

## Histopathological and Immunohistochemical Studies on the Tongue of Albino Mice Offspring from Cadmium Toxicated Dams Treated with Vitamin C and / or *Nigella Sativa*

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**Abstract:** Performance of the current study utilized a well elicited CD-1 seventy five (75) albino mice, fifty (50) of which were adult mature females, while the remaining twenty five (25) were adult mature males. The females albino mice were divided into 5 groups, 10 females in each group separated from males for one month, all groups were get the drug doses twice a week for one month before gestation and during pregnancy until delivery. For breeding purposes each 2 females were mated with 1 male from 5 pm. until 9 am. at the following day. Female mice were classified into five groups. **Group I;** (control group, dams were injected intramuscularly( i.m) with 0.5 ml of sterile normal saline); **group II;** (dams were injected i.m. with 5 mg/kg body weight of cadmium chloride (Cd Cl<sub>2</sub>) dissolved in 0.5 ml of sterile normal saline); **group III;** (were injected i.m. with Cd Cl<sub>2</sub> as in group II, plus i.m. injection of 10 mg/ kg body weight of vitamin C. **group IV;** (were injected i.m. with Cd Cl<sub>2</sub> as in group II and given 50 mg/kg body weight of *Nigella sativa* orally) and **group V;** (were injected i.m. with Cd Cl<sub>2</sub> and vitamin C as in group III and *Nigella sativa* as same as in group IV). After delivery, heads of pups were decapitated. The heads were fixed in Bouin's fixative and prepared routinely for paraffin sectioning and staining for histopathological and immunohistochemical investigations. All control group animals were normally developed, as well as the pups of the remaining groups which treated with vitamin C and / or *Nigella sativa* with cadmium. Immunohistochemical examination of transforming growth factor alpha(TGF- $\alpha$ ) revealed that, control pups (**group I**) expressed a strong immune reaction in the lingual epithelium and lingual muscles. Cadmium treated mice (**group II**) revealed degenerative changes in the lining epithelium of the tongue and its muscles. The intermuscular connective tissue was oedematous. This group showed negative immune TGF- $\alpha$  reaction. Cadmium and vitamin C treated animals (**group III**) expressed moderate TGF- $\alpha$  reaction in the tongue epithelium and faint reaction in the underlying connective tissue cells. Animals treated with cadmium and *Nigella sativa* (**group IV**) expressed the same immune reaction as in group III. Cadmium, vitamin C and *Nigella sativa* treated pups (**group V**) expressed strong immune reaction of TGF- $\alpha$  similar to that of control group.

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### 1.Introduction

The cadmium is one of common environmental pollutants; it is an important metal and has several uses in industry such as electroplating, soldering, batteries, painting and as a plastic stabilizer. The cadmium toxicity considered as an environmental disease which resulted from the cumulative absorption of small amounts of cadmium until toxic levels are reached in the body which results in a toxic state which is linked with a number of health problems. Cadmium is one of teratogenic agents which interfere with, modify, or inhibit the proliferation of cells and normal growth of organs (Salvatori *et al.*, 2004). Vitamin C as an antioxidant and immune enhancer is one of the important water soluble vitamins and essential for collagen synthesis (Naidu, 2003). The *Nigella sativa* (common food) is immune enhancer as it enhances T-cell mediated immunity through the improvement of T-helper to suppressor T-cell ratio. *Nigella sativa* seeds

have an antioxidant actions and cytoprotective effect (Smith and Reynard, 1992 and Ali Blunden, 2003). The *Nigella sativa* has an antibacterial effect (Thompson and Goldin, 1995), also it has anticancer effect (Sadler, 1985). The transforming growth factor alpha ( TGF- $\alpha$  ) is known to regulate cell proliferation and differentiation in the embryo.No available studies were done according to our knowledge to investigate the effects of cadmium chloride administration on the tongue development of the albino mice offspring subsequent to intramuscular injection of the drug to their mothers (dams) and to study the effect of vitamin C and *Nigella sativa* as an antioxidants and immune enhancers on the effect of cadmium on the development of the tongue in mice offspring. This promoted the present research, which will be investigated histopathologically and immunohistochemically.

### 2.Material and methods

## 2.1 Experimental-animals:

The animals used in this study were the CD-1 albino mice. The experimental work has done in the pre-clinical farm of the Contractor Company for Biological Products and Vaccine, Cairo where the animals produced and live in the same place. Seventy five (75) mature albino mice of five to six weeks of age, were well elected as fifty (50) mature females weighed 26-28g., and twenty five (25) mature males weighed 28-30g.

## 2.2 Grouping of the experiment:

### Group I:

The animals were injected intramuscularly in the thigh with 0.5 ml of sterile normal saline twice a week and used as a control group.

### Group II:

The animals were injected intramuscularly with 5mg/ kg body weight of cadmium chloride (El Nasr pharmaceutical chemicals Co. Abu Zaabal, Egypt) dissolved in 0.5 ml of sterile normal saline, twice a week. The experimental dose was determined by using dose of 10 mg/ kg body weight, all treated animals were died. Therefore, the sublethal dose which used in the current study was 5mg/kg body weight.

### Group III:

The animals have been injected with cadmium chloride as same as group II and also were injected by intramuscular injection of 10mg/ kg body weight of vitamin C (Cevaryl 1000 mg/5 ml ampoule, Memphis Co. for pharm.& chemical indust. Cairo, Egypt) two times per week.

### Group IV:

The animals were injected with cadmium chloride as in group II and were also given 50 mg/ kg body weight of *Nigella sativa* oil orally twice a week. The *Nigella sativa* oil (Baraka 450 mg capsules, Pharco pharmaceuticals, Alexandria, Egypt) was given orally by injection through plastic syringe with blunt needle into the pharynx.

### Group V:

The animals were treated as in group III and have been also given the *Nigella sativa*, as in group IV. All groups of animals have been administrated the doses for one month before pregnancy, and during gestation period till delivery.

## 2.3 Investigations of pregnancy:

After one month of drug administration, each two females were housed together with one male in a single cage, for breeding purposes. Animals were mated from 5.00 pm. until 9.00 am. at the following day when the males were separated from females. The technique used for determination of pregnancy was based on the vaginal smears, this day was determined the day 0 of gestation. All groups were given the drugs doses as the regime which has mentioned before for the whole pregnancy time until delivery. Each female was

separated in a single cage near to delivery to conserve the offspring. All mice were maintained in a controlled environment and were provided with adequate laboratory diet and water.

## 2.4 Obtaining-of-specimens-and-tissue-preparation:

After delivery, pups were collected, and sacrificed by decapitation using a sharp blade. The heads of each litter were fixed in Bouin's fixative, prepared routinely for paraffin embedding, and sectioned step serially parallel to the coronal plane for histopathological and-immunohistochemical-studies.

## 2.5 Histopathological examination

5-6µm step-serial sections were stained with Haematoxylin and Eosin (Bancroft *et al.*, 1994).

## 2.6 Immunohistochemical-examination

For the demonstration of transforming growth factor alpha (TGF- $\alpha$ ), tissue sections of 5-6 µm thick were cut from paraffin blocks and placed on positively charged (opti plus) slides for staining procedures. The avidin-biotin peroxidase complex method (ABC) outlined by Ramos-Vara (2005) was used. The transforming growth factor alpha receptors (TGF- $\alpha$ ) were used in addition to a polyclonal rabbit anti-human p- catenin. All antibodies were in the form of pre diluted antibody (conc. 1-50, Dako), which were ready to be used for the reaction procedures. Control slides were prepared using the same method omitting either primary or secondary antibodies.

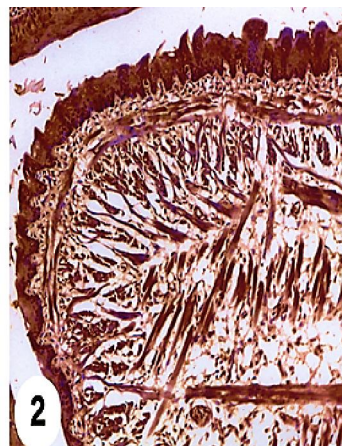
## 3.Results-and-Discussion

Histological examination of tongue from **control (sterile normal saline treated group);group I;** revealed normal epithelial covering with regular orientations formed of stratified squamous keratinized epithelium of 4-5 layers. The dorsum of the tongue carried prominent filiform and fungiform papillae. The tongue shown well-developed interlacing bundles of skeletal muscle fibers, with wellformed intermuscular connective tissue (**Figure 1**).TGF- $\alpha$  attributed strong immune staining reaction of the covering epithelium and underlying mesenchymal tissue cells. The skeletal muscles expressed strong immune staining reaction for TGF- $\alpha$  (**Figure 2**).The same finding was reviewed by numerous investigators; **Fraser (1971); Fitchett and Hay (1989), and Gibbins et al. (1999)**.Tongue from **cadmium toxicated group; group II;** revealed atrophied, hyalinized, and irregularly appeared covering epithelium with decreased collagenous density. The intermuscular connective tissue revealed signs of oedema (**Figure 3**). TGF- $\alpha$  denoted negative immune reaction in the covering epithelium and the underlying mesenchymal tissue cells and muscles (**Figure 4**).These results were comparable with investigations which studied the relation between TGF- $\alpha$  and tongue formation (**Shiang et al., 1993; Shaw et al., 1996; Bryant et al., 2001; Abbott and Best, 2005 and Alexander, 2006**).

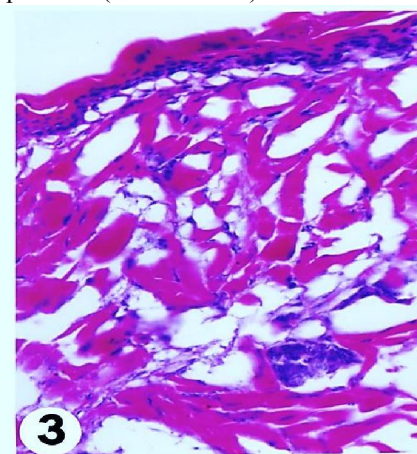
Considering tongue from **cadmium and vit.C treated group; group III**; There were signs of tissue regeneration in the form of re-epithelization of all epithelial layers, formation of wellformed keratine layer all over the surface while some degenerative changes in the underlying mesenchymal tissue cells and muscles were still present (**Figure 5**). TGF- $\alpha$  reported mild immune reaction in the covering epithelium and the underlying mesenchymal tissue cells and muscles (**Figure 6**). Which revealed the protective effect of vitamin C as an antioxidant against cytotoxic effect of cadmium (**Akhere et al., 2008 and Sema et al., 2008**). Regarding tongue from **cadmium and Nigella sativa treated group;group IV**; There were apparently healthy covering keratinized epithelium and muscles, while the connective tissue showed inflammatory cell infiltration (**Figure 7**). TGF- $\alpha$  exhibited moderate immune reaction in the covering epithelium and the underlying mesenchymal tissue cells and muscles (**Figure 8**).which revealed the repairing effect of Nigella sativa on the adverse effect of cadmium on the tongue of the mice (**Kantre et al., 2005 and Massadeh et al., 2007**)Tongue from **cadmium, vit.C, and Nigella sativa treated group; group V**; showed normal epithelial covering of keratinized stratified epithelium with underlying normal density of the connective tissue and muscle fibers (**Figure 9**). TGF- $\alpha$  recorded strong staining reaction in the covering epithelium and the underlying mesenchymal tissue cells and lingual muscles (**Figure 10**).These result were comparable with the results of the studies that reported the protective effect of vitamin C and *Nigella sativa* against cytotoxicity and embryotoxicity of cadmium on the mice (**Barrandon and Green; 1987, Kantre et al., 2005; Massadeh et al., 2007 and Sema et al., 2008**).



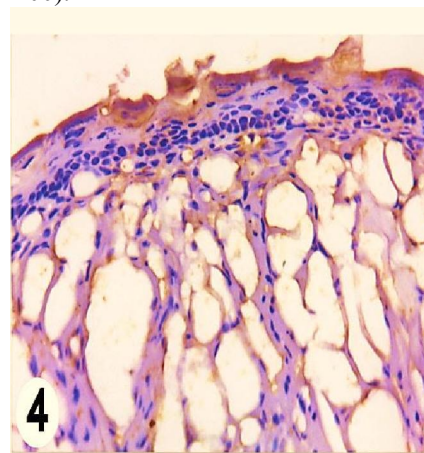
**Fig. 1:** Photomicrograph of mice offspring tongue from **group I**; showing normal epithelial covering, the tongue is formed of dense muscles and intermuscular connective tissue (H&E X400).



**Fig. 2:** Photomicrograph of mice offspring tongue from **group I**; showing, strong transforming growth factor alpha expression (TGF- $\alpha$  X400).

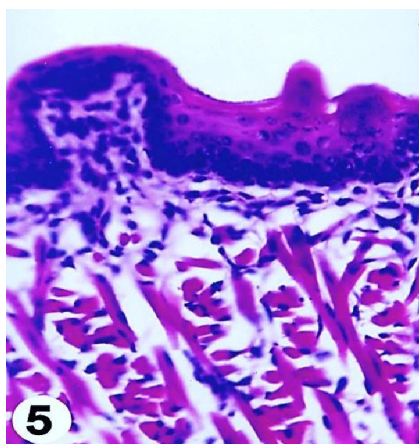


**Fig. 3:** Photomicrograph of mice offspring tongue from **group II**; showing atrophied, hyalinized, and irregularly appeared covering epithelium and muscles, the intermuscular connective tissue is edematous, (H&E X400).

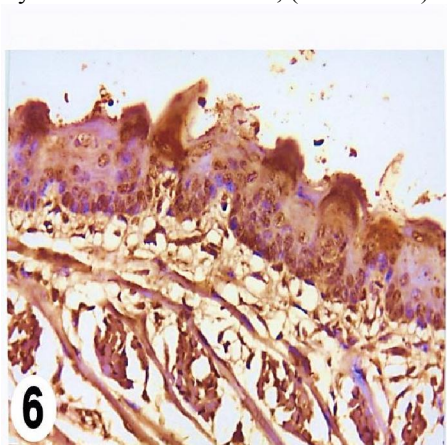


**Fig. 4:** Photomicrograph of mice offspring tongue from **group II**; showing, negative TGF- $\alpha$  staining reaction, (TGF- $\alpha$  X400).

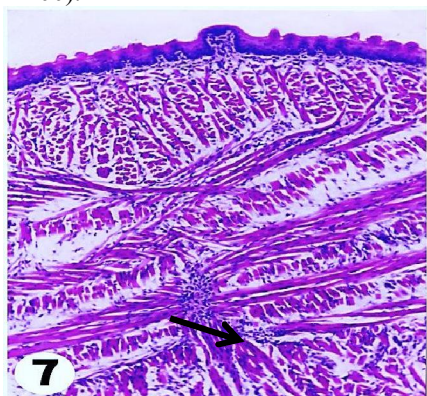




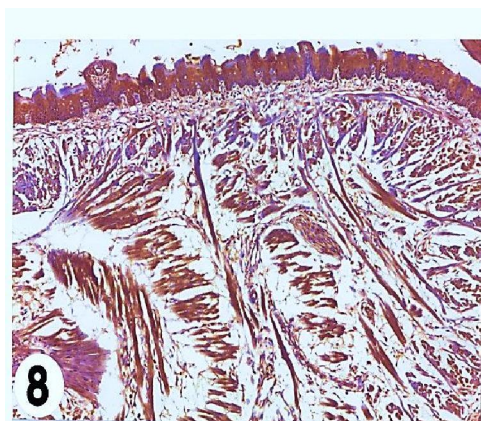
**Fig. 5:** Photomicrograph of mice offspring tongue from **group III**; showing tissue regeneration in the form of re-epithelization of all epithelial layers and formation of wellformed keratine layer all over the surface with some degenerative changes in the underlying mesenchymal tissue and muscles, (H&E X400).



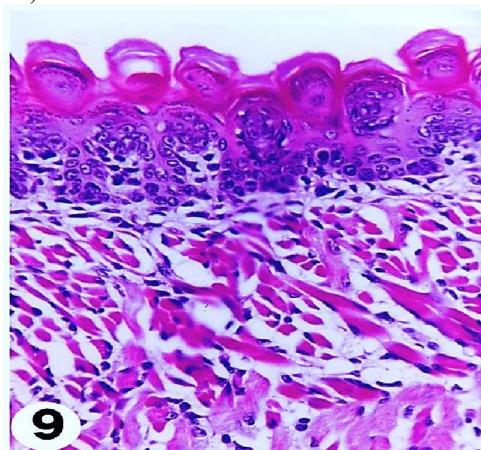
**Fig. 6:** Photomicrograph of mice offspring tongue from **group III**; showing, mild immune reaction, (TGF- $\alpha$  X400).



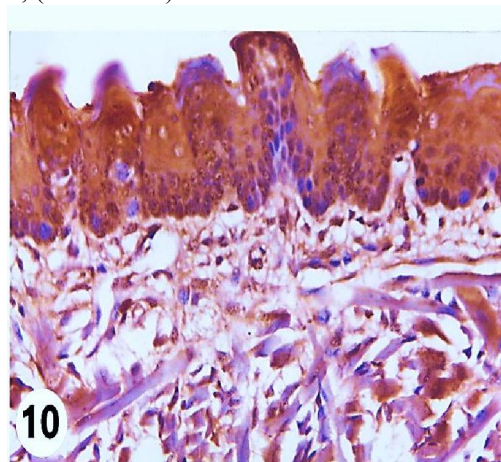
**Fig. 7:** Photomicrograph of mice offspring tongue from **group VI**; showing apparently healthy covering keratinized epithelium while the connective tissue shows inflammatory cell infiltration (arrow),(H&E X200).



**Fig. 8:** Photomicrograph of mice offspring tongue from **group VI**; showing moderate immune reaction, (TGF- $\alpha$  X200).



**Fig. 9:** Photomicrograph of mice offspring tongue from **group V**; showing normal epithelial covering of keratinized stratified epithelium with underlying normal density of the connective tissue and muscle fibers, (H&E X400).



**Fig. 10:** Photomicrograph of mice offspring tongue from **group V**; showing strong staining reaction, (TGF- $\alpha$  X400).

#### 4. Conclusion:

Cadmium chloride prevents normal epithelization of the tongue together with normal density of the underlying muscle and collagen. Furthermore, the drug adversely affected transforming growth factor alpha formation. Cadmium might be associated with altered expression of TGF- $\alpha$ . Vitamin C has an antagonizing effect of the cytotoxic effect of cadmium, also *Nigella sativa* has a protective effect against the embryotoxic effect of cadmium, while vitamin C and *Nigella sativa* together had overcome the adverse effect of cadmium on the tongue structure and expression of transforming growth factor alpha.

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