) (28).

Zinc was used in the form of zinc chloride and dissolved in distilled water at concentrations of 30 mg Zn/dm^3.

After the exposure termination, the animals were sacrificed, tongues from all groups were dissected and each tongue was cut longitudinally into two halves. One half (n=10) of each specimen were prepared for light microscopic examination using routine H & E stain.

2.1 Light Microscopy

Specimens were fixed in 10% formalin for 24 hours, washed under running tap water over night and then dehydrated through ascending grades of alcohol, cleared in xylol and embedded in paraffin. Sections of 5 µ thickness were cut, mounted on glass slides and stained with haematoxylin and eosin.

Prepared slides were evaluated under light microscope by 2 independent specialists (AAA, SA) after discussing and agree the evaluation criteria. The following histopathological changes were evaluated as follow:

1- Shape of filiform papilla: (0) normal, (1) Atrophic, (2) Hypertrophic, (3) Ill-defined.
2- Keratin layer: (0) Normal, (1) Hyper-Orthkeratosis, (2) Hyper-parakeratosis.
3- Thickness of epithelium: (0) Normal, (1) atrophic, (2) Hypertrophic.
4- Presence of epithelial dysplasia: (0) No epithelial dysplasia, (1) Mild epithelial dysplasia, (2) Moderate epithelial dysplasia, (3) Severe epithelial dysplasia.
5- Presence of Inflammatory cell infiltration: (0) No inflammation, (1) Acute inflammatory cell infiltration, (2) Chronic inflammatory cell infiltration.
6- Blood vessels: (0) Normal, (1) dilated blood vessels.

2.2 Ethical Approval

Research complies with the commonly-accepted '3Rs' for doing research on animals. Before starting this study, the research project was approved by University of Dammam Standing Committee for Research Ethics on living creatures.

2.3 Statistical Analysis

Data analysis was performed by using SPSS-19.0. All data variables were presented in terms of frequencies and percentages due to categorical type. Chi-square test was applied to see any significance of the effect of zinc chloride

and cadmium chloride alone and in combination in relation with the control group based on papilla examination and histopathological features. P-value less than 0.05 was considered statistically significant result.

3. RESULTS

The dorsal surface of rat's tongue of control group(group I) revealed evenly distributed prominent filiform papillae, regular in size, shape, and orientation with normal covering keratinized stratified squamous epithelium, well-formed connective tissue core, muscles and blood vessels (Fig. 1).

The dorsal surface of therat's tongue of the zinc group (group II) reveals almost normal histology of filiform papillae similar to those of the control group (Table 1).

The dorsal surface of rat's tongue in cadmium toxicity group (group III) revealed abnormal shape of filiform papillae, hyperkeratosis, acanthosis, epithelial dysplasia and inflammatory cell infiltration with dilation of blood vessels in 70% (7 cases), 70% (7 cases), 50% (5 cases), 30% (3 cases), 70% (7 cases), and 70% (7 cases) of the total 10 cases of this group respectively (Figs. 1 and 2). Differences between the histopathological features in group III and other groups were statistically significant ($p<0.005$).

The dorsal surface of the rat's tongue of cadmium and zinc group showed abnormal shape of filiform papillae (70%) without any changes at the cell level in the epithelium and the connective tissue core (Table 1).

4. DISCUSSION

The results of the present study highlighted two points, the toxic effect of cadmium chloride on the tongue mucosa of rat and the modulatory effect of zinc chloride.

With regard to the first point of this paper, results of this study demonstrated that cadmium chloride induced degenerative changes in the epithelium and the connective tissue components of the rats tongue which illustrated ill-defined shape of filiform papilla, hyperkeratosis and acanthosis of tongue epithelium, epithelial dysplasia, inflammatory cell infiltration and dilated blood vessels in the subjacent connective tissue. Similar results was reported by Picoli et al. [29] and Ribas et al. [30] who observed epithelial hypotrophy in the floor of the mouth and maxillary molar junctional epithelium of the rats due to direct action of cadmium in oral mucosal cells. Also, Deveci and Deveci [31] reported increase in keratinization and hypertropy in epithelium, the underlying connective tissue depicted dilated blood vessels and mononuclear cell infiltration of the esophagus of rats exposed to cadmium chloride.

Cadmium exposure induced metallothionein synthesis and consequent displacement of zinc and copper of the metallothionein channels [32]. When the amount of cadmium exceeds the amount of metallothionein, cadmium begins its toxicant effects. Cadmium is known to cause adverse effects on numerous cell processes from lead metabolism interruption to the eventual death of cells [33]. Cadmium-induced oxidative destruction of membrane polyunsaturated fatty acids [34], leading to loss of membranes fluidity, full in their potentials increased their permeability [35]. Lipid peroxidation seemed to be the major mechanism of free radical toxicity. Cadmium-induced lipid peroxidation was reported in various organs such as liver, testes, heart, lung and brain [36,37]. Cadmium was shown to cause oxidative stresses that could directly or indirectly produce major interrelated derangements of cell metabolism including peroxidation of lipid and damage to membrane ion transport systems [38,39]. Cadmium inhibits Na^+/K^+-ATPase (40) as well as Ca^{2+}-ATPase leading to an increase in the intracellular calcium concentration [41,42].

Cadmium is toxic to cellular processes by disrupting mitochondrial function [43] leading to inadequate energy supply. Cadmium disturbed the integrity of mitochondrial membranes concomitantly decreasing ATP/ADP ration. Also, cadmium stimulated lipid peroxidation in mitochondria [44]. Cadmium-induced collapse in the electrochemical gradient resulted from alteration in the activity of the respiratory chain and/or citric acid cycle [45]. Also, cadmium can interfere with the transportation and metabolism of many essential metals, such as iron, copper and zinc [46]. These facts may explain the histopathological changes found in the results of this study.

Some specimens in our study demonstrated that cadmium-induced epithelial dysplasia, at the dorsal surface of rat tongue. These finding are in agreement with that illustrated in the epithelium of the ventral rat prostate [47]. Cd-treated rats

showed an immunoexpression increase of Lysophosphatidic acid (LPA)-1 receptor, an increment of cell proliferation, a decrease of apoptosis, and an increase of angiogenesis in dysplastic lesions [48]. LPA-1 is associated with prostate cancer development [49,50]. Animal and occupational studies have been suggested that cadmium is carcinogenic [51,52].

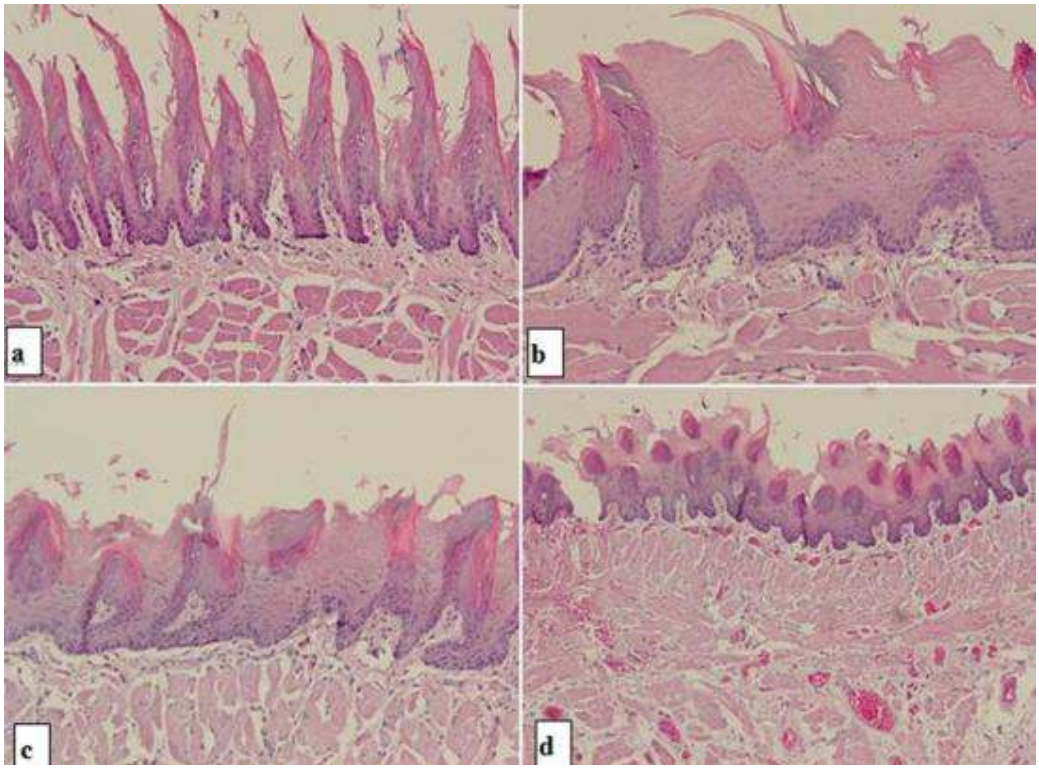

Fig. 1. Microscopic photo showing: (a) normal filiform papillae with thin smooth keratinized epithelium covering dorsal surface of rat's tongue. Control group, H&E X100. (b) Acanthosis with hyperorthokeratosis and mild inflammatory cell infiltration, H&E X200. (c) Abnormal shape of surface of the filiform papillae with abnormal architecture of the rete ridges H&E X100. (d) Dilated blood vessels in the subjacent connective tissue H&E X100

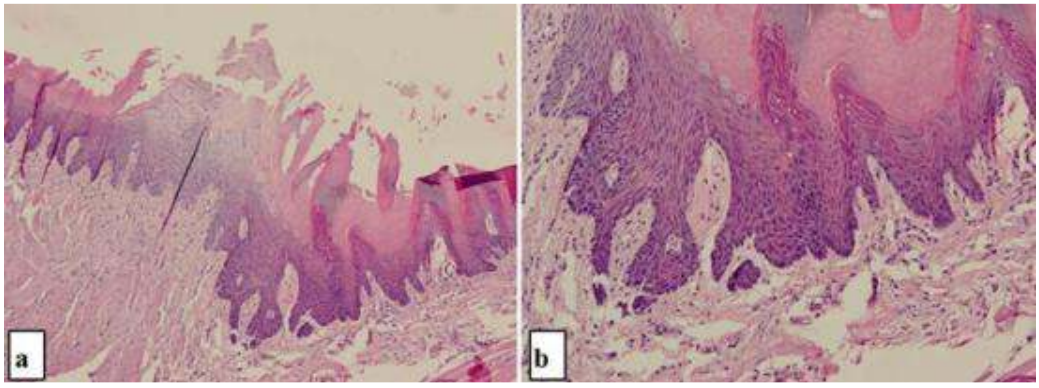

Fig. 2. Microscopic photo showing dysplastic squamous stratified epithelium. (a) H&E X100. (b) H&E X200

Table 1. Histopathological differences among different groups

Criteria	Group I		Group II		Group III		Group IV	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Shape of filiform papilla	10 100%	0 0%	8 80%	2 20%	3 30%	7 70%	3 30%	7 70%
Keratin layer	10 100%	0 0%	10 100%	0 0%	3 30%	7 70%	10 100%	0 0%
Thickness of epithelium	10 100%	0 0%	10 100%	0 0%	5 50%	5 50%	10 100%	0 0%
Presence of epithelial dysplasia	10 100%	0 0%	10 100%	0 0%	7 70%	3 30%	10 100%	0 0%
Presence of Inflammatory cell infiltration	10 100%	0 0%	10 100%	0 0%	3 30%	7 70%	10 100%	0 0%
Blood vessels	10 100%	0 0%	10 100%	0 0%	3 30%	7 70%	9 90%	1 10%

With regard to the second point of this study, the present study demonstrated that zinc supplementation had a protective effect against cadmium induced cytotoxicity on filiform papillae.

Simultaneous administration of zinc together with cadmium appeared to be extremely helpful in protection against cadmium toxicity. Histologically, compared to those treated with cadmium alone, the filiform papillae had abnormal appearances in 70% of the total cases of this group but it did not show any pathological changes at the cell level. They had normal epithelium; the covering keratized epithelium had no atrophy, hypertrophy or dysplasia. The connective tissue core did not show inflammatory cell infiltration or dilated blood vessels. This protective effect of zinc seemed to be associated with induction of metallothionein synthesis [53]. Metallothionein a low molecular weight, cystene rich protein contributes to protection against cadmium-induced cytotoxicity by sequestering cadmium. Each molecule of metallothionein could bind seven atoms of cadmium [54], resulting in diminished distribution of cadmium to the critical oarticulate fraction and decreasing the high molecular weight in the cytosol where the cadmium exihibits its cytotoxicity [55]. metallothionein could prevent both cadmium-induced acidification and disruption of ionic balance across plasma membranes [53].

Also, the protective effect of zinc could be due to non-metallothionein mechanisms, which include competition of zinc with cadmium either at the cell surface or at intercellular sites where cadmium exhibits its toxicity [23]. Zinc might block entry of cadmium via voltage-sensitive channels [56]. Zinc might repress the putative orphan receptor for cadmium and abolish its responsiveness to cadmium [57]. In addition, zinc had an antioxidant effect and had a role against lipid peroxidation through increasing intracellular content of glutathione [58,34].

5. CONCLUSIONS

We could conclude that:

- Intoxication caused by cadmium chloride in rat tongue led to histopathological alteration of the filiform papillae at the light microscopic level with changes of the covering squamous stratified epithelium.
- Simultaneously, administration of zinc chloride together with cadmium chloride could have a modulatory effect against cadmium toxicity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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