# Original Research

# The effect of lead toxicity on albino rats' filiform and fungiform papillae and the possible protective role of honey and black seed

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## ABSTRACT

**Aims:** (1) To investigate the histological changes in lingual papillae following lead toxicity in experimental rats. (2) To assess the possible protective effects of honey and black seed using light microscopy (LM) and scanning electron microscopy (SEM). **Materials and Methods:** Forty adult male albino rats were divided into four groups, 10 rats/group. Group I (control): 1 ml distilled water. Group II (lead): 16.5 mg lead acetate. Group III (honey + lead): 16.5 mg lead acetate + 50 mg/kg honey. Group IV (black seed + lead): 16.5 mg lead acetate + 50 mg/kg black seed aqueous suspension. All doses were given by oral intubation daily/6 weeks. After 6 weeks, rats were terminated, and tongues were decimated for LM and SEM. **Results:** True filiform papillae in Group II were distorted. Some appeared shorter while others had eroded tips and hyperkeratosis. Areas of epithelial erosion with complete loss of papillae and keratin were seen in some samples. Disfigured fungiform papillae with swollen taste buds were seen. In Group III, filiform and fungiform papillae appeared close to normal with minimal changes. However, in Group IV papillae were distorted with hyperkeratosis and limited areas of epithelial erosions were seen. **Conclusion:** The atrophy observed in Group II could be explained by lead-induced oxidative stress. Lead toxicity had a dual effect where increased free radical formation together with depletion of endogenous antioxidant enzymes resulted in inflammation and increased cell injury. Lead causes anemia and decreased salivary secretion, which may explain the epithelial erosion. Honey exerted its protective role through restoring enzymatic activity and through its antioxidant mechanism. Black seed offered lower protection than honey. Increased dose and/or duration of co-administration of black seed may improve protection.

KEY WORDS: Antioxidant, black seed, epithelial erosion, filiform papillae, fungiform papillae, honey, occupational lead toxicity, scanning electron microscopy, taste bud

## INTRODUCTION

Lead is a poisonous heavy metal, which is widely used in many industries. Lead toxicity is a serious occupational hazard, which occurs due to accumulative absorption of little amounts of lead into the body until toxic levels are reached. The symptoms of lead toxicity are usually nonspecific thus making its diagnosis difficult [1].

Many workers in the lead-based industries are ignorant of the dangerous effects of lead and so do not take proper precautions while handling it, leading to higher levels of exposure. Lead industries include; lead production and soldering, manufacturing of batteries, ceramics, plastics and bullets as well as metal radiator repair and recycling lead cables. Some construction work like demolition of old buildings, scraping of lead-based paint, repair of bridges, water tanks, welding of lead painted metal and furniture refinishing may also expose workers to considerable amounts of lead [2].

Oxidative stress is the main contributor to lead toxicity as lead directly and/or indirectly can change the antioxidant balance in biological tissues [3].

Although the biological mechanisms linking lead exposure and dental disease are not completely clear, it has been suggested that lead disrupts salivary gland function, thereby increasing the risk of dental caries. Studies on rats have shown that lead exposure during the prenatal period resulted in significantly higher levels of dental caries and markedly reduced salivary flow rate [4]. Similarly, adult rats exposed to lead through drinking water showed decreased salivary calcium and protein concentrations, increased lipid peroxidation and a decrease in total antioxidant capacity in salivary gland tissue. This indicates the presence of lead-induced oxidative stress [5].

Regarding the oral manifestations of lead exposure in humans; ulcerative stomatitis, gray spots on the buccal mucosa, a heavy coating of the tongue surface, and tremor of the extended

tongue have been documented [1]. Bluish-red or deep blue linear pigmentation of the gingival margin may be present [6], in addition to mucous pigmentation, gingivitis, tongue burning, and reduced taste perception [7], Clinical studies show that occupational lead exposure is a major cause of ulceration, fissuring and epithelial desquamation of the tongue, palate and other parts of the oral mucosa. Lead also increases the incidence of periodontal diseases (gingivitis and periodontitis), caries index and dental abrasions [8]. The histological effects of lead exposure have been the focus of substantial experimental research throughout the years. However, the study of the histological effects of occupational lead toxicity on the lingual papillae has not drawn much attention.

It has been known since ancient times that honey has protective and curative properties [9]. Honey is a remarkable liquid, prepared by honeybees from the natural solutions called nectar obtained from various flowers [10]. Honey contains a number of enzymes (like catalase and glucose oxidase) and free amino acids. It also contains some vitamins like riboflavin (vitamin B2), ascorbic acid (vitamin C), minerals like iron and calcium, antioxidants (such as flavonoids, vitamin C, catalase enzyme, polyphenols) and antibacterial agents like hydrogen peroxide [11]. Its high viscosity, acidic pH, high osmolarity, and rich nutritional properties can inhibit bacterial growth and enhance healing [12]. Honey is an extremely complex natural liquid that is reported to contain at least 181 substances. It is a supersaturated solution of sugars, of which fructose (38%) and glucose (31%) are the main contributors. A study investigated the protective effect of honey against lead toxicity in rats. The results showed a significant decrease of lead uptake in blood and tissues and a marked recovery in the biochemical alterations caused by lead [13].

Nigella sativa is a flowering plant belonging to the family ranunculaceae, the fruit is a capsule made of united follicles, each containing many small triangular black seeds with a pungent smell and a bitter taste. In folk medicine, the parts of the plant most commonly used for the therapeutic purposes are the seeds, which may be ingested with food as a spice or mixed with honey. The seeds are known as black seeds while in Arabia they are called "Habatul-baraka" or "Al Haba-alsawda, and in India and South Asia, it is called Kalonji [14].

Black seeds are famous for their effect on the respiratory system and have been long used in the treatment of cough, asthma, bronchitis, influenza and as a respiratory stimulant. They are also used in the treatment of chronic headache, fever, rheumatism, and eczema. Black seeds have been reported to contain essential oil, fixed oil, flavonoids, saponins, alkaloids, and proteins. The main component of black seed and its essential oil is thymoquinone, which is the primary active ingredient. Thin-layer chromatography screening methods, showed that thymoquinone and the components carvacrol, t-anethole and 4-terpineol demonstrated respectable radical scavenging property. These four constituents and the essential oil of black seed possessed variable antioxidant activity when tested in the diphenylpicrylhydracyl assay for non-specific hydrogen atom or electron donating activity. They were also effective OH radical scavenging agents in the assay for non-

enzymatic lipid peroxidation in liposomes and the deoxyribose degradation assay [15].

It was reported that the black seed contains over 100 valuable nutrients. They help to regulate the metabolism, carry toxins to the skin's surface for elimination, balance insulin levels, adjust cholesterol, improve body circulation, and stimulate healthy liver function. Proximate analysis of whole mature black seeds showed that the lipid content is 34.49-38.72%, carbohydrates 23.5-33.2%, crude protein 20-27%, ash 3.77-4.92%, and moisture content 5.52-7.43% [16].

The cytoprotective effect of black seed against lead-induced hepatorenal damage was studied. The co-administration of black seed showed marked reduction in damaged areas in both kidney and liver and increase in inflammatory infiltrate [17]. Since honey and black seed showed great potential to counteract the adverse effect of occupational lead toxicity; therefore, the aim of this study was assess the possible protective effects of honey and black seed against lead-induced toxicity in albino rats' filiform and fungiform papillae using light and scanning electron microscopy (SEM).

In humans; numerous filiform (hair like) papillae cover the anterior part of the tongue and consist of cone-shaped projections, each with a core of connective tissue and a keratinized epithelium. They are highly abrasive during mastication compressing the food bolus against the palate [18].

The rat filiform papillae have been classified into three distinct types by many researchers. A previous study defined the three types of filiform papillae on the rat tongue as simple conical papillae, giant conical papillae, and true filiform papillae [19]. The simple conical papillae cover the anterior two-thirds of the tongue (body of the tongue). It forms a curved conical structure ending in a strong, cornified spine [20]. The giant conical papillae form 7 to 10 rows, in the intermolar eminence and separate the simple conical papillae from the true filiform papillae. The true filiform papillae occupy the posterior one-third of the tongue (base of tongue). All of the filiform papillae possess a convex as well as a concave surface [19].

The second type of the lingual papillae is the isolated fungiform (fungus like) papillae found scattered between filiform papillae. In human, they are elevated, mushroom-shaped papillae which appear red because of their relatively thin non-keratinized epithelium overlying a highly vascular connective tissue core. Taste buds are normally present in the epithelium of the superior surface [18]. The dorsum of the rat tongue however is keratinized and so the rat fungiform papilla is covered by a thin layer of keratin [21]. It was also reported that every single fungiform papilla has a single taste bud in 99% of rats [22].

## MATERIALS AND METHODS

### Animals

Forty adult male albino rats weighing between 180 g and 200 g were kept under normal healthy conditions in the Animal House

of the Medical Research Center in Ain-Shams University for the duration of the experiment (March-April 2011). The rats were housed in wire mesh cages (five rats per cage) under controlled temperature and dark-light cycle. They were fed a standardized diet and tap water was available ad libitum.

## Materials

Lead: Inorganic lead acetate trihydrate powder was purchased from Sigma Chemical Company. Honey and black seed: Pure unprocessed honey and black seed were purchased from Imtanan Health Shop®. The black seed was washed under running water, dried in the sun and ground into a fine powder before use.

## Experimental Design

After a week of acclimatization, the albino rats were randomly divided into four groups, 10 rats in each group. The groups were classified into: Group I (control): Animals were given 1 ml distilled water daily by oral intubation. Group II (lead): Animals received 16.5 mg of lead acetate per day, which is equivalent to the occupational lead exposure (500 ppm)[23]. The dose was given in 1ml of distilled water by oral intubation. Group III (lead + honey): Animals received 16.5 mg of lead acetate each day followed directly by a honey dose equivalent to 50 mg/kg dissolved in 1 ml of distilled water by oral intubation [24]. Group IV (lead + black seed): Animals received 16.5 mg of lead acetate each day followed directly by a black seed dose equivalent to 50 mg/kg [24] suspended in 1ml of distilled water by oral intubation. The experiment was well-tolerated by all the rats and at the end of the 6 weeks duration; the rats were terminated by an overdose of anesthesia. Tongues were immediately collected. Tongues of each group were randomly chosen, so that five tongues were processed for light microscopic examination and another five tongues were processed for scanning electron microscopic examination. Disposal of the dead animals was done in Ain Shams incinerator.

## Light Microscopic Preparation

Tongue specimens were immediately fixed in 10% formalin solution for at least a week then the specimens were washed properly under running water, dehydrated by transferring them through ascending concentrations of alcohol 50%, 60%, 80%, 90%, 96%, and then absolute alcohol. The specimens were then transferred to xylol to clear them from alcohol. The tongues were then infiltrated in paraffin wax and embedded in the center of paraffin wax blocks. The embedded specimens were sectioned by the microtome (4-6 $\mu$m thick) and then transferred in descending concentrations of alcohol 96%, 70% then in distilled water. Finally, the sections were stained by hematoxylin and eosin stain [25] and examined by the light microscope. Examination of the true filiform papillae and fungiform papillae was carried out.

## Scanning Electron Microscopic Preparation

Tongue specimens were fixed in buffered glutaraldehyde (2.5% glutaraldehyde in 0.1 M phosphate buffer) with a pH 7.2, for 1.5-2 h, and then rinsed 2 times in phosphate buffer 15 min for each. The samples were placed in post-fixative (osmium in 0.1 M phosphate buffer) with a pH 7.2 for 2 h. The traces of unbound osmium were rinsed with two changes of buffer, 15 min for each.

Dehydration was then done in an increasing series of ethanol with concentrations (40-60-80-95-100%), 15 min for each step. The next step was drying by critical point drying. The final step in processing was the coating by Baltec 030 sputter coater [26]. Specimens were then examined by the SEM. The search was approved from the Research Ethics Committee of the Faculty of Dentistry-Ain Shams University.

## RESULTS

### Light Microscopy (LM)

True filiform papillae: Group I: The dorsal surface of the control tongues in Group I revealed evenly distributed true filiform papillae regular in size, shape and orientation with normal covering keratinized stratified squamous epithelium. Individual papillae appeared long and slender with a thread like shape. The convex and concave sides were distinctly observed so that the tips of the papillae were pointed backwards toward the base of the tongue [Figure 1]. Group II: These papillae appeared clearly distorted. Most papillae lost their normal thread like shape while others appeared much shorter. Loss of the convex and concave sides of keratin was also apparent together with eroded tips and hyperkeratosis. Thinning of the epithelium and reduced connective tissue papillae were also seen [Figure 2]. In some samples, areas of epithelial erosion with complete loss of the papillae and their covering keratin was seen. In these areas, remnants of thin irregular epithelium resting on an intact basement membrane were observed [Figure 3]. Group III: Most of these papillae had pointed tips while few had blunt tips. The epithelium appeared normal, but hyperkeratosis was observed [Figure 4]. Group IV: Most of these papillae did not portray the typical thread shape of normal true filiform papillae. They appeared shorter and thicker at the base together with hyperkeratosis [Figure 5].
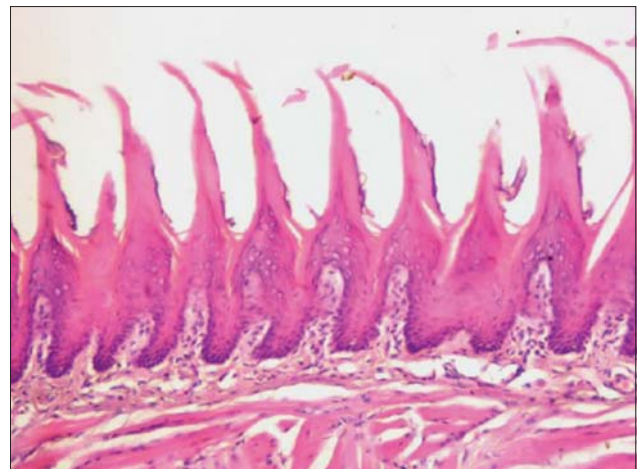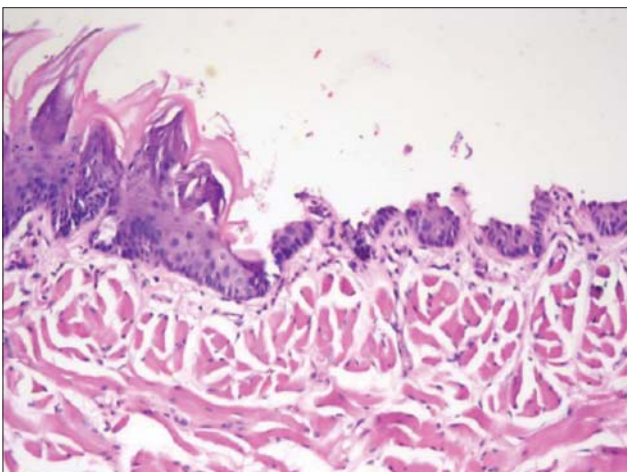


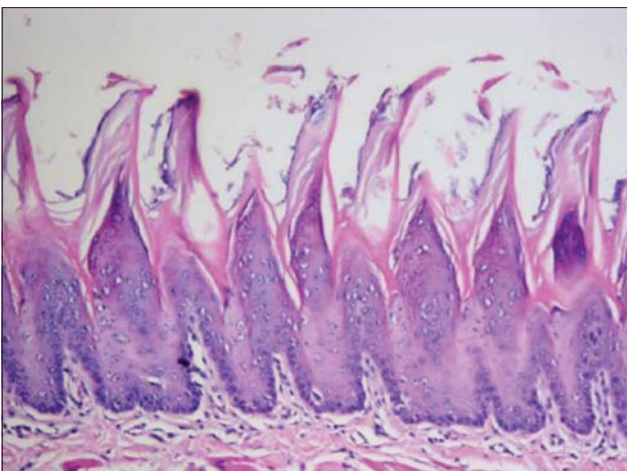**Figure 1:** Photomicrograph of true filiform papillae of Group I

Although most samples showed absence of areas of epithelial erosion, few samples showed some limited areas of epithelial erosions.
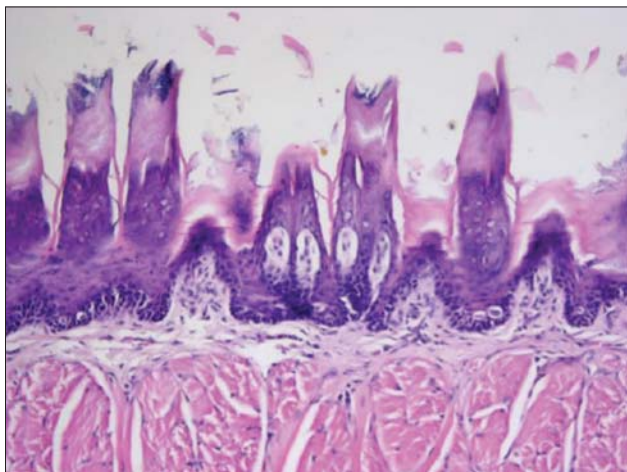


**Figure 2:** Photomicrograph of true filiform papillae of Group II



**Figure 3:** Photomicrograph of true filiform papillae of Group II showing epithelial erosion



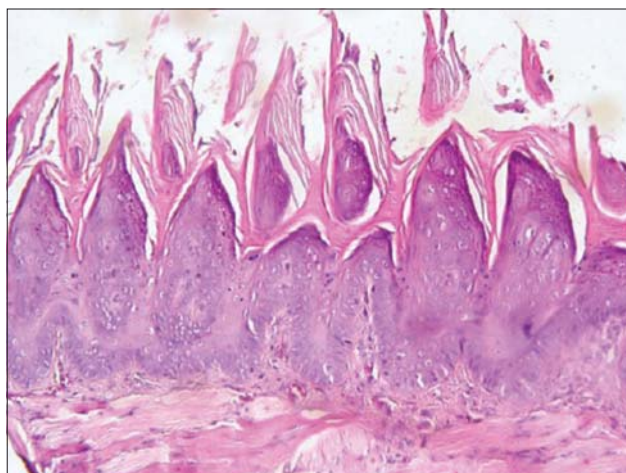**Figure 4:** Photomicrograph of true filiform papillae of Group III
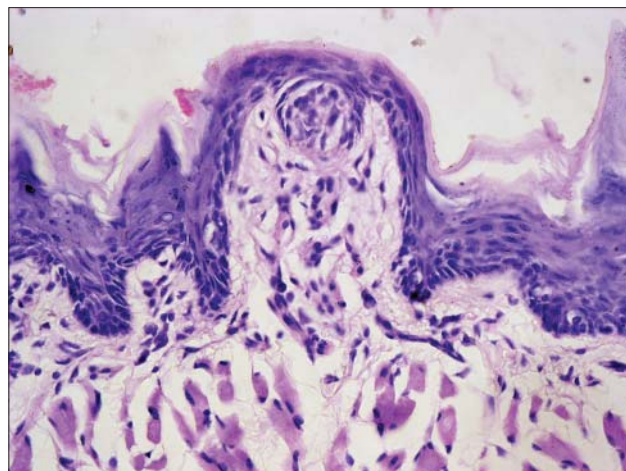
## Fungiform Papillae

*Group I*

These papillae showed the characteristic mushroom-shape and were elevated above the surface of the tongue. They were found scattered in between filiform papillae. Fungiform papillae were covered by keratinized stratified squamous epithelium. A thin uniform layer of keratin could be seen on the most superficial epithelial layer. A single well-defined barrel-shaped taste bud was observed on the dorsal surface. The lamina propria was formed of well-defined connective tissue papillae, with short secondary papillae [Figure 6].

*Group II*

The fungiform papillae were apparently shorter and distorted. The taste bud appeared shorter, swollen while its cells appeared somewhat separated. Some taste bud cells showed cytoplasmic halo. Detached keratin on adjacent epithelium could be noticed [Figure 7].



**Figure 5:** Photomicrograph of true filiform papillae of Group IV



**Figure 6:** Photomicrograph of fungiform papilla of Group I

*Group III*

The fungiform papillae closely resembled those of Group I with no obvious distortion. The taste bud appeared rounded with normal cells. Hyperkeratosis and detachment of keratin of adjacent epithelium could be seen [Figure 8].
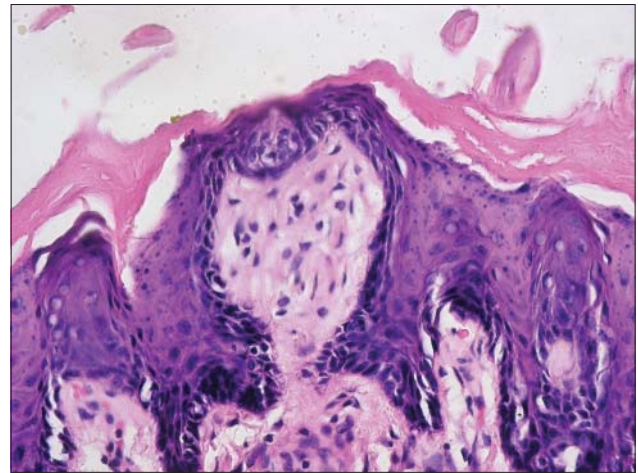
*Group IV*

The fungiform papilla appeared distorted and somewhat shorter than that of Group I. The taste bud appeared rounded with normal cells. Hyperkeratosis and separation of keratin could be seen, especially on the sides of the fungiform papillae [Figure 9].

**SEM**

True filiform papillae: Group I: These papillae appeared closely packed, thread-like in shape with a nearly regular orientation [Figure 10]. Group II: Decreased papillary density of the true filiform papillae was noticed here unlike the closely packed picture of Group I. Most papillae did not present the typical thread shape where most papillae appeared short and bent with

hyperkeratotic tips [Figure 11]. Group III: Closely resembled that of Group I with a thread-like shape, regular orientation, normal interpapillary distances and no bending of tips
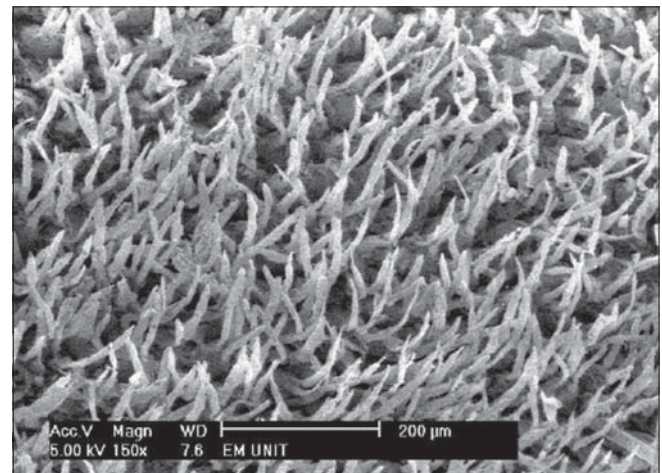


**Figure 9:** Photomicrograph of fungiform papilla of Group IV



**Figure 10:** Scanning electron micrograph of true filiform papillae of Group I
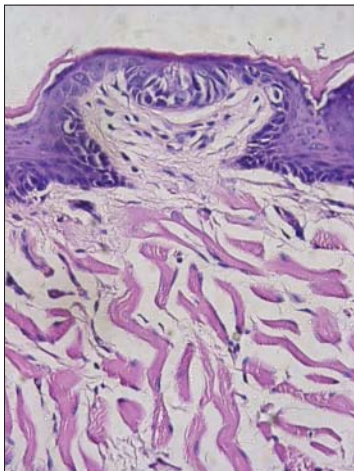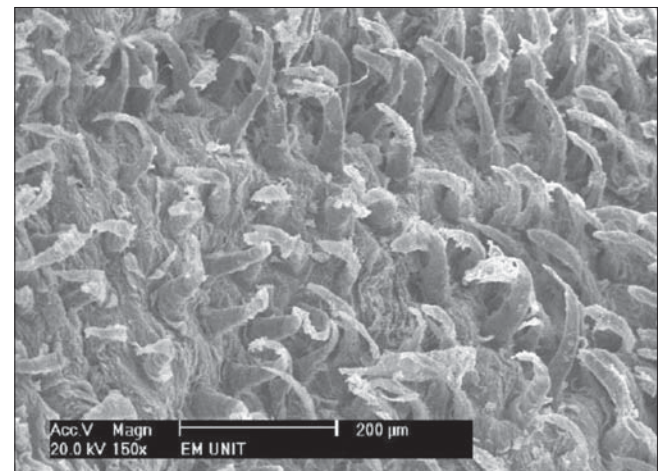


**Figure 7:** Photomicrograph of fungiform papilla of Group II
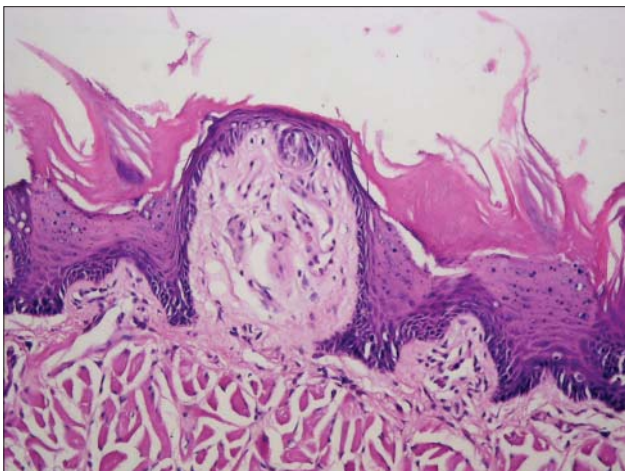


**Figure 8:** Photomicrograph of fungiform papilla of Group III



**Figure 11:** Scanning electron micrograph of true filiform papillae of Group II

[Figure 12]. Group IV: Most of these papillae resembled those of Group I; however, bending of the tips was seen while other areas showed apparent decreased papillary density [Figure 13].
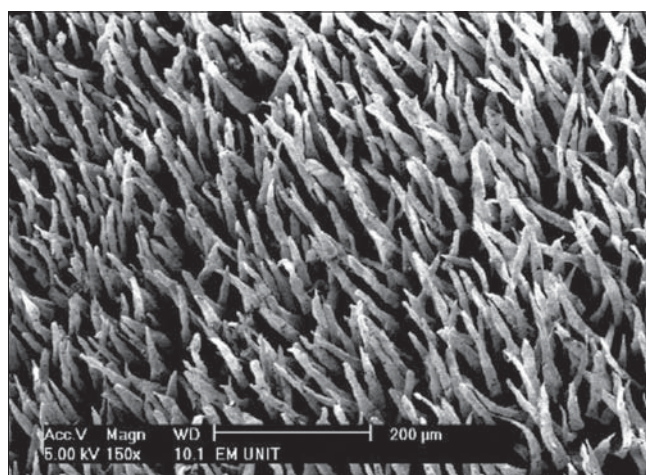
## Fungiform Papillae

### Group I

The fungiform papillae appeared rounded with a smooth surface. A centrally located well-defined regular taste bud was seen surrounded by a shallow indentation [Figure 14]. Group II: The fungiform papillae appeared less prominent and clearly distorted. The surface seemed irregular and wrinkled and the typical mushroom-shape could not be distinguished. The taste bud on most of the fungiform papillae was rarely encountered as it appeared rather smooth and ill-defined [Figure 15]. Group III: The fungiform papillae appeared regular with a well-defined outline, nearly similar to that of Group I. However, it was less projecting over the surface. The surface appeared smooth with a well-defined taste bud [Figure 16]. Group IV: These papillae appeared somewhat

distorted with a similar picture to that of fungiform papillae in Group II, but with a less wrinkly surface. The taste bud was regular in shape [Figure 17].
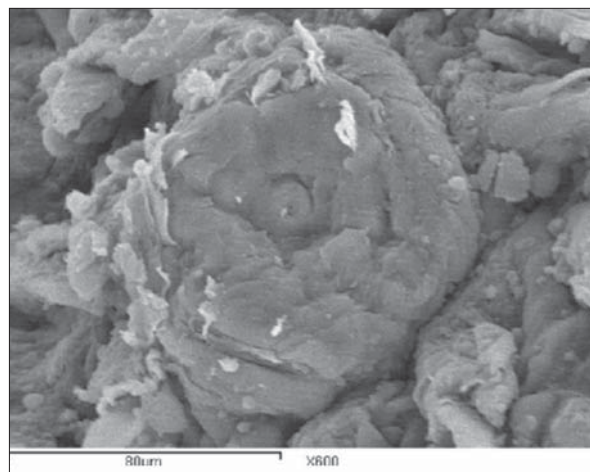


**Figure 14:** Scanning electron micrograph of fungiform papillae of Group I



**Figure 12:** Scanning electron micrograph of true filiform papillae of Group III
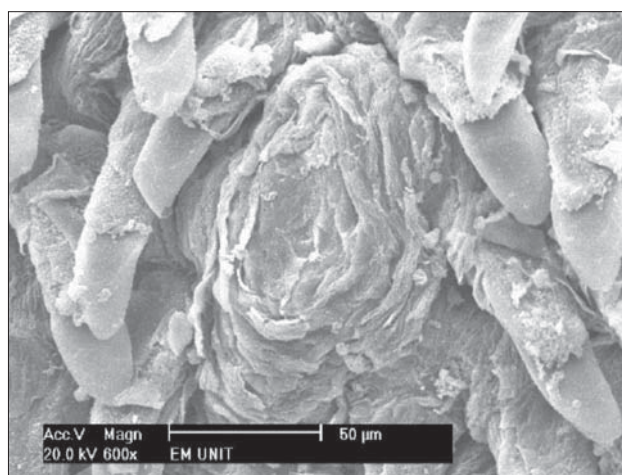


**Figure 15:** Scanning electron micrograph of fungiform papillae of Group II



**Figure 13:** Scanning electron micrograph of true filiform papillae of Group IV



**Figure 16:** Scanning electron micrograph of fungiform papillae of Group III

**Figure 17:** Scanning electron micrograph of fungiform papillae of Group IV

## DISCUSSION

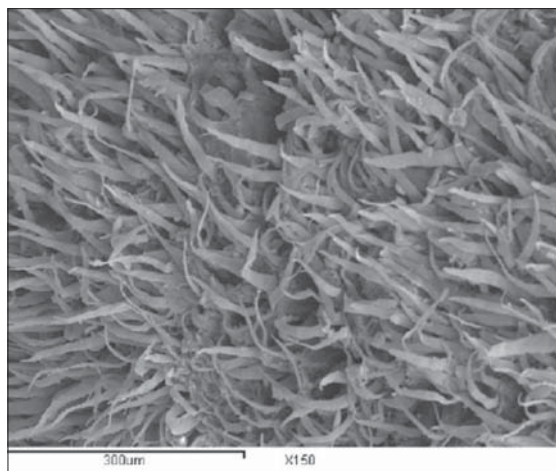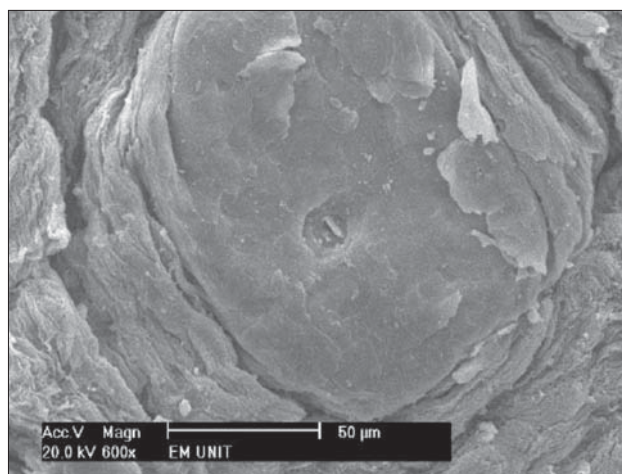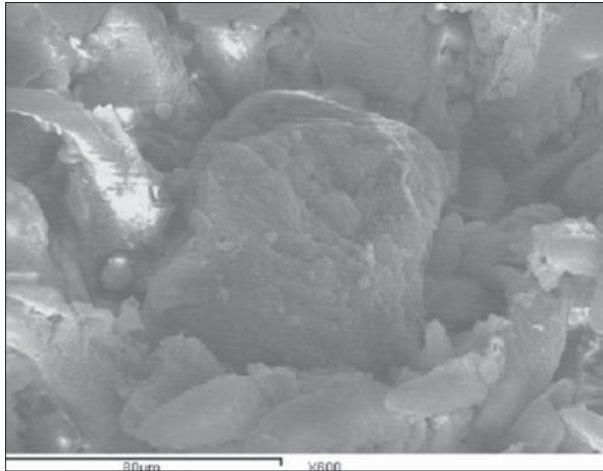There has been a growing interest to evaluate the use of natural remedies to protect against day to day health problems. This was the inspiration behind the present study. Occupational lead exposure presents a serious health hazard to workers in lead-based industries. Great efforts are required to increase awareness of workers at risk. Adequate prevention programs demonstrating good occupational hygiene and emphasizing proper precautions taken during handling of this toxic metal are also needed [27].

In addition, the use of natural antioxidants has a great potential to protect against lead-induced toxic effects on tissues. If honey or black seed prove to be helpful in reducing these toxic effects, then many workers will benefit from regular consumption of such natural remedies without suffering needless side-effects of prescribed medication.

The tongue is considered as a mirror of the general health, especially the filiform papillae. Cells of these papillae have a high metabolic activity and so any enzymatic disturbance, vascular changes or nutritional deficiency result in their atrophy. They undergo loss and atrophic changes faster and earlier before any other papillae [28].

Lingual atrophy may be a side-effect of a number of medications such as antibiotics, or may be due to cancer, diabetes, chemotherapeutic agents or metal toxicity [29]. This is why lingual papillae were the tissue of choice in the current study.

The light microscopic results of the present work reflected atrophic changes in the three types of filiform papillae of Group II. This is in agreement with other clinical studies that reported that lead toxicity causes lingual atrophy [30]. The lingual atrophy appeared in the form of distorted shape of papillae, shortening, increased interpapillary distances, thinning of the epithelium and some areas of degeneration of basal and parabasal epithelial cells. The SEM also confirmed this atrophy in the form of apparent thinning of papillae, tearing of keratin,

decreased papillary density in addition to areas completely devoid of papillae. This is in agreement with a clinical study, which reported that chronic lead exposure caused ulceration and desquamation of the tongues of workers in lead-based industries [8].

The true filiform papillae showed more histological changes compared to the other two variants. These changes included shortening, loss of thread shape and erosion of papillae tips, along with areas of complete epithelial erosion. Other studies also reported similar effects of lead on different tissues; where a dose of 100 ppm was given to rats in drinking water for 28 days. The results revealed desquamation, erosion and ulceration of the surface epithelium of intestinal villi. The study attributed this atrophy to the irritative effect of lead on the mucosa of the intestine [31].

This atrophy could be explained by lead-induced oxidative stress. Oxidative stress can be defined as an imbalance between the production of reactive oxygen species and antioxidant defense [32]. Much of lead's toxicity can be attributed to distortion of enzymes and structural proteins [33]. This can be further explained by a biochemical analysis study, which assessed the lead-induced oxidative stress on gastric ulcers [34]. Lead was given to rats at a dose of 100 mg/L in drinking water for 15 weeks. The results showed that lead exposure aggravated gastric ulcer by interfering with oxidative metabolism and increasing lipid peroxidation. It was found that lead has a dual effect as it increased free radical formation together with depletion of endogenous antioxidant enzymes which are free radical scavengers. Therefore, accumulation of free radicals resulted in inflammation and increased cell injury. This mechanism is well-known to cause cell injury in many tissues [3,35,36] and may be the cause of the lingual atrophy observed in the current study.

It is worth mentioning here that patients with atrophic tongue are reported to suffer from lowered rates of saliva secretion [29]. A research studied the oxidative effect of lead toxicity on rat submandibular gland and saliva using 100 mg/kg of lead acetate. After 2 h the gland and saliva were collected for evaluation. Results conveyed decreased secretary function and altered salivary composition, which were attributed to increased oxidative stress [37]. Hence, it can be suggested that the indirect effect of lead on decreased salivary function may be another cause of the lingual atrophy seen in the present study.

Furthermore, another cause of lingual atrophy is anemia where changes like dryness of the mouth, glossitis and patchy atrophy of filiform papillae have been reported [38]. A review on the effects of lead on the body stated that lead causes impairment of detoxification of environmental toxins and reduces hemoglobin synthesis; thus, decreasing oxygen transport to all tissues, ultimately causing anemia [39]. This is in agreement with another study, which stated that in chronic lead toxicity, anemia is a common clinical finding [40]. Again this association may explain the atrophic changes observed in this experimental model where lead-induced anemia may have caused a similar picture to lingual atrophy in anemia.

It was reported that during the process of atrophy the filiform papillae are affected first, followed by fungiform papillae [41]. In the present work, the fungiform papillae of Group II were apparently shorter, with a swollen taste bud. The SEM confirmed this apparent distortion in shape together with wrinkling of the surface and ill-defined taste buds. These results are similar to another research which evaluated the effect of heavy metal (manganese) poisoning on the fungiform papillae of mice [42]. Both light and SEM results showed similarity to the present findings. The authors suggested that the swelling of taste buds in fungiform papillae would decrease function of these taste buds and probably inducing changes in food intake behavior as a consequence.

This is also in agreement with another analytical study in which; clinical evaluation of 70 subjects working in lead mines was done together with measurement of blood lead level [7]. The study reported reduced taste perception and tongue burning in workers. The study attributed these changes mainly due to the direct contact of the lead fumes with the oral mucosa.

When honey was given with lead in Group III, a dose of 50 mg/kg of honey was given diluted in 1ml of distilled water [24]. This low dilution was chosen to facilitate administration and to activate the antioxidant activity of honey. In a study to evaluate the effect of dilution of honey on its antioxidant activity, it was found that excessive increase in honey dilution lowered antioxidant capacity [43].

Simultaneous administration of honey with lead in the present study in Group III appeared to ameliorate lead-induced histological alterations on the lingual papillae. In this group, the filiform papillae showed minimal changes when seen by both light and scanning microscopy. No areas of epithelial erosions were seen in Group III by LM while by SEM; the papillary density appeared increased compared to Group II, but apparently less than Group I.

In one study, the biochemical parameters were evaluated when honey was given with lead to rats using a dose of 10 mg/kg lead and 200 mg/kg honey both given for 7 weeks [13]. The results showed significant recovery in the biochemical alterations caused by lead toxicity upon consumption of honey. These findings suggested that honey may exert a protective role against lead toxicity. Three constituents of honey were suggested to have an antioxidant role namely; thiamin, riboflavin, and ascorbic acid. Thiamin inhibits or interferes with lead absorption due to complex formation between lead and thiamin. Ascorbic acid has also been reported to act as a detoxifying agent by forming poorly ionized, but soluble compounds with lead [13]. This improvement in the biochemical analysis upon honey consumption is in agreement with the results of the current study where a marked improvement on the light microscopic level was observed.

Another research on the protective effect of honey on lead-induced oxidative stress in rat liver was conducted. Lead was given as (0.2%) in drinking water while 1.5 ml/kg honey was given orally for 4 weeks. The biochemical analysis showed that honey significantly increased antioxidant enzyme activity. The histopathological examination revealed that honey diminished the adverse effects of lead in rat liver, restoring normal liver architecture. The authors suggested that honey exerted its protective role through restoring enzymatic activity and through its antioxidant mechanism [44].

Honey is a potent source of iron, copper, and manganese. When these elements are combined they aid in hemoglobin synthesis. Honey is therefore a powerful weapon against anemia [45]. Thus it maybe suggested that the anti-anemic effect of honey may provide an alternative or an additional mechanism to explain the protective effect of honey against the lead-induced lingual atrophy.

It was reported that in cases of regeneration of atrophied lingual papillae; the fungiform papillae regenerate first followed by regeneration of filiform [41]. This may explain the present findings where honey caused marked regeneration of the fungiform papillae. This was observed by both light and SEM where the fungiform papillae of Group III showed a close picture to that of Group I.

Black seed was added to basal diets in many studies [17,46] however, this method was avoided in the present study to standardize individual doses for each rat. Other studies used ethanol extracts of black seed [47] or essential oil [48] to test their effectiveness. Meanwhile in the present study it was preferred to use whole crushed black seed in an aqueous suspension to benefit from all its constituents especially that the whole seeds were traditionally used and not the black seed extracts or oil [49].

In one study, black seed showed a protective effect on the liver and kidney in lead intoxicated rats. The results showed that black seed successfully protected the liver and kidney tissue from damaging effects of lead toxicity and was attributed to its antioxidant activity [17].

By light and SEM, the atrophic histological changes of the lingual papillae were slightly less obvious in Group IV, which received black seed and lead, compared to Group II, which took the lead only. However, the improvement in the histological picture observed in Group IV was much less than in Group III, which received honey with lead and gave better results. Few filiform papillae of Group IV were shorter or ill-defined in shape when seen by LM. Although most of the true filiform papillae in this group were intact and had normal epithelium, few areas of epithelial erosions were noticed in some samples.

In a previous study, black seed completely prevented the formation of epithelial erosions and ulcerations of mucosa of the stomach caused by alcohol [50]. This difference in efficiency of black seed observed in that study and this current study, maybe due to the different doses used in each study and timing of administration. For instance, in the above mentioned study, black seed was given as a pretreatment at doses of 250-500 mg/kg 30 min before administration of alcohol which might have a topical or direct effect on the gastric mucosa [50].

Meanwhile, in the current study a dose of 50 mg/kg black seed suspended in 1 ml of distilled water was administered by oral intubation together with the lead dose at the same time, meaning that the black seed here has more of a systemic effect on lingual papillae.

As mentioned earlier the lead-induced lingual atrophy maybe attributed to the effect of lead toxicity on salivary gland structure and function. Black seed was shown to improve degenerative changes of aged parotid glands in rats [51]. A dose of 300 mg/kg was given to aged rats for 3 months. Their histopathological results showed that black seed decreased age changes in the parotid gland and maintained normal architecture of its structure. The authors stated that black seed had a cytoprotective effect against the degenerative changes of age and increased the activity of parenchymal cells of rat parotid gland. It is thus suggested that in the present study; the observed improvement in the histological picture in Group IV may be caused either by the direct effect of black seed on cells of the lingual papillae or indirectly through its effect on salivary gland components or both.

Some studies showed that black seed exerted a dose-dependent protection against various insults. For instance, a study tested the protective ability of different doses of black seed oil (0.5, 1.0 or 2.0 ml/kg against the nephrotoxic effect of gentamycin on the rat kidney. The results showed that treatment with black seed oil produced a dose-dependent amelioration of the biochemical and histological changes caused by gentamycin. Furthermore, there was a statistically significant higher protection observed at the two higher doses [52].

Other studies also showed dose and duration dependent effects of black seed. One study investigated the effect of different doses of black seed given for variable durations on serum lipids. Four doses of black seed were used (100, 200, 400, and 600 mg/kg/day) and each dose group was further subdivided into three duration subgroups 1, 2, and 4 weeks. The results showed that the 400-600 mg/kg doses showed a significant decrease in the levels of serum cholesterol after 1 week while after 2 and 4 weeks all doses from 200 to 600 mg were better than control [53]. From the above-mentioned studies it maybe suggested that varying the doses and/or duration of black seed co-administration with lead may render better protective effects in future research.

Although black seed offered some protection against the lead-induced lingual atrophy yet the protection offered by honey appeared more superior. However this is in contradiction with another study, which compared the effects of honey and black seed against the toxic effect of carbon tetrachloride on rat liver both biochemically and histopathological. The study used the same dose as in the present study. The results showed marked improvement in the antioxidant status and histopathological findings in both honey and black seed. However, black seed offered more protection than honey [24].

Nevertheless, other studies are in agreement with the results of the current study where honey offered higher protection than black seed. For example, a research investigated the effect of honey and/or black seed in modulating the heart disorder induced by food additives in male rats. The results showed that both offered remarkable protection against the toxic effects of these food additives on the heart. However, the treatment by honey was more effective than black seed [54]. Further research is required using different forms of black seed, increased doses and/or durations to test whether these would provide better ameliorative effects.

## CONCLUSIONS AND RECOMMENDATIONS

The results of the current study showed that the occupational lead dose causes detrimental effects on lingual papillae of albino rats. Filiform papillae appeared to be the more sensitive to lead toxicity than fungiform papillae. Based on the results of this preliminary research, honey appeared to be more protective than black seed against lead-induced changes. Further experiments are needed to investigate the use of honey or black seed preparations with defined composition to assess the exact mechanism involved in this amelioration. Further research is needed to test whether the obtained results will also be achieved in human workers.

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