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AGING-RELATED MOROLOPHGICAL CHANGES IN TASTE BUDS OF AGED RATS: AMELIORATIVE ROLE OF GREEN TEA AND GARLIC

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Green tea is popular drink and rich in strong antioxidants, flavonoids. Also, garlic is important dietary and has medicinal role for centuries and possess both antioxidant and anti-inflammatory action in several oxidative stress conditions. Four groups of both male and female rats [young (3 months-old), aged (26 months-old), aged+green tea (200 mg/kg) and aged+garlic (100 mg/kg)] were used. Histological and ultrastructural changes in the taste buds of the circumvallate papilla examined.The histopathological changes in the taste buds of aged animals appeared as degenerative changes in the cells of the buds and some buds appeared empty. The administration of aged rats with green tea relatively remedied the destructive effect of aging in both male and female rats. Garlic treatment of aged male and female rats showed great improvement in the taste buds. This improvement represented as the normally appearance of taste buds cells in the manner resemble that in young control group. In conclusion, this study showed that aging process induced destructive changes in taste buds and suggests that the use of both green tea and garlic as natural products may inhibit these changes which related to aging.

INTRODUCTION

As a unique feature of aging process, includes many physiological actions to maintain homeostasis and resulting in death in the life cycle of virtually all multicellular organisms. Some lack sufficient empirical support to merit serious consideration to explain the aging process [1].
It was proved that the oxidative stress theory of aging is the accepted one for the aging molecular basis [2]. The hypothesis of this theory explored the different links between longevity, oxidative stress and the diseases related to aging process.Salmon et al. [3] found that hydroxyl ($OH^{\cdot}$) and hydroperoxyl (HO_2) free radicals (the by-products of normal oxygen-utilizing metabolic processes) may play an important role in the process

of aging. A slight modification was added to this theory to maximize the role of mitochondria as the site of free radical production.

In many cases, a taste defect is truly considering a primary defect in olfaction. In addition to smell dysfunction, prior upper respiratory infection, head injury, drug use, are the most frequent causes of taste dysfunction. Indeed, gustatory dysfunction may be related to the normal process of aging[4].The sense of the molecules that can be tasted through the taste cells collected in the taste buds on the tongue, palate, pharynx and the upper third of the esophagus. The tongue has its taste buds primarily in papillae, in each taste bud there are four types of cells that can be morphologically identified: basal, dark, light and intermediate cells. Basal cells are believed to be stem cells which are small circular in the base of the bud and the other cell types derived from it. Taste cells are short-lived and are constantly replenished or regenerated [5].

One of the most popular drinks consumed worldwide is tea. Tea, from the plant *Camellia sinensis*, as green, black or Oolong tea is consumed in different parts of the world. However, the most significant effects of green tea consumption and drinking were observed consistently on human health [6]. Polyphenols are known as catechins which considered as antioxidants. Tea catechins include epicatechin (EC), epigallocatechin (EGC), EC gallate (ECG) and EGC gallate (EGCG). The most active antioxidant of the tea catechins is EGCG which responsible for the "green tea effect" [7].

Garlic (*Allium sativum*) has an important nutritional and natural role for centuries and even today. Garlic has been known since ancient times as a seasoning and flavoring agent for food. The pharmacological and antioxidant effects of garlic are hypolipidemic, hypoglycemic, anticoagulant, antihypertensive, antimicrobial, anticancer and antitumor, as treatment for heavy metal poisoning, hepatoprotective and as an immunomodulator [8].

Organo sulfur compounds, present in garlic responsible for the garlic antioxidant effect [9]. Allicin (allyl 2-propenethiosulphinate or diallylthiosulphinate) is considered the principal bioactive compound present in aqueous garlic extract. Banerjee and Maulik [10] found that, in garlic powder the activity and composition of allinase nearly identical to those of fresh garlic. They also reported that, the drying and dehydration temperature does not exceed 60°C because allinase is inactivated above this degree. Allinase enzyme present in garlic is activated when garlic is chopped or crushed and acts on alliin (present in intact garlic) to produce allicin which is the most active antioxidant [11].

MATERIAL AND METHODS

Animals

In this study a total number of 160 rats (80 males and 80 females) were used. 20 males and 20 females with initial ages of 3 months (young), 60 males and 60 females with initial age of 22 months (aged). They were purchased from Assiut University Joint Animal Breeding Unit. Care and treatment of animals was approved and practices were performed according to approval of ethics regulation at Assiut University. The animals were kept at temperature of 23 ± 2 C° and light cycle of 12:12 hours light: dark. All animals were given free access to standard chow and tap water [12].

Green tea and garlic preparations:

The leaves of green tea (*Camellia Sinensis)* were boiled in distilled water (1:10 w/v) 5 min twice. The solution was called unfiltered, and then filtrates were combined, concentrated, and lyophilized. Ten grams of lyophilized aqueous extract were soaked in 1 liter of boiling distilled water to make 1% solution. The solution designated as green tea extract (GTE) was orally administered to rats by gastric tube at a dose of 200 mg/Kg [13].

Garlic (Tomex 200 mg tab) was dissolved in distilled water and was given orally to rats by gastric tube at a dose of 100 mg/kg [14].

Experimental Design and procedures

The animals were divided into 4 groups for each gender, 20 rats for each; group 1; (3 mon- old) served as young control group (Ycont), group2; (26 mon-old) served as old group (Ag), group 3; (26 mon-old) orally received 200 mg/kg green tea extract per day (Ag+GT), and group 4; (26 mon-old) orally received 100 mg/kg garlic per day (Ag+Gr). The administration of green tea and garlic was repeated daily for 4 months.

Histological study

For the histological examinations, pieces of the tongue were fixed in formal alcohol. Blocks were made and paraffin sections were cut 5-7 μm thick and stained with haematoxylin and eosin. Sections were examined using light microscope and photographed. All methods were applied according to [15].

Transmission Electron Microscope study

For transmission electron microscopy, the posterior parts of the tongue were fixed in 2.5% glutaraldehyde in cacodylate buffer. The specimens were washed in cacodylate buffer (0.1 M, pH 7.2) for 1-3 hours and then post fixed in 1% osmium tetraoxide for 2 hours. The specimens were placed in propylene oxide for 60 minutes, then in pure epon 812 and incubated in a special polymerization incubator (one day at 37 °C, second day at 45 °C and then three days at 60 °C). Semithin sections were obtained and stained with toluidine blue and examined using a light microscope. Representative fields of semithin sections were selected. Ultrathin sections were mounted in copper grids and stained with uranyle acetate, lead citrate and investigated with TEM [12, 16].

RESULTS

Male groups

In young control group, H and E sections in the circumvallate papilla showed that the taste buds appear pale oval structures in the epithelium. They composed of many spindle shape dark and light cells with vesicular nuclei. These cells converge at the apical taste pore. Also, the buds have basal cells (fig. 1a). The semithin sections revealed the light cells with round vesicular nuclei. The dark cells were elongated with dark stained cytoplasm. The basal cells were distributed at the basal part of the bud (fig. 2a). Electron microscopic examination revealed that the light cells have electron lucent cytoplasm with vesicular nuclei. The dark cells appeared with electron dense cytoplasm and many with oval vesicular nuclei (fig. 3a).**In aged rats**, few buds were empty and others contained degenerated cells and mitotic division was clearly observed in the basal cells (fig. 1b). Semithin sections revealed the presence of many taste buds with few numbers of cells. These cells were widely separated by spaces. Some of these cells had dark irregular nuclei. Mitotic divisions were observed in the basal cells (figs. 2b).Electron microscopic examination of aged male taste buds showed the irregular distribution of electron dense dark cells. These cells have had many processes. Their nuclei were irregular with peripherally located chromatin. The electron lucent light cells were also widely separated and many nuclei appeared with lysis (fig. 3b). **In Aged rats treated with green tea**, the cells have had vesicular nuclei. Mitotic division was observed in the basal cells. Few taste buds were small with few cells as shown in H&E and semithin sections (figs. 1c and 2c). Electron micrographs showed light and dark cells. These cells had vesicular nuclei and the cells were separated by many spaces (fig.

3c). **Aged rats treated with garlic,** the majority of the taste buds of the rats treated with garlic appeared normally. The buds were filled with spindle shape cells and mitotic divisions were observed in the basal cells (fig. 1d). Semithin sections showed normal appearance of the taste buds. The buds composed of light, dark and basal cells (fig. 2d).With electron microscopic examination, the taste buds of aged male rats treated with garlic showed the normal appearance except few cells appeared with small and dark nuclei (fig. 3d).

Female groups

In young control group, haematoxylin and eosin stained sections showed that the buds appear as pale oval or round structures filled with cells. The cells were spindle in shape and converge at the apical taste pore (fig. 1e). Semithin sections showed the light, dark and basal cells of the taste buds. The light cells had rounded vesicular nuclei and pale cytoplasm. Dark cells had oval vesicular nuclei and darkly stained cytoplasm. The longitudinal section showed taste pore filled with cellular microvilli (fig. 2e). Electron microscope showed that taste buds of young control female rats have dark cells with electron dense cytoplasm and light cells with electron lucent cytoplasm. The cells had nuclei with dispersed chromatin (fig. 3e).**In Aged group,** in haematoxylin and eosin stained sections; some empty taste buds are present. Other buds have small number of cells. These cells had deeply stained nuclei .Some taste buds had degenerated cells (fig. 1f). Semithin sections showed few numbers of cells in taste buds were widely separated. The buds were surrounded by dark stained irregular apoptotic cells (fig. 2f). Electron micrographs showed few numbers of dark and light cells widely separated (figs. 3f). **In Aged rats treated with green tea,** Haematoxylin and eosin stained sections showed few taste buds with dark stained cells and others had few numbers of cells. These dark cells had deeply stained nuclei. The other cells were nearly normal (fig. 1g). Semithin sections revealed the presence of dark and light cells with vesicular nuclei. Some cells had deeply stained nuclei. The same spaces are present between cells (fig. 2g).Electron micrographs showed longitudinally cut taste buds containing light and dark cells with tapering end to the pore and the cells had vesicular nuclei (fig. 3g).**InAged rats treated with garlic,** haematoxylin and eosin stained sections showed that the taste buds composed of spindle shaped cells with vesicular nuclei (fig. 1h). Semithin sections revealed the normal appearance of taste buds. The cells had vesicular nuclei. Mitosis observed in some basal cells (fig. 2h).The electron micrographs showed normal dark and light cells with microvilli in the taste pore (fig. 3h).

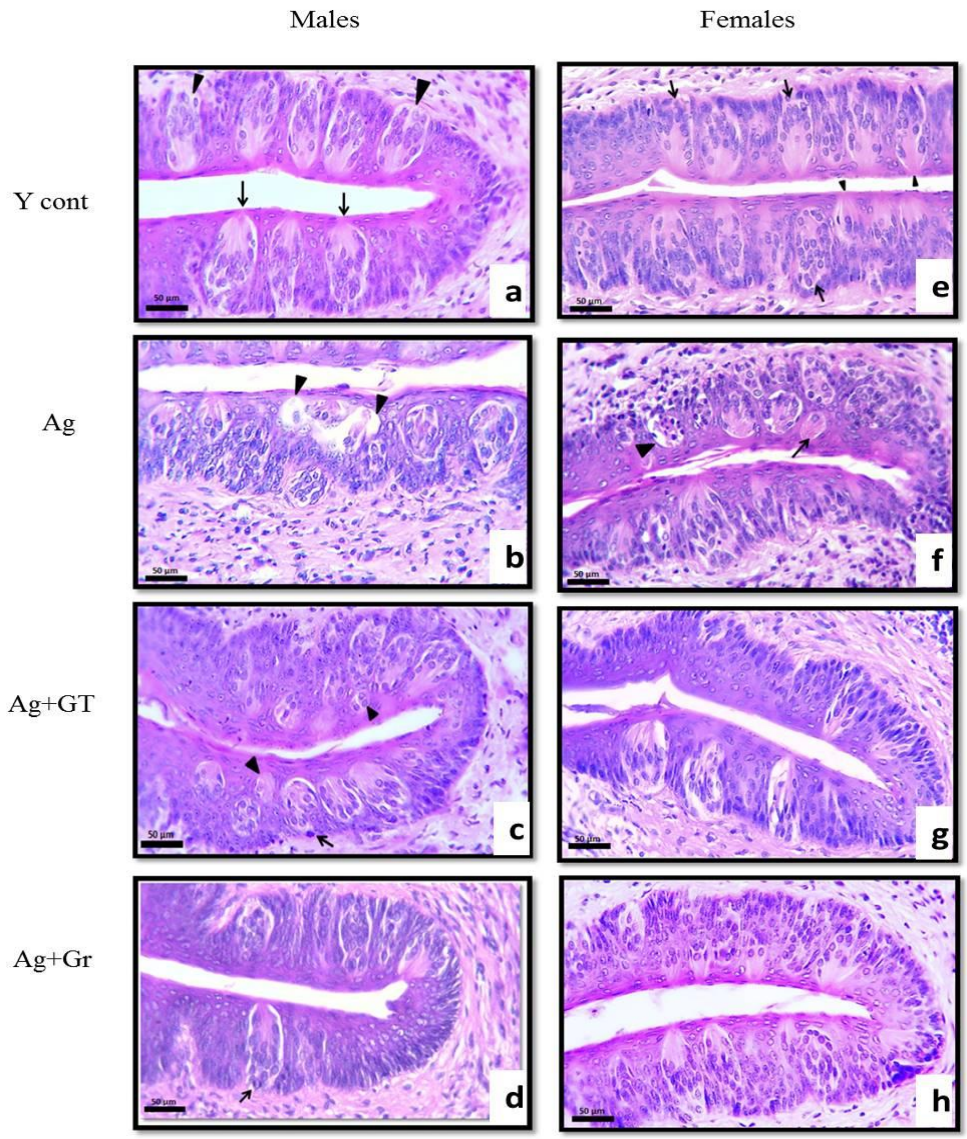

Figure 1. Photomicrographs of taste buds in circumvallate papillae of male and female rats.For males (a-d).**a:** in Y cont group; taste buds (▲), taste pores (↑). **b:**in Ag group; empty taste bud (▲).**c:**in Ag+GT group; small bud with few cells (▲), mitosis in basal cell (↑). **d**: in Ag+Gr group; mitosis in basal cell (↑). For females (e-h).**e:** in Y cont group; taste buds (↑), taste pore (▲). **f:**in Ag group; taste buds with few cells, degenerated cells (▲). **g:**in Ag+GT group; normally appeared taste buds. **h**: in Ag+Gr group; normal taste buds. (H&E, Bar = 50µm)

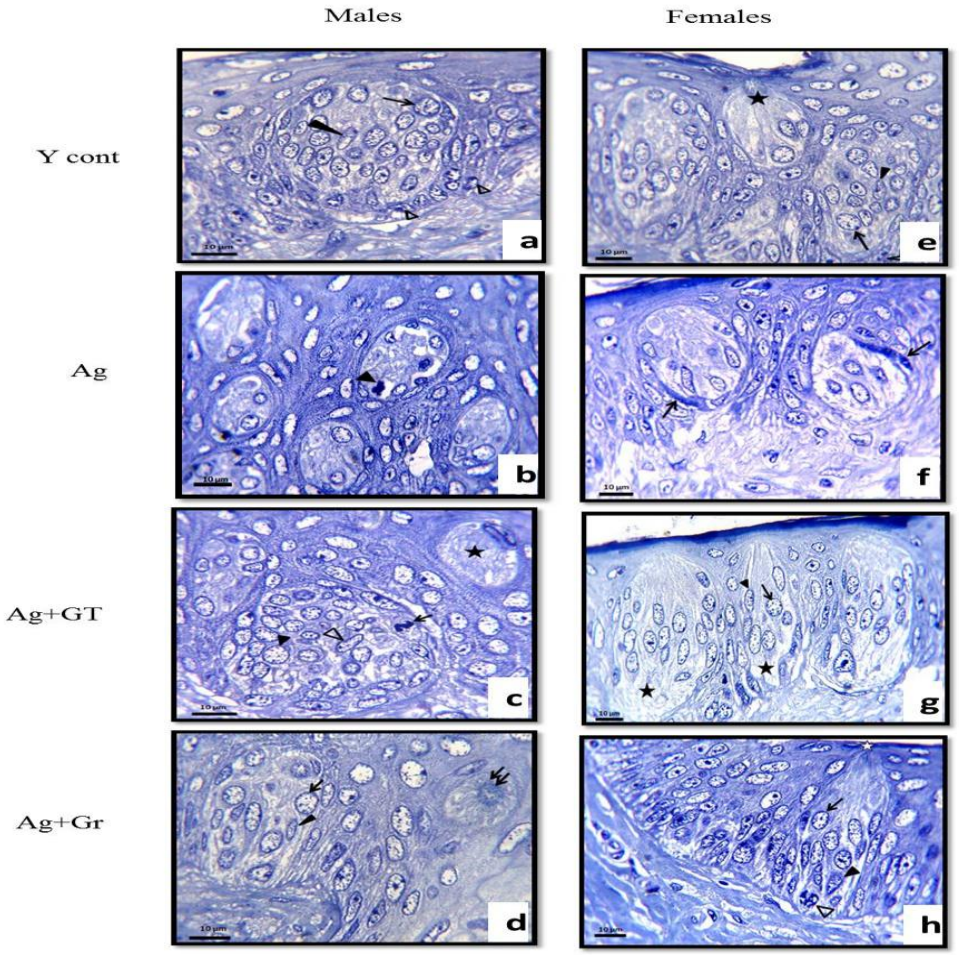

Figure 2. Photomicrographs in semithin sections of taste buds of male and female rats.For males (a-d).**a:**in Y cont group; light cell (↑), dark cell (▲), basal cell (Δ).**b:**in Ag group;small dark irregular nuclei and mitosis in basal (▲).**c:**in Ag+GT group; light cell (▲), dark cell (Δ), separation between few taste cells (⁎).**d**: in Ag+Grgroup;light cell (↑), dark cell (▲), microvilli in taste pore (↑↑).For females (e-h).**e:** in Y cont group; light cell (↑), dark cell (▲), basal cell (Δ), microvilli in taste pore (⁎). **f:**in Ag group; dark stained apoptotic irregular cells (↑).**g:**inAg+GT group; light cell (↑), dark cell (▲), separation between cells (⁎). **h**: in Ag+Gr group; light cells (↑), dark cell (▲), mitosis in basal cell (Δ), taste pore (⁎). (Toluidine blue, Bar = 10µm)

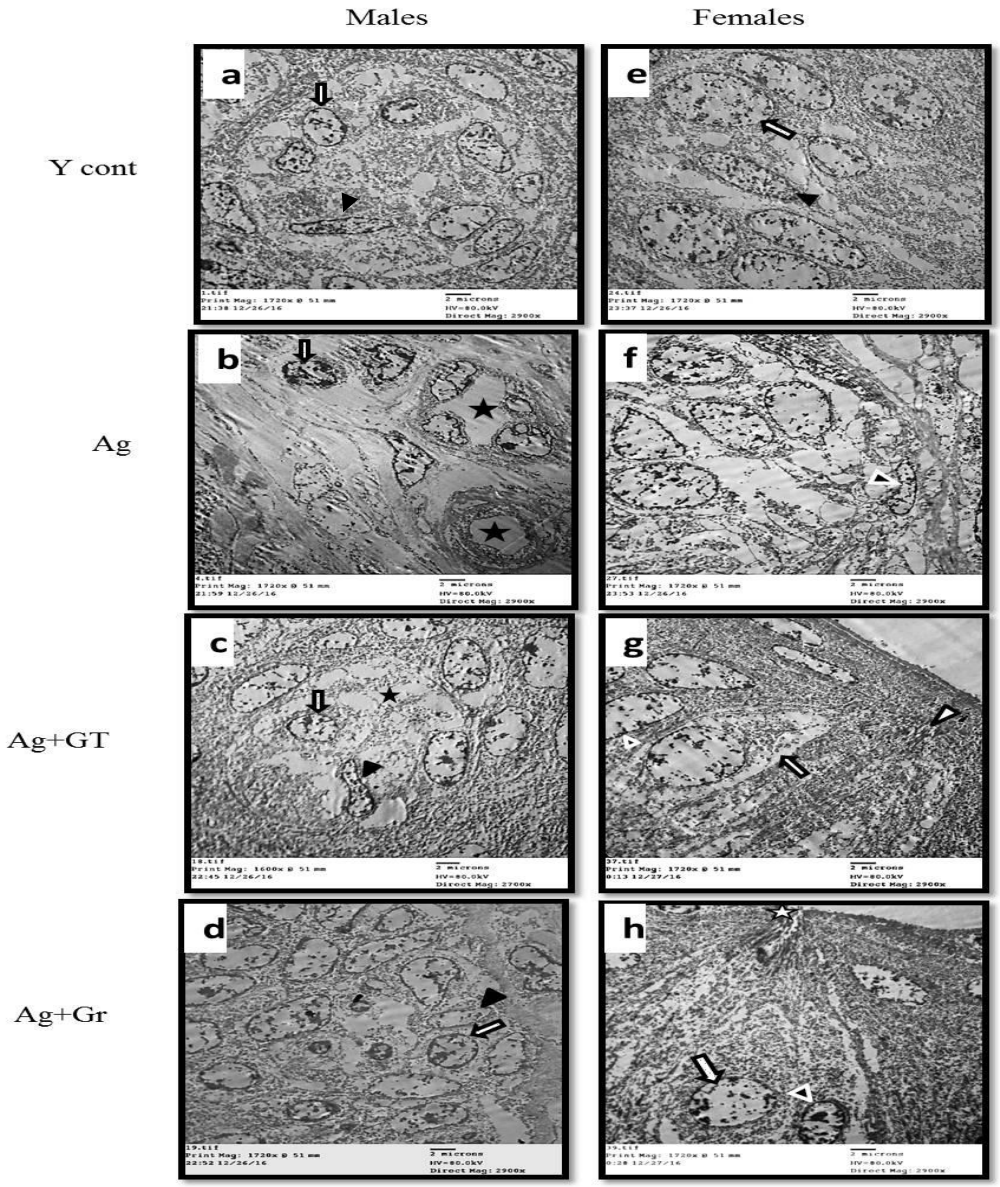

Figure 3. Electronmicrographs of taste buds of male and female rats. For males (a-d).**a:** in Y cont group; light cell (↑), dark cell (▲).**b:**in Ag group;dark cell with processes and dense cytoplasm (↑), microvilli in taste pore (*).**c:**in Ag+GT group; light cell (↑), dark cell (▲), spaces between cells (*).**d**: in Ag+Gr group; light cell (↑), dark cell (▲). For females (e-h).**e:** in Y cont group; light cell (↑), dark cell (▲).**f:** in Ag group; dark cell (▲), spaces between cells.**g:**inAg+GT group;light cell (↑), dark cell (▲). **h**: in Ag+Gr group;light cell (↑), dark cell (▲), taste pore(*). X2900

DISCUSSION

Aging is a complex natural process potentially involving every molecule, cell, and organ in the body. In its broadest sense, aging merely refers to changes that occur during the lifespan. Aging leads to an accumulating loss of body functions, which ultimately increases the probability of death, as we get older. El-Sokkary *et al* .[17] reported that, in old rats, aging results in an inhibition of lymphocytic functions in the spleen and thymus gland. Also, aging induced severe morphological changes in pituitary gland of rats [18].This study was done also to evaluate the effect of aging on rat taste buds and the protective effect of green tea and garlic on the induced structural changes using light and electron microscopy.

In the current wok, we observed light, dark and basal cells in the taste buds. The light cells have large rounded nuclei and light cytoplasm. Dark cells have dark cytoplasm and smaller nuclei. Yilmaz and Toprak[19] and Dmitrieva[20] observed two types of taste bud cells, dark and light. These cells were located in low 2/3 of taste buds. In other studies, Kinnamon*et al.*[21] and Cano *et al.*[22] observed three types of cells dark, light and intermediate in taste buds of mouse and rats.

In our study, the basal cells appeared in the basal part of the buds and were more prominent in the aged and treated groups. This clears the activity of basal cells to renew degenerated cells and to restore the normal structure of the buds. These observations come in line with Feng *et al.*[23, 24] who cleared that the taste buds are onion-shaped epithelial structures contain 50–100 tightly packed cells. These cells are taste receptor, supporting and basal cells. Taste receptor cells detect nutrient and transmit the sensation information to gustatory nerve endings in the buds. Supporting cells clear the excess of neurotransmitters after their release from taste receptor cells. Basal cells are the precursor cells which differentiate into the other mature cells.

The electron microscopic observation revealed the presence of dark and light cells depending on their electron density. The differentiation was difficult but the dark cells appeared irregular as having processes. But the light cells if it is cut longitudinally it will appear with tapering ends only. The cytoplasmic appearance was similar and organelles were few and less apparent in both light and dark cells. Pumplin *et al.*[25] concluded that, light cells are spindle shaped cells with smooth circular outlines seen in light micrographs of rat’s vallate taste buds.This result confirmed by the electron microscopic examination which showed that

large round nuclei are in light cells while dark cell had smaller, more irregularly shaped nuclei and lateral projection of cytoplasm. They showed also that the light cells were surrounded by lateral projections of dark cells. These lateral projections were sheet like not finger like. The light cells had extensions of cytoplasm from nuclear region toward the apical regions of the taste buds. They added that the cell organelles are similar in both. They also observed desmosomes between dark cells and light cells and also between similar types of cells.

In this study, the pathological changes in the taste buds of aged animals appeared as degenerative changes in the cells of the buds and some buds appeared empty. The changes of aged buds observed by many researchers were different. Shimizu [26] and Gao *et al*. [27] showed that in the 74 to 85 year old age, there was a decrease in the average number of taste buds in human circumvallate papillae. Feng et al. [24] and Kano *et al.*[28] noticed that in the epithelium in the elderly the density of taste buds was decreased. Their findings come in line with our results in the rats. In another study, Conger and Wells [29] showed that the number of taste buds in mouse circumvallate papillae and the number of cells in each taste buds decrease with age. Also Shin *et al.* [30] detected a significance reduction in circumvallate taste bud size and number in aged mice.

The degenerative changes observed in the aged male and female rats in our study confirmed the report of Feng *et al.*[24] who showed that various diseases in many patients develop taste disorders, including taste loss and taste distortion. They added that the decrease in taste function also occurs during aging. They suggest that alteration of taste bud homeostasis may be the cause of taste dysfunction with diseases and aging. Mojet *et al.* [31] concluded that age effects were found for age, but not for gender. They also showed that the age effect found could be attributed predominately to a generic taste loss. Many studies suggest that the taste cell lineage specification may be affected in aged mice and rats [30, 32, 23]. They added that the cells expressing toll-like receptor 3 (TLR3) and some taste modulators were decreased in aged mice. Jang *et al.*[33]; Jones and Rando [34] reported that it is generally believed that the number and/or proliferative activity of adult tissue stem cells decrease with age. Feng *et al.* [23, 24] reported that the decrease in taste function in the elderly is accompanied by reduced number of taste buds and taste cells. The number of proliferating taste progenitor/stem cells also seems to decrease in old age.

In this study, we observed apoptotic bodies in aged rats. This is come with the suggestion of Zeng and Ookley[35] who mentioned that the

apoptosis is a major mechanism for cell death in taste buds. The studies of Zeng *et al.*[36] confirmed that aged taste cells may use regulators for apoptosis (Bax- and caspase-dependent pathway) to commit cell death.

In the current study, the administration of aged rats with green tea relatively remedied the destructive effect of aging in both male and female rats. This improvement represented in light and electron microscopic examination as the presence of light and dark cells with vesicular nuclei and the basal cells with apparent mitotic division. This improvement supposed to be due to the antioxidant properties of green tea. These results supported by those of Pandey and Rizvi [37] who cleared that the consumption of foods containing antioxidant has been implicated to play a possible role in the prevention of chronic and age-related diseases. They added that the consumption of green tea resulted in a significant increase in plasma antioxidant activity associated with an increase in the concentration of catechins in plasma. Several studies proved that tea catechins exerted antioxidant activity by scavenging free radicals and chelating redox-active transition metal ions [38- 42]. Antioxidants properties of tea polyphenols act by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. They may also function as antioxidants indirectly through 1) inhibition of the redox-sensitive transcription factors, nuclear factor-B and activator protein-1; 2) inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, cyclooxygenases, lipoxygenases and xanthine oxidase; and 3) induction of phase II and antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases [43].

In our study, Garlic treatment of aged male and female rats showed great improvement in the taste buds. This improvement represented as the normally appearance of taste buds cells in the manner resemble that in young control group. Sohal and Weindruch [44] previously explained that loss of function related to senescence is due to rise of molecular oxidative damage. Garlic as an antioxidant decreased to a great extends the damage induced by oxidative stress which resulted from aging process. Škrovánková *et al.*[45] found that garlic contains important biologically active compounds such as phytoncides, antioxidants and others. It contains besides polyphenols also large amount of sulfur compounds that contribute together to overall antioxidant capacity. In the pathophysiology, garlic found to be effective against diseases of which oxygen free radicals have been implicated. Effectiveness of garlic may be due to its ability to scavenge oxygen free radicals [46, 47]. Queiroz, *et al.*[48] cleared that garlic extracts exhibit significant protective effects against DNA damage induced by H_2O_2 and HNE (4-hydroxynonenal)

which might be related to antioxidant activity [49]. Recently, Matsutomo *et al.* [50] showed that N-trans-Feruloyltyramine isolated as the P-selectin expression suppressor from garlic, has been reported to show significant antioxidant and anti-inflammatory activities.

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التغيرات المورفولوجية المتعلقة بالشيخوخة في براعم التذوق فى الفئران المسنة: الدور المحسن للشاي الأخضر والثوم

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الشاي الأخضر مشروب شعبي غني بمضادات الأكسدة القوية. من المعروف أن الثوم من المحتويات المهمة في الوجبات حيث أن له دوراً طبياً منذ قرون لإحتوائه على مضادات الأكسدة كما أنه مضاد قوي للإلتهابات في كثير من حالات جهد الأكسدة . تم في هذه الدراسة استخدام أربعة مجموعات من كل من ذكور و إناث الفئران كان تصميمها كالأتي: ١- مجموعة صغيرة السن ضابطة وعمرها ثلاثة شهور. ٢- مجموعة مسنة عمرها ٢٦ شهرا. ٣- مجموعة مسنة أعطيت الشاي الأخضر عن طريق الفم يومياً ولمدة أربعة أشهربجرعة قدرها ٢٠٠ ملليجرام/كجم. ٤- مجموعة مسنة أعطيت الثوم عن طريق **الفم يومياً** ولمدة أربعة أشهر بجرعة قدرها ١٠٠ ملليجرام/كجم. تم في هذه الدراسة الفحص المجهري بالميكروسكوب الضوئي والإليكتروني النافذ لمعرفة التغيرات في براعم التذوق في الحلمات الدائرية الموجودة على لسان الحيوانات موضع الدراسة. أوضحت النتائج أن هناك تغيرات نسيجية مرضية في براعم التذوق في الحيوانات المسنة والتي تمثلت في التدمير الخلوي لخلايا براعم التذوق إضافة إلى وجود بعض البراعم خالية تماما من محتواها الخلوي **و**ظهورها فارغة. أدى إعطاء الشاي الأخضر للحيوانات المسنة إلى معالجة نسبية للتغيرات الناتجة عن الشيخوخة في كل من ذكور و إناث الفئران . نتج عن إعطاء الثوم للحيوانات المسنة تحسناً كبيراً في براعم التذوق حيث ظهرت براعم التذوق في الحيوانات المسنة وكأنها تشبه مثيلاتها في الحيوانات صغيرة السن. تشير نتائج هذه الدراسة إلى أن الشيخوخة قد أحدثت تغيرات مدمرة في براعم التذوق كما أنها تقترح أن إعطاء الشاي الأخضر و الثوم كمنتجات طبيعية يمكنه أن يثبط ويخفف من هذه التغيرات المرتبطة بالتقدم في العمر.