

# Zinc Oxide Nanoparticles Attenuate Cadmium Chloride Induced Tongue Toxicity in Adult Male Albino Rats (Histological and Biochemical Study)

Original  
Article

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## ABSTRACT

**Introduction:** Cadmium is a toxic heavy metal naturally present in plant food and unavoidable pollutant for many dental procedures. This metal shares in toxic peroxidation and destruction of macromolecules in different tissues, besides inhibition of DNA repair enzymes. The tongue is one of the vital organs that could be affected by chronic cadmium toxicity, especially the filiform papillae and tongue skeletal muscles due to their high metabolic activity.

**Aim of Work:** To study the possible role of Zinc oxide nanoparticles (ZnO NPs) in attenuation of the structural, ultra-structural and biochemical changes in rats' tongues induced by Cadmium chloride.

**Materials and Methods:** Sixty adult male Albino rats were divided equally -according to the utilized substance- into three groups; control, Cadmium chloride (CdCl) and (CdCl+ ZnO NPs) groups; every group received the allocated materials for 4 weeks. Samples were processed for light, electron microscopic examinations and biochemical analysis (malondialdehyde; reduced glutathione; catalase; superoxide dismutase; glutathione peroxidase).

**Results:** Cadmium produced various apparent histopathological and biochemical alterations in tongue tissues in the form of filiform papillae with blunted ends, and thick keratin plaques. Epithelial cell layers appeared with vacuolated cytoplasm and dark nuclei. Degenerated muscle fibrils with wide spaces between them and many collagen fibers. Significant decrease of tongue reduced GSH, SOD, GPx and Cat enzymes activity and significant increase in MDA level in Cadmium chloride group as compared to control group. ZnO NPs attenuated these changes and revealed pointed tipped filiform papillae, nearly normal cells and Parallel arrangements of myofibrils. The levels of reduced GSH, SOD, GPx, Cat and MDA were insignificant compared to control group.

**Conclusion:** Zinc oxide nanoparticles (ZnO NPs) attenuated the structural and biochemical changes in rats' tongues induced by Cadmium chloride.

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**Key Words:** Cadmium, histology, tongue, zinc oxide nanoparticles.

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## INTRODUCTION

Cadmium (Cd) is one of the non-essential toxic heavy metals naturally present in the environment and human food. It is poisonous and its exposure induces various health damages<sup>[1,2]</sup>. It is a fixed constituent of prosthesis used in various dental procedures such as dental amalgam, metallic dental bridges and dental acrylic-based resin<sup>[3]</sup>. Cadmium, copper, zinc, silver, and nickel, are more liable to be released from dental alloys<sup>[4]</sup>. Increased salivary acidity enhances its elution from these dental prosthesis leading to exaggeration of its harm<sup>[5-7]</sup>.

Cadmium induces peroxidation and release of reactive oxygen species (ROS) that destroy cellular macromolecules and leads to the death of cells by altering the antioxidant system within them<sup>[8]</sup>. It also inhibits DNA repair enzymes in a Swedish population-based sample<sup>[9]</sup>. An ultra-low concentration of cadmium induce embryopathy and affects each step of embryo development<sup>[10,11]</sup>.

Zinc oxide Nanoparticles (ZnO NPs) are verified to be a Generally Recognized As Safe material (GRAS) by The Food and Drug Administration Agency, and extensively used in many fields<sup>[12]</sup>. Zinc shares many enzymes in producing metallothionein (cadmium sequesters) that antagonizes cadmium induced tumors<sup>[13,14]</sup>.

Tongue is a vital organ for taste, mastication, and speech. Due to their high metabolic activity, the filiform papillae are usually affected by any nutritional deficiency, vascular changes or enzymatic disturbance leading to their atrophy<sup>[15]</sup>.

The direct effect of cadmium on several tissues composing the tongue (in particular) had not been investigated yet. So, this work aimed to study the possible role of Zinc oxide nanoparticles (ZnO NPs) in attenuation of the structural, ultra-structural and biochemical changes in rats' tongues induced by Cadmium chloride.

## MATERIAL AND METHODS

### Chemicals

Cadmium chloride CdCl<sub>2</sub> (CAS no 10108642) was obtained from (Sigma-Aldrich, Steinheim, Germany) in form of solid beads which were grounded into powder to adjust the dose.

Zinc oxide NPs (CAS no.1314-13-2) was obtained as dispersion from (Sigma-Aldrich, Steinheim, Germany); concentration 50% in H<sub>2</sub>O, average NP size <40 nm, hydrodynamic diameter <100 nm.

### Experimental animals

Sixty adult male Wister albino rats weighing 180-200 g were utilized and fed ordinary diet and water, given ad-libitum and kept in plastic cages in the animal residence belonging to the Faculty of Medicine, Zagazig University, with suitable temperature and controlled light-dark cycle. Care provided to rats occurred according to the Ethical Committee of Zagazig University.

### Experimental design

After one-week adaptation, the rats were distributed into three groups:

(i) Control group (36 rats) divided into three groups (12 rats each):

ia: fed on a balanced diet for 4 weeks.

ib: were administered intraperitoneal injection of 0.25 ml distilled water once daily for 4 weeks, (the solvent of both Cadmium chloride and ZnO NPs).

ic: ZnO NPs group given 5 mg/kg/day ZnO NPs melted in 0.25 ml distilled water intraperitoneally for 4 weeks<sup>[16,17,18]</sup>.

(ii) Cadmium chloride group (12 rats) given Cadmium chloride orally 4.4 mg/kg bw/ day (1/20 of LD<sub>50</sub>) melted in distilled water for 4 weeks<sup>[19]</sup>.

(iii) Cadmium chloride+ ZnO NPs group (12 rats) were given a mixture of Cadmium chloride and ZnO NPs daily liquefied in distilled water as previously prescribed doses for 4 weeks.

After experiment completion; rats were sacrificed by injecting intraperitoneal thiopental 50 mg/kg. Each rat was placed on surface in a way that it can be gripped and a rod was pressed firmly across the posterior aspect of the neck. Tongues were carefully dissected and cut; pieces of them were used for histopathological preparations and other pieces were frozen at -80°C until being used for biochemical analyses.

### Histopathological study

#### Haematoxylin and Eosin (H&E) stain

The fixative used was 10% buffered formalin. Then, the specimens been processed to obtain 5-µm-thick paraffin sections for H&E stain<sup>[20]</sup>.

### Ultrastructure study

#### Transmission electron microscopy (TEM)

Phosphate-buffered glutaraldehyde and osmium tetroxide were used as fixative. Specimens were then dehydrated and embedded in epoxy resin. Cutting by (Leica ultra-cut UCT), and staining by uranyl acetate and lead citrate<sup>[21]</sup>. Processed specimens were snapped by a JEOL JEM 2100 electron microscope (Jeol Ltd, Tokyo, Japan).

#### Scanning electron microscopy (SEM)

The steps were; fixation, dehydration, and drying using liquid CO<sub>2</sub> in a drying apparatus<sup>[22]</sup>. Mounting was performed on aluminum stubs for scanning in SEM (JEOL JSM- 6510 LV electron microscope; Jeol Ltd, Tokyo, Japan).

TEM and SEM were conducted in the Electron Microscope Research Center belonging to the Faculty of Agriculture, El Mansoura University, Egypt.

Determination of oxidative stress & antioxidant markers Malondialdehyde (MDA); the lipid peroxidation marker was measured in tongue homogenate (nmol/g. tissue) according to Okhawa *et al*<sup>[23]</sup>. The following antioxidant markers: Reduced glutathione (GSH) (mmol/g)<sup>[24]</sup>, Catalase (CAT) activity (U/g tissue)<sup>[25]</sup>, Superoxide dismutase (SOD) (U/gm tissue)<sup>[26]</sup> and Glutathione peroxidase (GPx) (U/gm tissue)<sup>[27]</sup> been measured using their relevant kit (Biodiagnostic, Cairo, Egypt).

#### Statistical Analysis

MDA: malondialdehyde; GSH: reduced glutathione; Cat: catalase; SOD: superoxide dismutase and GPx: glutathione peroxidase levels were analyzed. Data was analyzed using SPSS statistical analysis software version 20. Values were expressed as means ± standard deviation (SD). ANOVA test to compare groups, followed by Tukey's post-hoc test were used. The probability values (*p*) less than 0.05 was considered significant and less than 0.001 as highly significant.

## RESULTS

As all control groups yielded similar morphological and biochemical results, only group ia will be discussed.

There was total affection of cadmium chloride in all specimens of cadmium chloride group and extended in the whole layers. Cadmium chloride+ ZnO NPs specimens showed much improvement in the whole layers except for few pathologies in focal layers were still presented.

#### Gross Examination results

Gross visual tongue examination of Cadmium chloride group revealed thick white plaques covering a wide area of the surface. Some sort of flaccidity with obvious tongue edema compared with the control group were noticed.

### **Light microscope results**

H&E-stained sections of the tongues of the control group showed filiform papillae covering the dorsal surface with pointed tips and thin smooth keratin coat. The tongue skeletal muscle fibers were organized in different directions; some of them appeared longitudinal showing parallel bundles of muscle fibers, others seemed polygonal with eosinophilic cytoplasm and peripherally positioned nuclei (Figures 1 A,B). Cadmium chloride group revealed filiform papillae with blunted ends, and thick keratin plaques. Some papillae appeared having cells with vacuolated cytoplasm and darkly stained nuclei; however, others were seen with separated and distorted keratin layers. Some areas appeared with increased thickness of the stratified squamous epithelium and increased mitotic activity. Congested blood vessels and mono-cellular infiltrating cells in the connective tissue were also seen. Marked separation between muscle bundles with severe congestion, red blood cells extravasation and fatty infiltrations were noticed. Loss of striations was noted in some of these fibers (Figures 1 C-K). Cadmium chloride+ ZnO NPs group revealed pointed tipped filiform papillae with keratin covering. The underlying connective tissue contained skeletal muscle fibers organized in different directions (Figures 1 L,M).

### **Transmission electron microscope results**

Examination of tongue sections of the control group revealed the columnar stratum basale cells lying on the basement membrane. The polyhedral stratum spinosum cells were settled in several layers and attached by desmosomes. Parallel organization of myofibrils with dark and light bands appeared in the muscle fibers. Many mitochondria located between the myofibrils were observed. Connective tissue cells and nerve fibers were also seen (Figures 2 A-C). Cadmium chloride group showed disfigured stratum basale cells lying on distorted basement membrane with profuse connective tissue cells in the lamina propria. The cells of stratum spinosum seemed to have irregular dark nuclei, large cytoplasmic vacuoles and disjointed by broad intercellular spaces. Some phagocytic

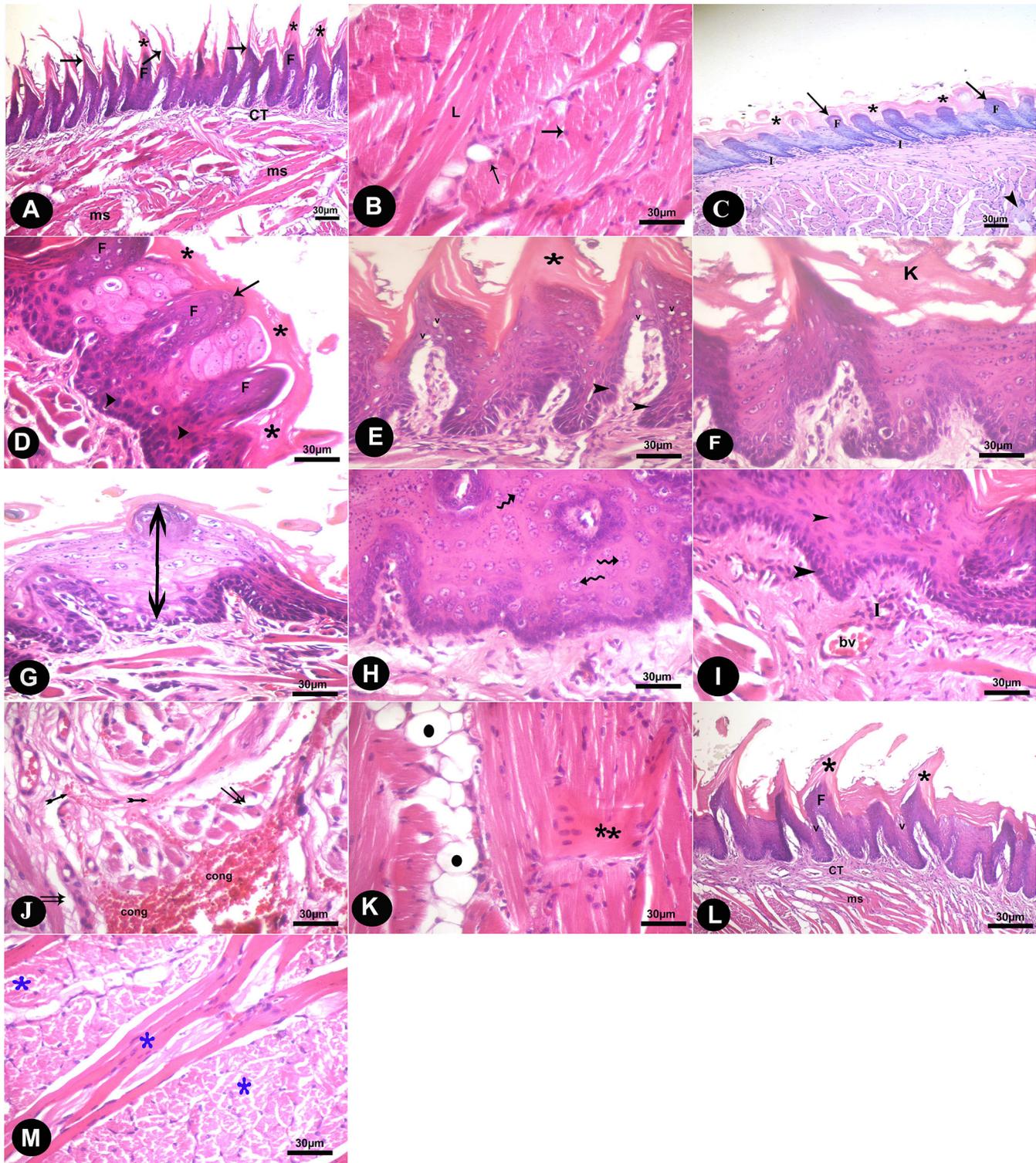
cells having lysosomes, irregular nuclei and cytoplasmic processes were obvious between stratum spinosum cells. Some muscle fibrils appeared degenerated with many wide spaces between them and many collagen fibers aggregated in some areas and dispersed in others were also obvious in the connective tissue (Figures 2 D-I). Cadmium chloride+ ZnO NPs group revealed stratum spinosum cells with desmosomal junction. Parallel arrangements of myofibrils with light and dark bands were obvious, however, some degenerated muscle fibers were seen between normal ones (Figures 2 J,K).

### **Scanning electron microscope results**

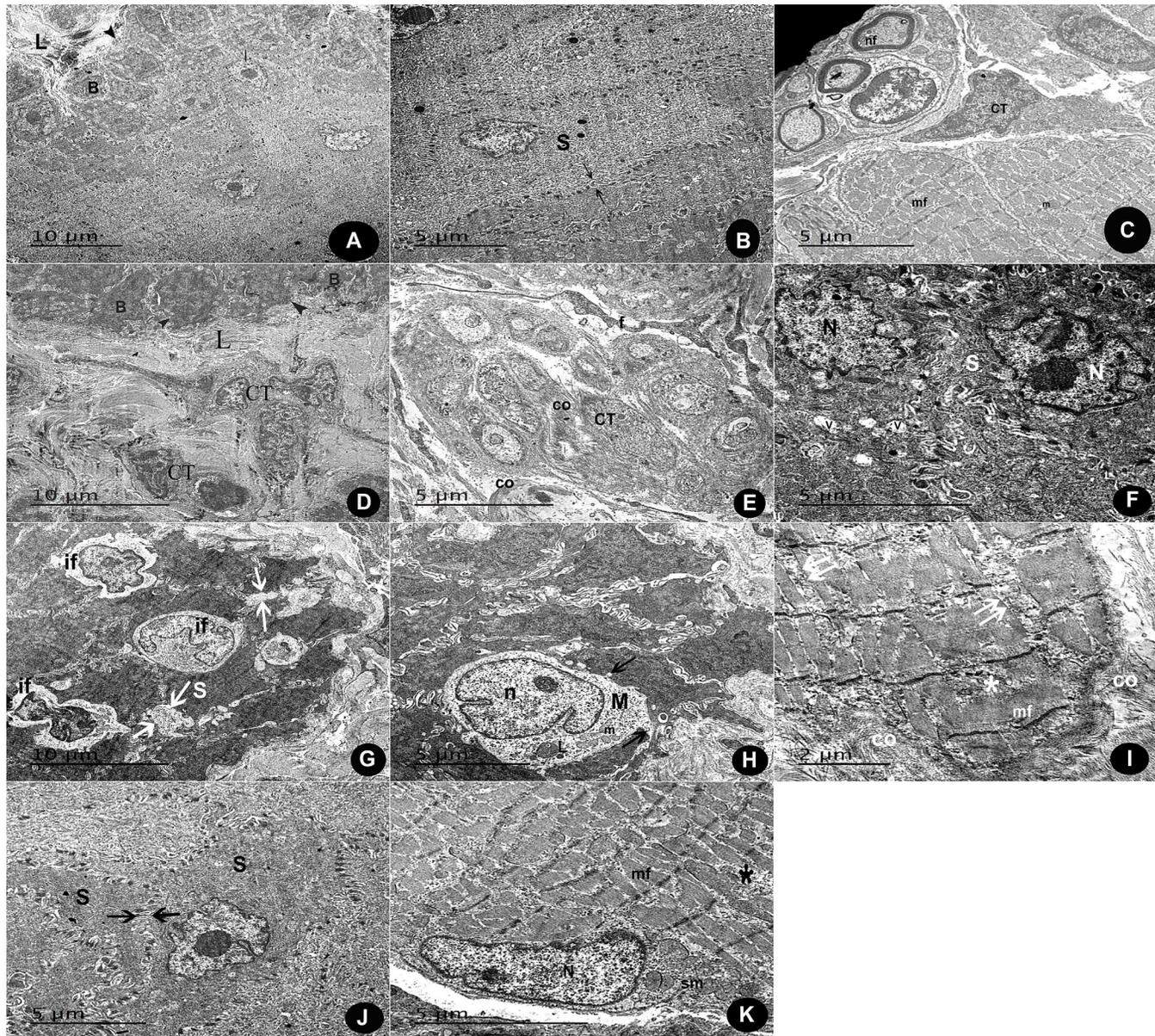
SEM micrographs of the dorsum of the tongue of the control group displayed long filiform papillae having tapered ends and oriented in one direction. The fungiform papillae displayed dome-like shape among the filiform papillae. They appeared with circular keratin packs and centrally located well-defined taste pore (Figure 3A). Cadmium chloride group were seen with filiform papillae pointed to diverse directions and some of them appeared short; others have blunt and disfigured ends. Parts of these papillae appeared with numerous poly-microbial grouping of cocci intermingled with inflammatory cells. The fungiform papillae seemed less prominent and distorted with wrinkled surfaces (Figures 3 B,C). Cadmium chloride+ ZnO NPs group showed long filiform papillae with tapered ends and oriented in one direction; few of them were in diverse directions. The fungiform papilla appeared with cauliflower shape and central taste pores (Figure 3D).

### **Tongue oxidative stress markers**

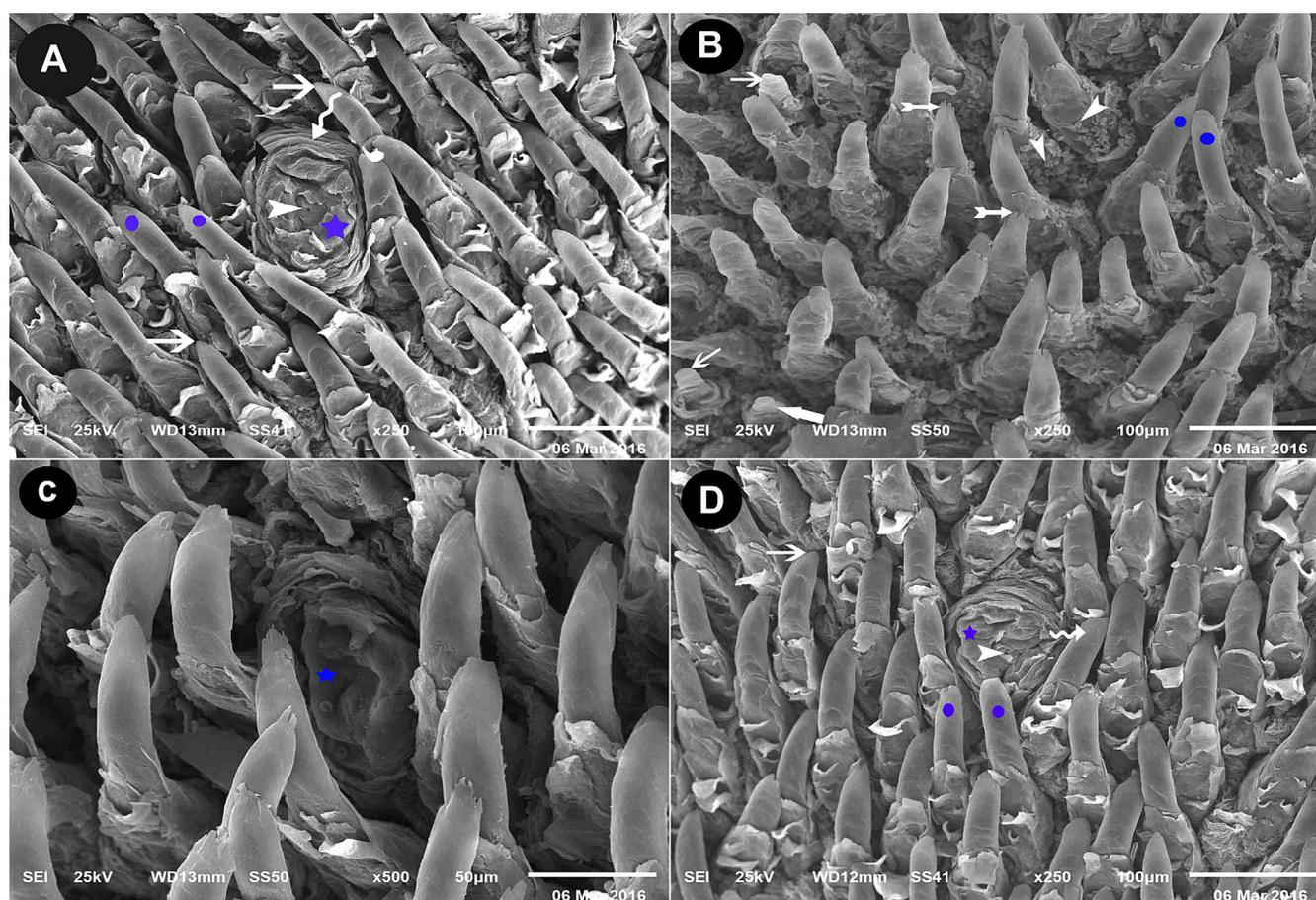
There was a significant decrease of tongue reduced GSH level, SOD, GPx and Cat enzymes activity in Cadmium chloride group as compared to control group. The same group showed significant increase in tongue MDA level compared to control group. The difference in levels of reduced glutathione, SOD, GPx, Cat and MDA in Cadmium chloride+ ZnO NPs group was insignificant compared to control group. (Table 1), Bar 1).



**Fig. 1:** Photomicrographs of H&E stained sections in the tongues of the studied group. A, B: control group. A: Filiform papillae (F) covering the dorsum of the tongue and having pointed tips (arrow) and thin smooth keratin covering (star). The connective tissue (CT) contains skeletal muscle fibers (ms). B: Skeletal muscle fibers organized in different directions; some of them appear longitudinal (L) showing parallel bundles of muscle fibers, others appear polygonal with eosinophilic cytoplasm and peripherally positioned nuclei (black arrow). C-K: Cadmium chloride group. C: Filiform papillae (F) with blunted ends (arrow), thick keratin covering (star), monocellular infiltrating cells (I) and muscle fibers with loss of striations (arrow head). D: Filiform papillae (F) with blunted ends (arrow), dark epithelial nuclei (arrowhead) and thick keratin covering (star). E: Papillae with vacuolated cytoplasm (v), dark epithelial nuclei (arrowhead) and thick keratin covering (star). F: Separated irregular keratin layer (K). G: Areas of thickened stratified epithelium (double-opposed arrows). H: Mitotic activity is obvious in many epithelial layers (wavy arrow). I: Dark epithelial nuclei (arrowhead), monocellular infiltrating cells (I) and congested blood vessels in the connective tissue layer (bv). J: Separated muscle fibers (double arrow). Severe congestion (cong) and extravasated red blood cells (tailed arrow). K: Fatty infiltrations (black circle) and muscle fibers with loss of striations (double star). L, M: Cadmium chloride+ ZnO NPs group. L: Filiform papillae cover the tongue dorsum and have pointed tips (arrow) with keratin covering (star). The underlying connective tissue (CT) contains skeletal muscle fibers (ms). M: Skeletal muscle fibers organized in different directions (blue asterisk). (H&E A, C x 100, B, D - K and M x 400, L x 200) scale bar 30  $\mu$ m.



**Fig. 2:** transmission electron microscope of tongue sections of the studied group. A-C: Control group. A: The columnar stratum basale cells (B) are lying on the basement membrane (arrowhead) that isolates it from the underlying lamina propria (L). B: The polyhedral stratum spinosum cells (S) are settled in several layers and attached to one another by desmosomes (2 opposing arrows). C: Parallel organization of myofibrils (mf) with dark and light bands, many mitochondria (m), connective tissue cells (CT) and nerve fibers (nf) are observed. D-I: Cadmium chloride group. D: Irregular shaped cells of stratum basale (B) rest on irregular basement membrane (arrowhead) with abundant connective tissue cells (CT) in the underlying lamina propria (L). E: lamina propria with fibroblast (f), collagen fibers (co) and connective tissue cells (CT). F: stratum spinosum cells (S) with irregular nuclei (N) and large cytoplasmic vacuoles (V). G: Polyhedral cells in the stratum spinosum (S) are disjointed by broad intercellular spaces (2 opposing arrows). Many inflammatory cells (if) appeared between these cells. H: phagocytic cell (M) is seen among stratum spinosum cells, it has an irregular nucleus (n), mitochondria (m), lysosomes (L) and cytoplasmic processes (arrow). I: Disorganized myofibrils (mf), degenerated myofibrils (\*). Wide spaces between muscle cells (double arrows) with many collagen fibers appeared in the connective tissue (co). J, K: Cadmium chloride+ ZnO NPs group. J: Group of stratum spinosum cells (S) with desmosomal junction (2 opposing arrows). K: Parallel organization of myofibrils (mf) with dark and light bands, nucleus (N), subsarcolemmal mitochondria (sm), degenerated muscle fibers (\*).



**Fig. 3:** Scanning electron micrograph of the tongues dorsum of adult male rats of all study groups. A: Control group. Long filiform papillae having tapered ends (arrow) and oriented in one direction (blue dots). The fungiform papillae (star) displayed dome-like shape among the filiform papillae. It appeared with circular keratin packs (wavy arrow) and centrally located well-defined taste pore (arrowhead). B, C: Cadmium chloride group. B: Filiform papillae oriented in different directions (circle). Some of them appear short (thick arrow). Others have blunt ends (arrow) and disfigured ends (bifid arrow). The filiform papillae appeared with polymicrobial grouping of cocci intermingled with inflammatory cells (arrowhead). C: The fungiform papilla (star) seemed less prominent and distorted with a wrinkled surface. D: Cadmium chloride+ ZnO NPs group. Long filiform papillae with tapered ends (arrow) and oriented in one direction (blue dots). Few of them are deviated in their directions (wavy arrow). The fungiform papilla appeared with cauliflower shape (star) and a central taste pore (arrowhead).

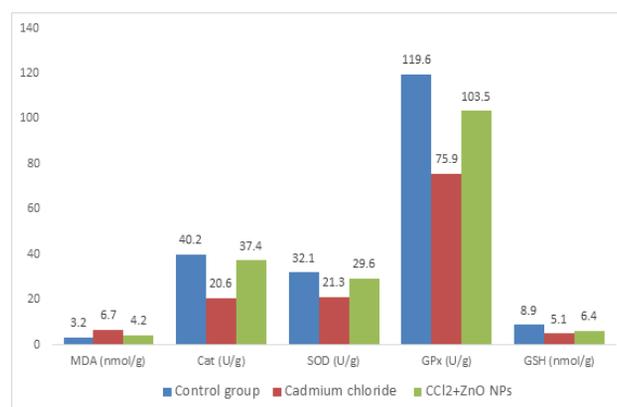
**Table 1:** oxidative stress markers among studied groups

	Group i Control group	Group ii Cadmium chloride	Group iii CdCl <sub>2</sub> +ZnO NPs
MDA (nmol/g)	3.2 ± 1.05	6.7 ± 1.07 <sup>a</sup>	4.2 ± 1.7 <sup>b</sup>
Cat (U/g)	40.2 ± 10.1	20.6 ± 5.9 <sup>a</sup>	37.4 ± 3.4 <sup>b</sup>
SOD (U/g)	32.1 ± 9.6	21.3 ± 6.1 <sup>a</sup>	29.6 ± 7.5 <sup>b</sup>
GPx (U/g)	119.6 ± 18.7	75.9 ± 10.8 <sup>a</sup>	103.5 ± 15.6 <sup>b</sup>
GSH (nmol/g)	8.9 ± 2.6	5.1 ± 2.0 <sup>a</sup>	6.4 ± 2.3 <sup>b</sup>

Values are expressed as mean ± standard deviation (SD) of n = 10 animals; a significant as compared with control group; b significant as compared with Cd Cl<sub>2</sub> -treated group. MDA: malondialdehyde; GSH: reduced glutathione; Cat: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase.

## DISCUSSION

In our study, histological examination of tongue sections from cadmium chloride group showed filiform papillae with blunted ends and surrounded by thick separated keratin layers with dark nuclei, and vacuolated cytoplasm. These findings go in context with the description of many researchers about cadmium hazards on mouth<sup>[28]</sup>, intestine<sup>[29]</sup> and esophagus of rats<sup>[30]</sup>. In trial to explain



**Bar 1:** oxidative stress markers among studied groups

these changes, Ribas, *et al.*<sup>[28]</sup> clarified that the epithelial hypertrophy in the mouth was due to the direct toxic impact of cadmium on oral mucosa. Osman, *et al.*<sup>[31]</sup> proposed that the filiform papillae undergo damage earlier than any other papillae. Arriazu, *et al.*<sup>[32]</sup> attributed cell proliferation to the increased Lysophosphatidic acid (LPA)-1 receptor (a marker for cell proliferation) caused by Cd exposure.

The present work showed vacuolated cytoplasm within the epithelial cells as well as distorted keratin layer upon CdCl administration. These findings might be in parallel to what have been proposed by Wang *et al.* that Cd in the cells triggers a generation of reactive oxygen species (ROS) such as intracellular MDA, H<sub>2</sub>O<sub>2</sub>, and super oxygen anion radicals which subsequently destroy the cell membrane structure, and result in an leakage of cytoplasm and disrupt the osmotic balance, thereby negatively affecting the intracellular physiological metabolic activities<sup>[33]</sup>. Morris-Wiman *et al.*<sup>[34]</sup> mentioned that the increased epidermal growth factor (EGF) is the main cause of the thickened keratin layers.

In the present study, degeneration and splitting of the skeletal muscle fibers were noticed in Cadmium chloride group. It has been explained by Cullen, *et al.*<sup>[35]</sup> who stated that separation of muscle fibers is a common response occurs when the fiber arrives to a critical size as oxygen supply and exchange of metabolites become inefficient. This may also be correlated to what we had visually observed regarding the flaccidity and loss of tone of the rats' tongues which was also affirmed by Yoo *et al.*,<sup>[36]</sup> who found that high levels of heavy metals raise the risk of sarcopenia, especially in older people. So this could be applied on the tongue as a muscular organ.

In the current work, inflammatory cells and fatty infiltrations seen between muscle fibers in Cadmium chloride group were hand in hand with Agency for Toxic Substances and Disease Registry<sup>[37]</sup> who also reported that cats exposed to high concentration of cadmium oxide fume for one day showed cell granulation and fatty infiltration. They proposed to be related to increased levels of pro-inflammatory cytokines caused by heavy metal poisoning, which consequently leads to the muscle fatigue associated with these conditions. Interestingly, Kim *et al.*<sup>[38]</sup> correlated intramuscular fat deposition to the occurrence of obstructive sleep apnea. They said that the increased intramuscular fat will change the tongue contour and decrease its contractile power, decreasing the ability of the tongue to act as a pharyngeal dilator muscle. Moreover, Bailey *et al.*<sup>[39]</sup> said that the intrinsic and extrinsic tongue muscles are co-opted during sleep to maintain the patency of the airway; and fat deposition at these serious junctions will prevent these mechanisms preventing sleep apnea.

Congestion and red blood cells extravasation were obvious between muscle fibers with loss of striations in some of them. These changes were in context with Suchismita and Abhik<sup>[29]</sup>. Niewenhuis<sup>[40]</sup> explained these findings as Cd increases both arterial blood pressure and microvascular permeability causing weakness in the junctional complexes between the capillary endothelial cells and thus leading to extravasation of blood cells.

Ultrastructural examination assured the pathological findings seen by light microscope. The most prominent changes were the irregular basal lamina and wide separations between the cellular elements in TEM results;

it may be a part of cellular damage that occurred through the generation of ROS, affection of DNA repair enzymes, up-regulation of cytokines and proto-oncogenes<sup>[41-43]</sup>. All of these might affect the synthesis and modifications of the basement membrane and trans-membrane proteins. Hall, 2016<sup>[44]</sup> stated that the heavy metals depress the cell membrane ionic pumps that enhance sodium permeability causing water to enter the cells by osmosis with resultant edema and cellular separation. This might explain the finding of gross tongue edema.

SEM examination of the Cadmium chloride group identified the filiform papillae in different directions unlike the control group, also most of them appeared with blunt tips. These changes mostly represent signs of degeneration as reported by Waleed, *et al.*<sup>[45]</sup> who observed the same findings as a consequence of lead toxicity. This could be attributed to the erosive and ulcerative changes caused by heavy metals that distort the structural protein enzymes within the mucosal cells<sup>[46]</sup>.

The poly-microbial grouping that was evident in Cadmium chloride group was suggested to be enhanced by anemia caused by heavy metals. This anemia predisposes to mouth dryness and glossitis<sup>[47]</sup>. Also, anemia leads to muscle hypoxia which decreases the contractile ability of tongue protruder and retractor muscles<sup>[48]</sup>.

On the other hand, Cadmium chloride+ ZnO NPs group revealed nearly normal filiform papillae with normal epithelial cells, nuclei, keratinization and no vacuolations were seen in the cytoplasm. No inflammatory cellular infiltration or dilated blood vessels were observed. Odessa, *et al.*<sup>[49]</sup> assured that zinc appeared to be more effective in protection against cadmium toxicity. Zn is a potent antioxidant metal; it is the core component of antioxidant enzymes such as superoxide dismutase (SOD). It raises mRNA expression of SOD, catalase and glutathione reductase<sup>[50]</sup>. In agreement with this, co-administration of ZnO NPs with CdCl<sub>2</sub> elicited significant drop in MDA levels in the tongue tissues and increased CAT, GPx, SOD activities, and GSH content.

The normal epithelial cellular findings in this group may be due to the anti-apoptotic effect of ZnO NPs by inhibiting caspase enzyme activity and cytochrome C<sup>[51]</sup>.

The most exciting explanation was by Kang *et al.*<sup>[52]</sup> who mentioned that the protecting value of zinc comes from the induction of metallothionein synthesis. Up to 20% of intracellular zinc is conjugated by metallothioneins (MTs) protecting the cells from apoptosis. Also, This tight Zn conjugation with MTs in each body cell is necessary to avoid oxidative stress within it, therefore, Zn has a preventive role against free radical formation and protects biological structures from being injured during inflammatory processes<sup>[53]</sup>. Some researchers added that MTs are the link between zinc and cellular redox status and this Zinc-Metallothionein Redox System not only play as an anti-oxidant and anti-inflammatory agents but also control cellular zinc homeostasis<sup>[54]</sup>.

The reduction in the inflammatory profiles that we noticed in ZnO NPs administered group was in accordance with Kim *et al.*<sup>[55]</sup>. They concluded that zinc containing herbal medicine could be used for the treatment of inflammation by regulating caspase-1 activity as ZnO NPs have been shown to reduce mRNA expression of inflammatory cytokines by inhibiting the activation of NF- $\kappa$ B (nuclear factor kappa B cells).

In the present work, we observed almost an absence of microbial colonization at the electron microscopic level in ZnO NPs given animals' specimens. This came in agreement with Siddiqi *et al.*<sup>[56]</sup> as they proven that Zn NPs have an antimicrobial activity. The action mechanism has been described as that ZnO NPs penetrate the bacterial cell walls via diffusion and cause disintegration of their cell membranes and accumulate in the cytoplasm where they interact with biomolecules leading to cell apoptosis and subsequently microbial cell death.

According to the previously analyzed data, it seems necessary for further future studies checking other involvement of ZnO NPs in various treatment plans for human.

## CONCLUSIONS

Zinc oxide nanoparticles (ZnO NPs) attenuated the structural and biochemical changes in rats' tongues induced by Cadmium chloride.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

## REFERENCES

1. Kumar, S., & Sharma, A.: Cadmium toxicity: effects on human reproduction and fertility. *Reviews on environmental health*, 2019; 34(4), 327-338.
2. Jarup L, Akesson A: Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.* 2009; 238: 201-208.
3. Minoia C, Ronchi A, Veronese I *et al.*: The confounding effects of intraoral metals in salivary biomarkers. *Occup. Environ. Med.* 2007; 64: 856.
4. Sakaguchi, R L. Powers, J M. Ferracane J. Craig's restorative dental materials-e-book. 14th ed. Elsevier Health Sciences, 2019; chapter 10 Pp. 222.
5. Alomary A, Al-Momani IF, Massadeh AM: Lead and cadmium in human teeth from Jordan by atomic absorption spectrometry: some factors influencing their concentrations. *Sci. Total Environ.* 2006; 369:69–75.
6. Dąbrowska E, Szyńska B, Kulikowska- Karpieńska E: Ultrastructural study of the submandibular gland of the rat after 6- month exposure to cadmium and zinc in drinking water. *Advances in Medical Sciences.* 2006;51:245-249.
7. Picoli LC, Lopes RA, Semprini M. *et al.*: Transmission electron microscopy study of the neonatal rat tongue mucosa treated with special attention to the bacteria on the epithelial cell membrane. *Int. J. Morphol.* 2006; 24(2):159-163.
8. Azhdari, A., Hosseini, S. A., & Farsi, S.: Antioxidant effect of high intensity interval training on cadmium-induced cardiotoxicity in rats. *Gene, Cell and Tissue* 2019; 6(3).
9. Schultze B, Lind PM, Larsson A, Lind L: Whole blood and serum concentrations of metals in a Swedish population-based sample. *Scand. J. Clin. Lab. Investig.* 2014; 74: 143–148.
10. Veeriah, V., Saran, U., Swaminathan, A., Balaguru, U. M., Thangaraj, P., Nagarajan, S. & Chatterjee, S: Cadmium-induced embryopathy: nitric oxide rescues teratogenic effects of cadmium. *Toxicological Sciences*, 2015; 144(1), 90-104.
11. Calle, J. S., Pachajoa, H., Charry, J. D. *et al.*: Teratogenic Effect of Cadmium: From The Developing Embryo To The Fetus. *Revista Colombiana de Salud Ocupacional.* 2016; 5(2), 21-26.
12. Espitia P, Soares N, Coimbra J *et al.*: Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications. *Food Bioprocess Technol.* 2012; 5:1447-1464.
13. Cortese, M.M., Suschek, C.V., Wetzel, W., Kröncke, K.D., Kolb-Bachofen, V.: Zinc protects endothelial cells from hydrogen peroxide via Nrf2-dependent stimulation of glutathione biosynthesis. *Free Radic. Biol. Med.* 2008; 44: 2002–2012.
14. Dreaden EC, Austin LA, Mackey MA, *et al*: Size matters: Gold nanoparticles in targeted cancer drug delivery. *Ther Deliv.* 2012; 3:457–478.
15. Eltokhey, H.M., Zahran, D.H. and Ezzat, A.M.: Tibolone improves the degenerative changes of tongue mucosa in ovariectomized female rats. *J.Cytol. Histol.* 2012; 3, 130.
16. Alkaladi, A., Abdelazim, A.M., Afifi, M.: Antidiabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats. *Int. J. Mol. Sci.* 2014;15 (2),2015–2023.
17. Reza, E.H., Kobra, S., Leila, S., Vahid, Y., Esmail, A.: Investigation of the zinc oxide nanoparticles effect on testosterone, cholesterol and cortisol in rats. *Res. J. Recent Sci.* 2014; 2277, 2502.
18. Mozaffari, Z., Parivar, K., Roodbari, N.H., Irani, S.: Histopathological evaluation of the toxic effects of zinc oxide (ZnO) nanoparticles on testicular tissue of NMRI adult mice. *Adv. Stud. Biol.* 2015; 7, 275–291.

19. Mahmoud, H.; Saliem, S. and Abo-Elfotoh: "Cadmium chloride toxicity and possible role of vitamin A." *Suez Canal Vet. Med.*, 2007; Vol. 2: 269278.
20. Bancroft, J., Layton, C.: *The Hematoxylin and eosin*. In: Suvarna SK, Layton C and Bancroft JD (ed). *Theory and Practice of histological techniques*. 7<sup>th</sup> ed. 2013; Churchill Livingstone of El Sevier, Philadelphia, Ch. 10 and 11, pp: 172-214.
21. Ayache, J.; Beaunier, L.; Boumendil, J.; Ehret, *et al.*: *Sample Preparation Handbook for Transmission Electron Microscopy Techniques*. Springer Science and Business Media. 2010; New York, NY, USA.
22. Martins, R. H. G., Goncalves, T. M., Madeira, S. L., Dias, N. H., & de Oliveira Semenzati, G: Scanning electron microscopy of the tongue, pharynx, and larynx of rats exposed to cigarette smoke. *Journal of Voice*, 2014; 28(3), 287-290.
23. Ohkawa, N. H. Ohishi, K. Yagi: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979; 95:351-358.
24. Beutler, O. E. Duron, B.M. Kelly: Improved method for the determination of blood glutathione. *J Lab Clin Med*. 1963; 61:882-888.
25. Goth. L.: A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta*. 1991; 196:143-151.
26. Nishikimi, N. M. Appaji, K. Yagi: The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun*. 1972; 46: 849-854.
27. Paglia, W.N. and Valentine: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. 1967; 70: 158-169.
28. Ribas JP, Lopes RA, Sala MA *et al.*: Effect of cadmium on rat maxillary molar junctional epithelium during lactation. *Int. J. Morphol*. 2004; 22: 257-262.
29. Suchismita Das I and Abhik Gupta: Cadmium Induced Histopathological Changes in the Intestine of Indian Flying Barb, *Esomus danricus*. *Current World Environment*. 2013; 8(2): 305-308.
30. Deveci E, Deveci S: The effects of cadmium chloride on the oesophagus of rats. *Int. J. Morphol*. 2011; 29: 678-680.
31. Osman HI, Abdel-Razek N, Koura SA: Histological changes of rat lingual papillae dueto chromium toxicity and the protective role of vitamin E. *Egypt Dent. J*. 2006; 52:193- 200.
32. Arriazu R, Durán E, Pozuelo JM, Santamaria L.: Expression of lysophosphatidic acid receptor 1 and relation with cell proliferation, apoptosis, and angiogenesis on preneoplastic changes induced by cadmium chloride in the rat ventral prostate. *PLoS One*. 2013; 8(2):e57742.
33. Wang, H. R., Che, Y. H., Wang, Z. H., Zhang, B. N., Huang, D., Feng, F., & Ao, H.: The multiple effects of hydrogen sulfide on cadmium toxicity in tobacco may be interacted with CaM signal transduction. *Journal of Hazardous Materials*, 2020; 403, 123651.
34. Morris-Wiman, J., Segó, R., Brinkley, L. and Dolce, C.: The effects of sialoadenectomy and exogenous EGF on taste bud morphology and maintenance. *Chem. Senses*. 2000; 25: 9-19.
35. Cullen MJ, Johnson MA, Mastaglia FL: Pathological reactions of skeletal muscle. In: Mastaglia FL, Walton JN, editors. *Skeletal muscle pathology*. Edinburgh: Churchill Livingstone; 2001: 123–84.
36. Yoo, J. I., Ha, Y. C., Lee, Y. K., & Koo, K. H.: High levels of heavy metals increase the prevalence of sarcopenia in the elderly population. *Journal of bone metabolism*, 2016; 23(2), 101-109.
37. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Cadmium*. Draft for public comment. US Department of Health and Human Services. 2008; Atlanta, US.
38. Kim, A. M., Keenan, B. T., Jackson, N. *et al.*: Tongue fat and its relationship to obstructive sleep apnea. *Sleep*, 2014; 37(10), 1639-1648.
39. Bailey EF, Janssen PL, Fregosi RF. PO<sub>2</sub>-dependent changes in intrinsic and extrinsic tongue muscle activities in the rat. *Am J Respir Crit Care Med* 2005;171:1403-7.
40. Niewenhuis RJ, Dimitriu C, Prozialeck WC.: Ultrastructural characterization of the early changes in intercellular junctions in response to cadmium (Cd<sup>2+</sup>) exposure in LLCPK1 cells. *Toxicol Appl Pharmacol*. 1997;142:1–12.
41. Mitra RS.: Protein synthesis in *Escherichia coli* during recovery from exposure to low levels of Cd<sup>2+</sup>. *Appl Environ Microbiol*. 1984; 47:1012–1016.
42. Abshire MK, Devor DE, Diwan BA, Shaughnessy JD Jr, Waalkes MP.: *In vitro* exposure to cadmium in rat L6 myoblasts can result in both enhancement and suppression of malignant progression *in vivo*. *Carcinogenesis*. 1996; 17:1349–1356.
43. Durnam DM, Palmiter RD.: Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. *J Biol Chem*. 1981; 256:5712–5716.
44. Hall, J. E.. *Guyton and Hall textbook of medical physiology*. 2016. 13th edition. Philadelphia, PA: Saunders Elsevier, Pp 314.
45. Waleed, O., Shawakh, E., Afaf, A., & Sahar, K: Protective role of honey after lead toxicity effect on albino rats lingual papillae. *Alexandria Dental Journal*, 2016; 41(2), 214-219.

46. Suradkar SG, Vihol PD, Patel JH, *et al.*: Pathomorphological changes in tissues of wistar rats by exposure of lead acetate. *Vet World*. 2010; 3:82-4.
47. Osaki T, Ueta E, Arisawa K, Kitamura Y, Matsugi N. The pathophysiology of glossal pain in patients with iron deficiency and anemia. *Am J Med Sci*. 1999;318: 324-9.
48. Fuller, D. D., & Fregosi, R. F.: Fatiguing contractions of tongue protruder and retractor muscles: influence of systemic hypoxia. *Journal of Applied Physiology*. 2000; 88(6):2123-2130.
49. Odessa Addison, Robin L. Marcus, Paul C. LaStayo and Alice S. Ryan: Intermuscular Fat: A Review of the Consequences and Causes. *International Journal of Endocrinology*. 2014; Article ID 309570.
50. Aitken, R.J., Roman, S.D.: Antioxidant systems and oxidative stress in the testes. *Oxid. Med. Cell. Longev*. 2008; 1:15–24.
51. Kaufmann SH, Hengartner MO. Programmed cell death: Alive and well in the new millennium. *Trends Cell Biol*. 2001;11(12):526–534.
52. Kang, M., Zhao, L., Ren, M., Deng, M., & Li, C.: Reduced metallothionein expression induced by zinc deficiency results in apoptosis in hepatic stellate cell line LX-2. *International Journal of Clinical and Experimental Medicine*, 2015; 8(11), 20603.
53. Chasapis, C. T., Loutsidou, A. C., Spiliopoulou, C. A., & Stefanidou, M. E.: Zinc and human health: an update. *Archives of toxicology*, 2012; 86(4), 521-534.
54. Drozd, A., Wojewska, D., Peris-Díaz, M. D., Jakimowicz, P., & Krężel, A.: Crosstalk of the structural and zinc buffering properties of mammalian metallothionein-2. *Metallomics*, 2018; 10(4), 595-613.
55. Kim, M. H., Seo, J. H., Kim, H. M., & Jeong, H. J.: Zinc oxide nanoparticles, a novel candidate for the treatment of allergic inflammatory diseases. *European journal of pharmacology*, 2014; 738, 31-39.
56. Siddiqi, K. S., Rahman, A., & Husen, A.: Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale research letters*, 2018; 13(1), 1-13.

## الملخص العربي

# جسيمات أكسيد الزنك النانوية تخفف من التسمم الناتج عن كلوريد الكاديوم في لسان ذكور الجرذان البيضاء البالغة (دراسة هستولوجية وكيميائية حيوية)

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**مقدمة:** يعد الكاديوم من المعادن الثقيلة السامة والمتواجدة طبيعياً في بعض الأغذية النباتية المصدر كما انه يعتبر أحد الملوثات التي لا يمكن تجنبها في العديد من اعمال طب الاسنان. يشارك هذا المعدن في اكسدة و تدمير العديد من جزيئات الأنسجة المختلفة إلى جانب تثبيط الانزيمات المصلحة للمادة الجينية. يعد اللسان من الأنسجة الحيوية التي يمكن ان تتأثر بالتعرض المزمن لسمية الكاديوم و بالأخص الحليمات الخيطية و العضلات الهيكلية نظراً لنشاطهم التمثيلي المستمر. **اهداف البحث:** لدراسة الدور المحتمل لجزيئات اكسيد الزنك النانوية في تخفيف التغيرات التركيبية والدقيقة والكيميائية الحيوية لألسنة الجرذان المسببة بواسطة كلوريد الكاديوم.

**طريقة البحث:** تم تجميع عدد ٦٠ جرذاً أبيضاً ذكراً و تقسيمهم وفقاً للمادة المستخدمة الى ٣ مجموعات: المجموعة الضابطة والمجموعة المتلقية لمادة كلوريد الكاديوم والمجموعة المتلقية لمادة كلوريد الكاديوم مع جزيئات اكسيد الزنك النانوية. كل مجموعة تلقت المادة المقررة لها لمدة ٤ اسابيع. تم تجميع عينات اللسان وتحضيرها للفحص بالمجهرين الضوئي والالكتروني وايضا لاختبار العمليات الحيوية الكيميائية من خلال تحليل مجموعة من الانزيمات (المالون داي الدهيد، الكاتاليز، الجلوتاثيون، فوق أكسيد الديسموتيز و الجلوتاثيون بيروكسيديز).

**نتائج البحث:** نتجت عن الكاديوم مجموعة من التأثيرات الضارة الهادمة لأنسجة اللسان وبعض التغيرات الكيميائية الحيوية مثل الحليمات الخيطية ذات النهايات المفالطة ولويحات الكيراتين السمكة. كما ظهرت خلايا النسيج الطلائي بفجوات سيتوبلازمية وأنوية غامقة. أيضاً ظهرت الليفات العضلية متحللة وبينها مسافات واسعة والكثير من ألياف الكولاجين. كما ظهر نقص كبير في مستوي إنزيمات الجلوتاثيون وفوق أكسيد الديسموتيز و الجلوتاثيون بيروكسيديز والكاتاليز إلى جانب زيادة كبيرة في المالون داي الدهيد مقارنة بالمجموعة الضابطة. كان لجسيمات أكسيد الزنك النانوية القدرة علي تخفيف هذه التغيرات حيث أظهرت الحليمات الخيطية بنهايات مدببة كما ظهرت الخلايا بشكل شبه طبيعي و الليفات العضلية ظهرت بترتيب متوازي. وكان مستوي إنزيمات الجلوتاثيون وفوق أكسيد الديسموتيز و الجلوتاثيون بيروكسيديز والكاتاليز و المالون داي الدهيد غير مختلف مقارنة بالمجموعة الضابطة.

**الخلاصة:** جزيئات اكسيد الزنك النانوية خففت التغيرات التركيبية والكيميائية الحيوية لألسنة الجرذان المسببة بواسطة كلوريد الكاديوم.