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**ORIGINAL ARTICLE**

# The Possible Protective Role of Vitamin E on the Induced Silver Nanoparticles Toxicity on Filiform and Circumvallate Tongue Papillae of Albino Rats Histological and Immunohistochemical Study

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**KEYWORDS**

Silver nanoparticles;
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**ABSTRACT:** Recently Silver nanoparticles (AgNPs) are widely used as in manufacturing of filters to purify drinking water, as a part of surgical prosthesis, splints, dental alloys and most importantly in the medical field as a bactericidal, fungicidal and as a therapeutic agent. Although these widely usage of AgNPs, can induce toxicity. to evaluate the protective role of vitamin E on the induced silver nanoparticles toxicity on filiform and circumvallate tongue papillae of albino rats. twenty-eight male albino rats weighing 200-250 grams were divided into; the control and the experimental groups, which is subdivided into, subgroup I receiving AgNPs and subgroup II receiving AgNPs and vitamin E. the experimental period were 28 days, then rats' tongue was dissected to be stained by H&E and examined immunohistochemically for BCL-2. Histological examination of the epithelium covering both filiform and circumvallate papillae of subgroup I showed few epithelial cells vacuolations and signs of degeneration. Meanwhile the taste buds' cells of the circumvallate papillae also showed signs of degeneration together with amalgamated, ill-defined serous acini of Von Ebner Salivary Gland (VESG). Subgroup II showed that the histological features of both tongue papillae were nearly comparable to that of the control group. Immunohistochemical examination showed minimum apoptotic changes in subgroup II as compared to subgroup I and nearly comparable to that of the control group. vitamin E showed an apparent protective role against the histological and apoptotic alterations caused by the toxic effect of AgNPs on both tongue papillae.

## INTRODUCTION

Nanotechnology is a unique and innovative scientific approach that includes designing and application of materials & devices at molecular level in nanometer size ranges in size from 1 to 100 nanometers (nm) [1].

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Using of nanoparticles is widely expanding and have been used in multiple applications, as health care, consumer products like cosmetics, food and feed, environmental health, and agricultural science. It also can be used in medicine including immunology, cardiology, endocrinology, ophthalmology, and pulmonology. This wide usage led to the necessity of appearance of some nanoparticles with some important criteria among which is their biocompatibility that led to the appearance of inorganic nanomaterials like silver nanoparticles (AgNPs) [2].

AgNPs have been used nowadays as a part of the dental field. Dental porcelain being superior in its esthetics and durability properties made it the best choice of material for ceramic restorations. However, conventional dental porcelain suffered from problems such as fragility, chipping, and fracture. This encourages researchers to develop new techniques to increase its toughness [3].

Despite AgNPs beneficial effects in many fields, it induced cytotoxicity and genotoxicity both in vivo and in vitro. This toxicity has been demonstrated by three main mechanisms of toxicity of Ag NPS have been proposed: oxidative stress, DNA damage and cytokine induction [4].and one of these mechanisms is the release of silver ions and production of reactive oxygen species (ROS). It has been shown that mitochondrial dysfunction as well as induction of reactive oxygen species (ROS) by AgNPs result in DNA damage and chromosomal aberrations [5].

Antioxidants either endogenous or exogenous are substances that can slow or prevent damage to cells caused by ROS, which the body makes as a consequence to environmental hazards. There are a lot of antioxidants that can be used like vitamin E which is the one of interest in this study [6].

Vitamin E is another antioxidant and is a fat-soluble vitamin which is stored naturally in the body. It is a family of 8 different compounds. The most potent of which is α-tocopherol. It is found in both natural and synthetic forms, of which the body more readily absorbs natural forms than synthetic. Since it is a fat-soluble vitamin, the best sources of vitamin E are foods high in fat, such as oils and nuts. Similar to vitamin C, vitamin E helps to manage the bodies' level of oxidative stress [7].

## MATERIALS AND METHODS

Silver oxide nanoparticles Purchased from Nano Gate., Egypt. Particles were prepared by chemical reduction method [8] with size 45 ±5 nm in aqueous nitrate buffer. A solution of $AgNO_3$ has been used as $Ag^{+1}$ ion precursor. The Polyvinylpyrrolidone (PVP) has been used as stabilizing agent and borohydrate as mild reducing agent. The color of the solution slowly turned into grayish yellow, indicating the reduction of the Ag ions to AgNPs.

Vitamin E was purchased from Pharco Pharmaceuticals, Cairo, Egypt in form of capsules of 1 gm concentration to be dissolved in 100 ml of olive oil.

### *Animals*

Twenty-eight adult male albino rats were used in this study (weighing +/- 200 gms) each. A license for animal was given by the ethical committee of Faculty of Dentistry Ain Shams University number 613-19/10/2017. Rats were housed in separate cages in the Animal House of "The Medical Research Centre" in Ain Shams University. Rats were kept under good ventilation and adequate stable diet consisting of fresh vegetables, dried bread and tap water ad libidum.

Rats were randomly divided into:

**Control group (C):** which was further subdivided into 2 subgroups each contained 7 rats corresponding to the experimental groups.

**Subgroup (C1):** Rats received intraperitoneal (IP) injections with aqueous nitrate buffer. The dose was 2 mg.kg body weight (B.W) daily for 28 days [9].

**Subgroup (C2):** Rats received through oral gavage olive oil in a dose of 2 mg once daily for 28 days.

**Experimental group** each contained 7 rats which was further subdivided into the following subgroups:

**Subgroup I:** Rats received (IP) injection with AgNPs. The dose was 2 mg.kg B.W once daily for 28 days [9].

**Subgroup II:** Rats received I.P injections with AgNPs, the dose was 2 mg.kg B.W once daily as the previous group, in addition to administration of vitamin E by oral gavage concomitantly, the dose was 100 mg.kg B.W daily for 28 days [10].

At the end of the experiment, all rats were sacrificed by overdose anesthesia. Rats' tongues were dissected and preserved. The specimens were processed to be stained by H&E stain to detect the histological changes and for immunohistochemical examination using BCL-2 stain to detect apoptotic changes.

Statistical analysis was done for the groups of this study where the mean surface area of positively immunohistochemical stained cells was analyzed using Statistical Package for Social Science software computer program version 23 (SPSS, Inc., Chicago, IL, USA). Data were presented in mean and standard deviation.

One way Analysis of variance (ANOVA) and tukey test were used for comparing data. P value less than 0.05 was considered statistically significant.

***Histological results***

***Control group***

Both control subgroups (C1, C2) showed the same histological results. The filiform papillae exhibited organized and conical shape appearance. The papilla appeared covered with keratinized stratified squamous epithelium underlyed with lamina propria of connective tissue (c.t.) which contains groups of mucous glands (Figure 1, A&B). Meanwhile for the circumvallate papilla (CVP), the epithelial covering showed the same histological features as the same mentioned in filiform papillae. (Figure 2 A&B). The taste buds appeared with normal barrel shaped with apparent normal cells. Von Ebner salivary glands showed well developed serous acini (Figure 3 A&B).

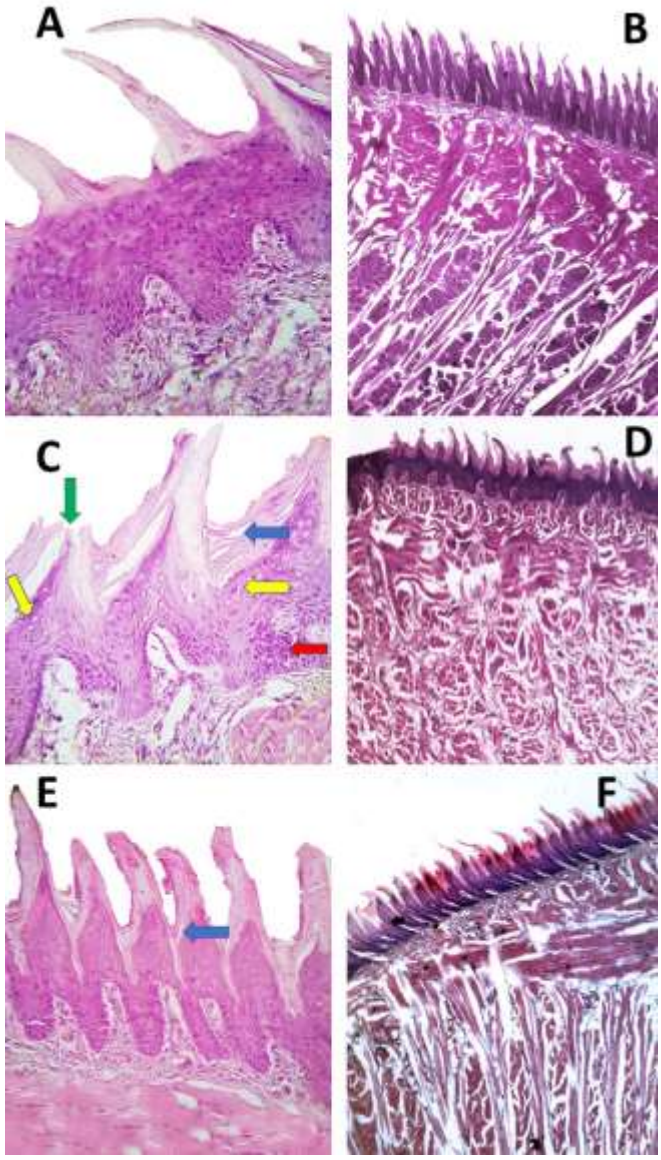


**Figure 1.** Photomicrographs of a section of rat tongue of experimental groups showing filiform papilla (A): control group specimens exhibiting conical shape papilla covered with keratinized stratified squamous epithelium underlined with histologically normal lamina propria of dense connective tissue. (C): experimental group specimens showing Subgroup I the papillae appeared irregularly arranged and the keratin layer showed some areas of separation (blue arrow) with few epithelial cells vacuolations (yellow arrow), Lymphocytic cells infiltration (red arrow), and loss of keratin thickness (green arrow) The underlying c.t. revealed some areas of separations. (E): subgroup II the papillae covered with uniform epithelium and keratin layer while the underlying c.t. appeared more or less uniform, and minor areas of separation in the keratin layer (blue arrow). (H&E, Orig. Mag.400x). (B): control group showing well organized papillae and tongue muscle fibers in the underlying c.t. (D): group I showing non-uniform configuration of the papillae. (F): showing properly arranged filiform papillae. (H&E, Orig. Mag.100x)

***Experimental group***

***subgroup I***

The filiform papillae appeared irregularly arranged and the keratin layer showed some areas of separation with few epithelial cell vacuolations observed specially in the prickle cells layers. The underlying c.t. revealed some areas of sepertaion**.** Also, lymphocytic cells infiltration within the

c.t. papilla was detected (Figure1, C&D). Affection was almost covering the whole papillae.

Circumvallate papilla showed apparent thinning of the covering epithelium and overlying keratin layer. There were areas of degenerated collagen fibers observed as areas of separation between the c.t. fibers and dilated blood vessels (Figure2 C&D). Some swollen taste buds appeared with degeneration of some taste cells, while some taste buds appeared atrophied with irregular outline (Figure3 C). The serous acini of VESG appeared as aggregations neighbored by tongue muscle fibers. There was apparent reduction in size of acini appeared as minor spaces in-between the acini. The serous acini appeared amalgamated with ill-defined cell borders and ill-defined secretory granules (Figure3 D).

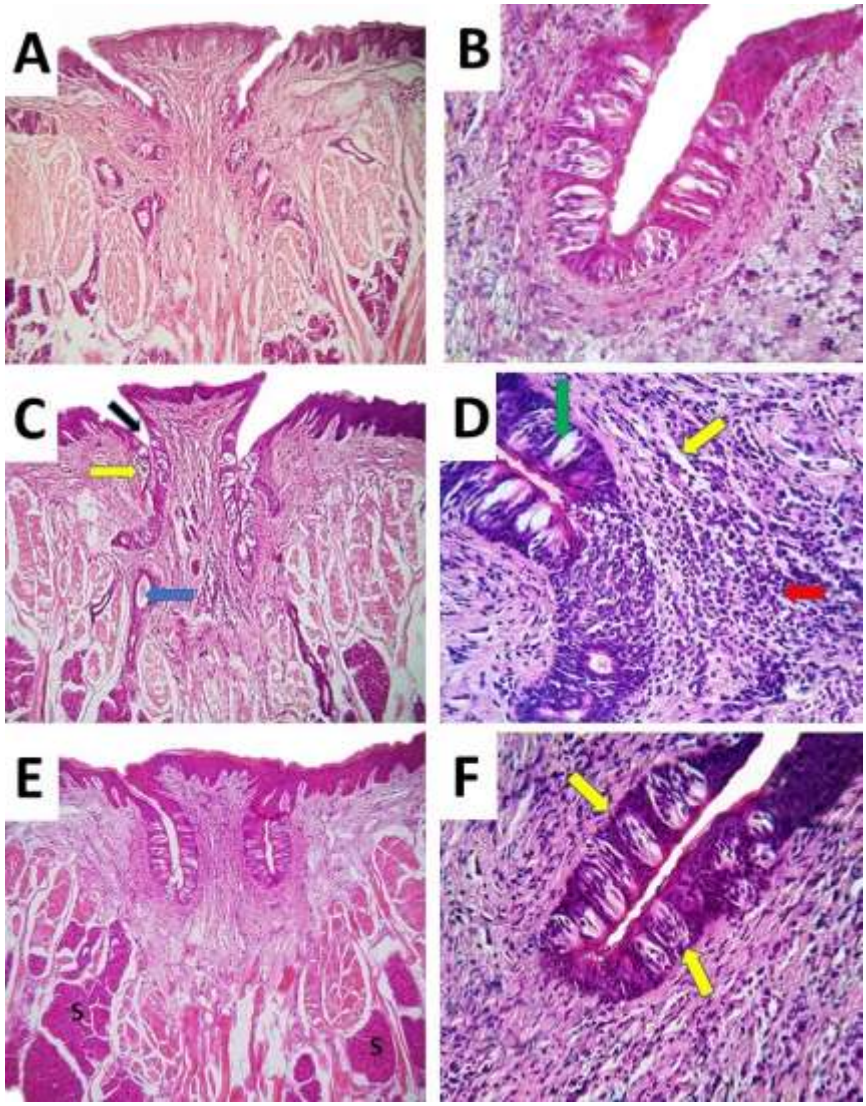


**Figure 2.** photomicrograph o of a section of the circumvallate papilla (CVP) of rate tongue showing (A): control group specimens the circumvallate papilla (CVP) surrounded by a trough and underlying developing VESG formed of branched serous acini within the c.t. (C): group I showing deformity in the general outline of CVP with narrowing and shortening of the trough (black arrow). Apparent Decrease in number of taste buds (yellow arrow), VESG excretory duct showed stagnant secretion (blue arrow) (E): group II showing normal outline with properly arranged taste buds on its sides and Von Ebner serous acini (S) underlying the papilla. (H&E, Orig. Mag.100x). (B): control group showing a barrel shaped taste buds with apparent normal taste cells. (D): group I showing some taste buds with separation and degeneration of some taste cells (yellow arrow), inflammatory cell infiltration (red arrow), areas of separations in the underlying collage fibers (green arrows) (F): group II showing some normal shaped taste buds and taste cells (yellow arrow). (H&E, Orig. Mag.400x)

***subgroup II***

The filiform papillae covered with uniform epithelium and keratin layer, while the underlying c.t. appeared more or less uniform with blood capillaries (Figure 1, E&F).

For the circumvallate papilla, it showed taste buds and taste cells which were regularly arranged at the walls of the trough. Meanwhile the underlying connective tissue appeared uniform with blood capillaries and few inflammatory cells infiltration. The VESG serous acini showed almost normal histological features (Figure2 E&F).

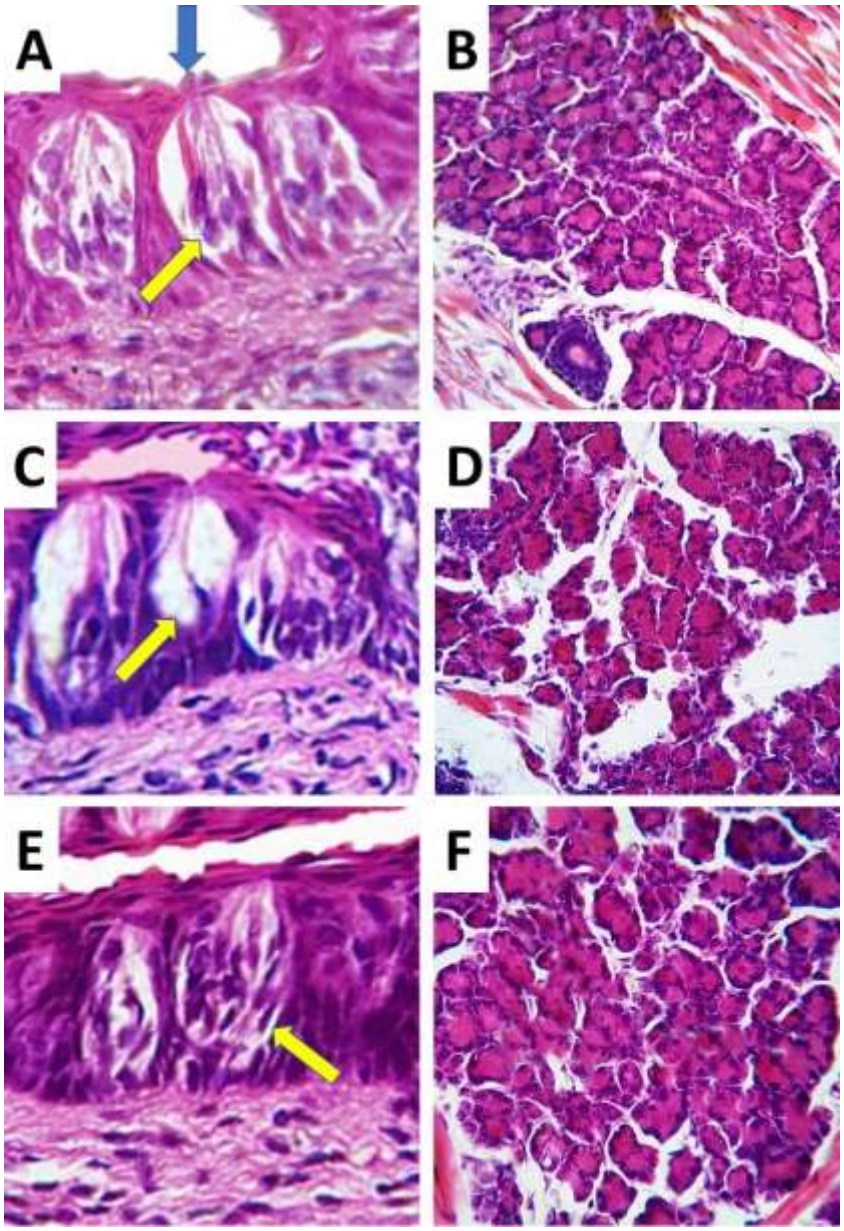


**Figure 3.** photomicrograph of higher digital magnification of taste buds of a section of rate tongue (A): control group showing a normal barrel shaped taste buds with taste cells having prominent nuclei (yellow arrow) and taste pore opening into the trough (blue arrow) (C): group I showing degeneration of some taste cells (yellow arrow) (E): group II showing almost normal shaped taste buds and taste cells (yellow arrow). (B): control group showing well developed serous acini surrounded by c.t. septa (D): subgroup I the serous acini of VESG appeared amalgamated with ill-defined cell borders (F): subgroup II VESG serous acini showed apparent normal histological features. (H&E, Orig. Mag.400x)

***Immunohistochemical results***

Immunohistochemical examination using BCL-2 stain to detect apoptotic changes, the bcl-2 gene was found encodes a membrane protein localized to the nuclear membrane, the inner surface of mitochondria and the endoplasmic reticulum. It is a cytoplasmic reaction targeting the basal cells of epithelium, the more reaction indicating less apoptotic changes. The cells with positive staining appeared with brownish staining meanwhile the rest of the section showed negative reaction which was counter stained blue.

***Control group***

Both control subgroups (C1, C2) showed the same immunohistochemical results. Examination of the filiform and Circumvallate papillae immunostained with bcl-2 demonstrated apparent decrease in the numbers of positively stained cells. (Figure 4 A&B).

***Subgroup I***

The immunostained sections of the filiform and Circumvallate papillae of this group apparently demonstrated no positively stained cells **(Figure4 C&D).**

***Subgroup II***

Examination of the immunohistochemical stained sections of the filiform and Circumvallate papillae of this group demonstrated increased number of positively stained cells (Figure 4 E&F).

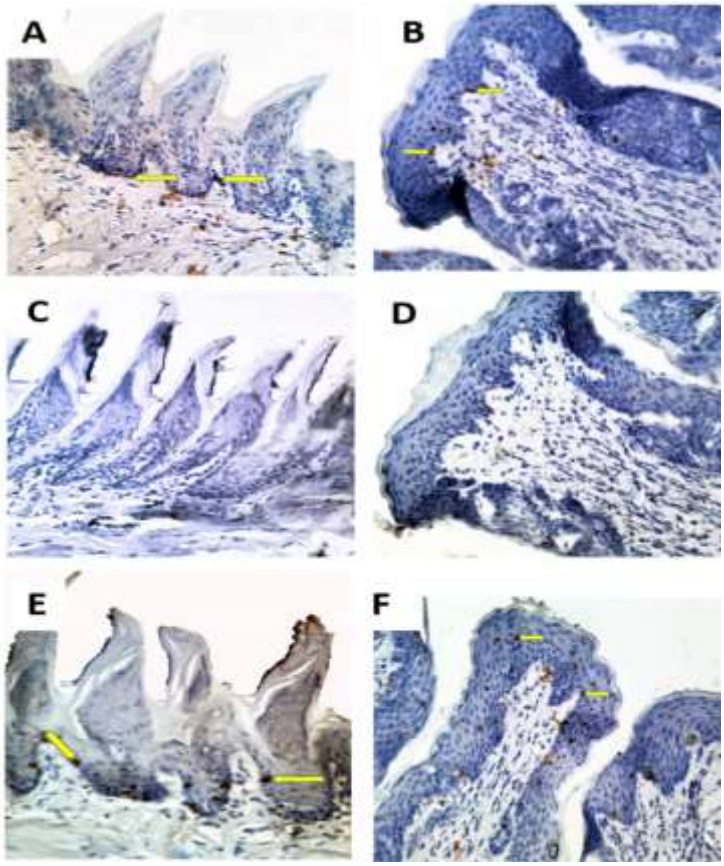


**Figure 4.** photomicrograph of control group specimens (antibcl-2, Orig. Mag.400x) showed the filiform (A) and circumvallate papillae (B) immunostained with bcl-2 demonstrated apparent decrease in the positively stained cells (yellow arrows). Photomicrograph of experimental group (antibcl-2, Orig. Mag.400x) showing Subgroup I: The immunostained sections of the filiform (C) and circumvallate papillae (D) showed almost no positively stained reactions to the cells. Subgroup II: The immunohistochemically stained sections of the filiform (E) and circumvallate (F) papillae showed increased number of the positively stained cells (yellow arrows).

***Statistical analysis***

Statistical analysis for the mean surface area of the positively stained cells with bcl-2 stain in different examined specimens showed the following:

***The filiform papillae***

One- way ANOVA statistical analysis revealed a statistically significant difference between groups (p<0.001). Subgroup I showed highly significant difference decrease compared to the control group with a p value <0.001. While subgroup II showed high significant difference, increase compared to the control group with a p value 0.001. subgroup II showed high significant difference increase compared to subgroup I with a p value <0.001.

***The circumvallate papillae***

One- way ANOVA statistical analysis revealed a statistically significant difference between groups (p<0.001). Subgroup I showed highly significant difference decrease compared to the control group with a p value <0.001, while subgroup II showed no significant difference increase compared to the control group with a p value =0.2. subgroup II showed high significant difference increase compared to the subgroup I with a p value <0.001.

**Table1.** (A) showing mean and standard deviation (SD) values and results of one way ANOVA followed by post-hoc tukey test for comparison between the mean surface areas of positively stained cells of filiform papillae in the different examined groups. (B) showing mean and standard deviation (SD) values and results of one way ANOVA followed by post-hoc tukey test for comparison between the mean surface areas of positively stained cells of circumvallate papillae in the different examined groups.

| A | Mean±SD | P value | ANOVA P value |
|---|---|---|---|
| **Control group** | 15719±2441.2 | | |
| | | | <0.001* |
| **AgNPs group** | 3933±587.5 | <0.001* | |
| **AgNPs+E group** | 42493±4074.4 | <0.001* | |
| **B** | | | |
| **Control group** | 23080±6621.5 | | |
| | | | <0.001* |
| **AgNPs group** | 1042±369.8 | <0.001* | |
| **AgNPs+E group** | 48262±8541.7 | 0.2 | |

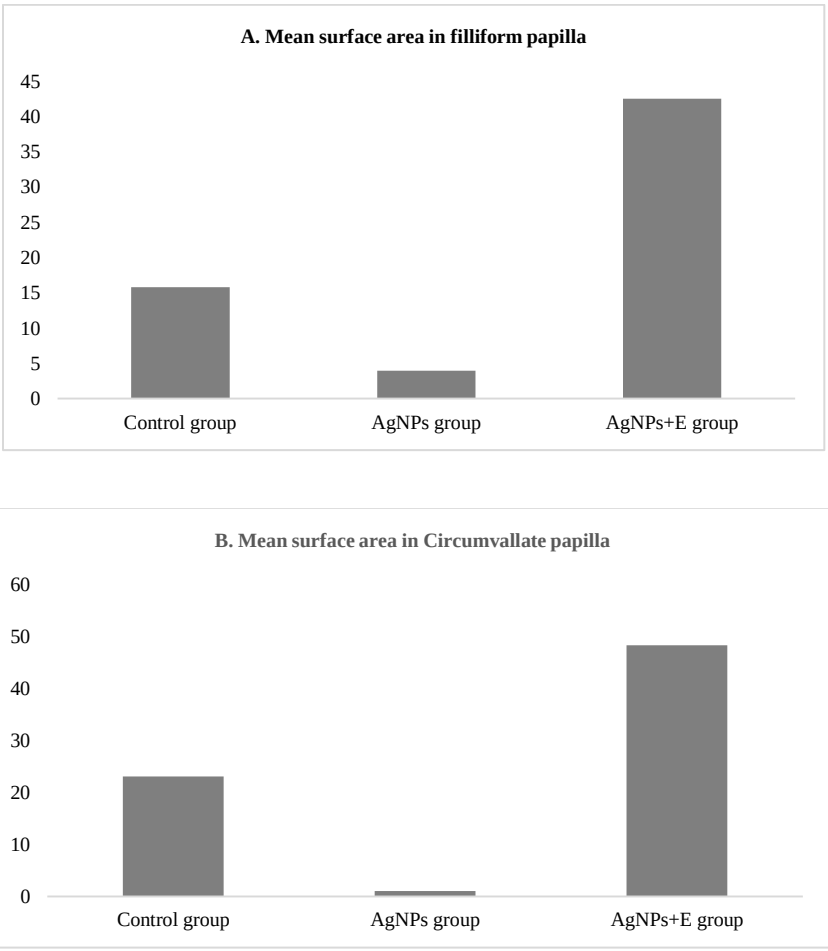


**Figure 5.** Bar chart (A) representing mean surface area of positively stained cells of filiform papillae in the different examined groups. Bar chart (B) representing mean surface area of positively stained cells of circumvallate papillae in the different examined groups.

## DISCUSSION

In the present study rats received (IP) injection with AgNPs, the dose was 2 mg.kg B.W once daily for 28 d. This dose was used to study the toxic effect of AgNPs on liver and bone marrow. Also, they stated that this dose is the maximum toxic dose to be given which if exceeded it will increase the mortality rate of rats [9].

In the present study, rats received vitamin E by oral gavage, the dose was 100 mg.kg B.W daily for 28 days. where it was founded that vitamin E with the previously mentioned dose minimized the histological and apoptotic changes demonstrated with lead toxicity on liver tissues [10].

In the present study, the experimental subgroup I, the filiform papillae showed some areas of separation between keratin layers. Where they found out that AgNPs can damage DNA and caused chromosomal aberrations by increasing production of ROS which eventually led to mitochondrial damage. Therefore, these factors may be considered the prime factors resulting in cell cycle arrest which eventually may lead to these separations [11].

Also, the circumvallate papilla showed some taste buds with atrophied and irregular outline while taste cells showed signs of degeneration. The authors studied the

effect of AgNPs which revealed a strong toxic and oxidative stress response with appearance of ROS [12].

The serous acini of VESG of subgroup I appeared amalgamated with ill-defined cell borders. That one of the likely mechanisms of AgNPs toxicity are the induction of ROS and the oxidative stress in cells and organs which could be the cause of these results [13].

The results of this study come in agreement with several studies that examined the effect of toxins on serous acini of submandibular glands and the subsequent production of ROS which resulted in mitochondrial damage as well as damage to other cell organelles specially the nucleus and the rough endoplasmic reticulum [14].

Concerning the filiform papillae of subgroup I, few epithelial cell vacuolations were observed specially in the prickle cells layer. The toxicity of AgNPs affecting the mitochondrial activity increased with the rise in the concentration of AgNPs which led to reduction in the mitochondrial function, necrosis and apoptosis of mitochondria suggesting the presence of these vacuolations is due to mitochondrial apoptosis [15].

Collagen fibers in the underlying c.t. of filiform and CVP papillae of subgroup I showed signs of degeneration leaving areas of separation. As they used a full- thickness excisional wound model in mice where the wounds were treated with AgNPs. It was founded that AgNPs addition led to suppresses the proliferation of fibroblasts, changed the normal phenotype of the fibroblasts, and thus decreased the fibroblasts' cell number [16].

In subgroup II receiving AgNPs and vitamin E, taste buds and taste cells showed normal histological features comparing to subgroup I. these findings were in accordance with the neuroprotective effect of vitamin E on single-walled carbon nanotube-induced neurotoxicity, which was shown in cultured PC12 cells, an in vitro model of neuronal cells. The presence of vitamin E inhibited the formation of ROS, suppressed the level of lipid peroxide, blocked the reduction in mitochondrial membrane potential and prevented apoptotic cell death of PC12 [17].

Furthermore, the von Ebner gland serous acini in subgroup II appeared with normal histological features comparing subgroup I. These findings could be clarified by the fact that the most efficient Non-enzymatic antioxidants include Vitamin E and C, that interact with the oxidative radicals thus protecting the cells from ROS [18].

Using of antioxidants as vitamin c reverse this toxic effect of silver nanoparticles by minimizing the severity of both histological and apoptotic changes in circumvallate and filiform tongue papillae [19].

In the present study using of bcl-2 to reveal the apoptotic changes were demonstrated in the experimental groups, the subgroup I showed almost no positively immunostained cells compared with the control and subgroup II which was also statistically confirmed with p value (P=<0.001). This over-expression of bcl-2 in subgroup II indicating less apoptotic changes demonstrated in subgroup II in comparison to the subgroup I. The effects of AgNPs on the baby hamster kidney (BHK21) cell lines to assess the mechanisms that AgNPs induced apoptosis by enhancing the production of ROS. The bcl-2 expression was found to be down-regulated in the cell lines exposed to AgNPs indicating more apoptotic and this was also confirmed by increased histological alterations [20].

In subgroups II, the taste buds and taste cells of CVP showed nearly normal histological features comparing to subgroup I. These results were in parallel with authors demonstrated that the supplementation of vitamin E significantly mitigated the damage caused by single-walled carbon nanotubes administrated by I.V injections on the central nervous system of mice. vitamin E was orally administrated with the doses of single-walled carbon nanotubes, to investigate the vitamins' protective effect. The results showed that the administration of vitamin E decreased the level of oxidative stress in the brain, suppressed brain cell damage, inflammation, and apoptosis [21].

Meanwhile in the filiform papillae of subgroup II, there was apparent reduction in the epithelial cells vacuolations in the overlying epithelial layers comparing to subgroup I. This finding could be explained that the effects of Ag on mitochondria can be blocked by the addition of antioxidant scavengers like vitamin E. Vitamin E play a major role in cellular protection against oxidative stress due to their ability to bind and neutralize ROS, which eventually led to

avoiding lipid oxidation and mitochondrial permeability [22].

There was apparent reduction in the lymphocytic cells' infiltration in the following study in the underlying c.t. of subgroup II comparing to subgroup I. This could be explained by the mechanism of protection of vitamin E involving ROS elimination, downregulation of T-helper 2 cells' responses and reduction of Ig production [23].

In conclusion, administration of AgNPs caused marked histological and apoptotic changes in circumvallate and filiform tongue papillae, meanwhile administration of antioxidants as vitamin E succeeded to overcome the effect of AgNPs by minimizing the severity of these histological and apoptotic changes.

**Grant Information**

These authors declare that no grants were involved in supporting this work.

**Conflict of interest**

No conflicts of interest.

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