Quest Journals
Journal of Medical and Dental Science Research
Volume 8~ Issue 8 (2021) pp: 07-17
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Histological and Ultra-Structural Effects of Antioxidant Vitamin C as anti-aging on Abnormal Alterations in Submandibular Salivary Gland of Wister Aged Mice

Aml Aljayer
Assistant Professor of Oral Biology of Faculty of Dentistry Gharyan University – Libya

ABSTRACT:
The current finding has been planned to estimate the biological impacts of the regular administration of antioxidant vitamin C on the submandibular gland of aged mice. ***Material & methods:*** *Thirty aged Wister male mice (12-15 months), weighing 300 to 350gs, were utilized in this research. The mice were separated randomly into double groups (15 mice/each). The firstgroup where animals administrated distilled water orally. The second group where animals administrated orally vitamin C about 100mg once daily for a month by using the syringe. After a month all mice were sacrificed.The samples were attained from the gland of the mice in the whole group. After that, the slides were prepared and observed by the microscope.* ***Results:*** *Histopathological findings in the group1 included; damage of parenchymal and connective tissues of glands. In group2, the acini and ducts appeared approximately normal. Ultra-structure inspection of the group1 showed some vacuoles in the parenchyma, as well as, distorted most organelles. In group2, the ultra-structural manifestations appeared nearly normal.* ***Conclusion:*** *Vitamin C revealed obvious improvement toreduce aging alterations of the salivary gland.*

KEYWORDS: *Histopathological and ultrastructural effects, Antioxidant Vitamin C, Submandibular salivary gland, Aged mice.*

Received 10 August, 2021; Revised: 24 August, 2021; Accepted 26 August, 2021

I. INTRODUCTION

Aging is a complex organic process related to inevitable and irreversible changes such as telomeres shortening and dysfunctions of macromolecules and organelles of the cells like mitochondria as a result of aggregation of the free radicals in the tissues leading to mitochondrial oxidative damage and finally aging of the cells (Signer and Morrison, 2013).

Antioxidant vitamin C (Vit C), is called ascorbic acid, synthesized by plants and some animals. However, apes and humans cannot create vitamin c inside their bodies as a result of the reduction of some oxidase enzymes which play important role in the formation of the vitamin. Thus, vit C has to be taken by natural food and drugs(Pham-Huy et al., 2008). Because vit C acts to the formation of the collagen, which constitutes the principal protein of connective tissues such as skin, bones, teeth and cartilage; consequently, ascorbic acid deficiency affected collagen synthesis(Naidu, 2003).

Michael, (2006)reported that people who took a diet with high ascorbic acid showed a lower risk of age-related disorders like cardiovascular diseases and cancer. Since vit C had a necessary factor in decreasing the defects in cellular molecules and increasing the activity of the immune cells. Many experiments demonstrated that topical application of vitamin C had antioxidant and anti-aging effects; as it could accelerate the production of Collagen I, III and important enzymes for the formation of collagen in the dermis(Haftek et al., 2008). These enzymes improved elasticity and reduced the wrinkling, erythema and pigmentations of skin in old people (Kerscher and Buntrock,2011).

Salivary glands as numerous organs of the bodyundergo alterations with continues life l. It has been detected thatsenescence decreasedthe number and size of glandularacini. In addition, increase the amount of fibrous, fatty tissues and inflammatory infiltration leading to a reduction in the quality and quantity of saliva (Dayan et al., 2000). Iwaki et al, (2006)showed that impairing denture fitting in edentulous patients was associated with xerostomia that was due to the presence of aging transformations in the major glands as acinar

atrophy, Interstitial fibrosis and inflammatory cell infiltration. Choi et al., (2013)reported that ultra-structural changes in sublingual glands of old rats were morphological alterations in organelles and secretory granules of acinar cells of secretory elements that led to a decrease in their abilities to synthesize and exert the secretions.

Although there are several studies related to the useful impacts of antioxidant vitamins,However, no previous researches had been handled forward on its anti-aging impact on the tissues of themouth. Consequently, the current work has been described to estimate histo-pathological and ultra-structural impacts of antioxidant vit C on the submandibular gland of elderly mice.

II. MATERIAL AND METHODS

Thirty geriatric white malemice (12-15 months), weighing 300 to 350gs, were used in the current research Themice were attained from the animal house, Faculty of Dentistry, Cairo University. The animals have stayed inplastic containers in healthy rooms. They had good water and rodent food. The practice was declared conforming to the instructions of the ethics committee of the Faculty of Dentistry, Cairo University. The animal was isolated randomly into double groups (15 rats/each): First group where animals administrated distilled water orally. The second group where animals administrated orally vitamin C about 100mg once daily for a month by using a syringe (Alshamsi, et al., 2006). After one month all mice were sacrificed. The samples were attained from the submandibular gland of the aged mice. Part of the specimens was inserted in formalin and was cut to about four to five microns' thick sections. Then slides were placed in Haematoxylin and Eosin (H&E) staining. Regarding, the other samples were cut one mm3 and prepared by glutaraldehyde solution. Finally, the slides were electron microscopically investigated.

III. THE FINDING

I) Histopathological results:

A) First group (geriatric mice):

By age, there was an alteration in the structure of the mice's salivary gland affecting both the parenchyma and connective tissue stroma. There were some alterations in the submaxillary gland of the old rat showed the acini and granular convoluted tubules of this group showed massive degenerations and shrinkages in the overall size **(Fig.1a).** In addition, there was cytoplasmic vacuolization, these vacuoles attained huge size at several sites of some parenchymal elements **(Figs.1a&2a).** There was a marked increase in the thickness and density of fibrous connective tissue septa in between the lobes and capsule **(Figs.2a&3a).** There were numerous chronic inflammatory cells spread in wide area of connective tissue and surrounded the blood capillaries **(Fig.3a).** Moreover, there was highly increase of the fatty tissues in the connective tissue septa **(Fig.4a).**

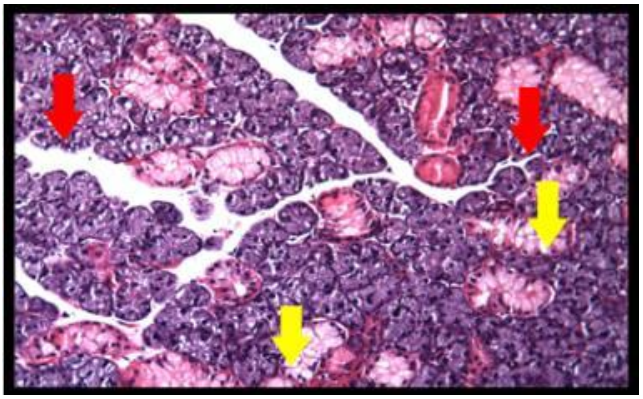
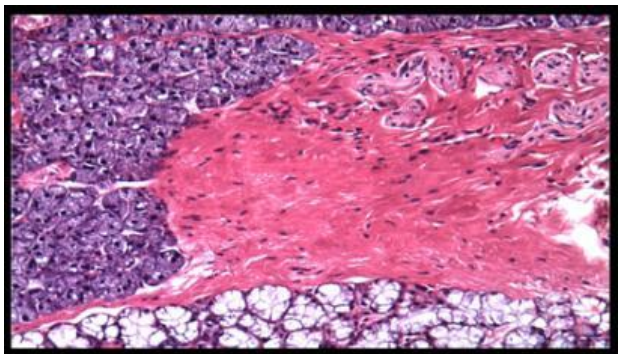


Fig.(1a): A Light microscopic picture of the submandibular salivary gland of aged mice (first group) displaying shrinkage& vacuolizations of serous (red arrow) and mucous acini (yellow arrow) (Hx. &E.X200)



(Fig 2a): A Light microscopic picture displaying a change in the structure and shape of the submaxillary gland of aged mice (first group) marked in both the parenchyma and connective tissue stroma as massive fibrosis (Hx. & E .X 200)

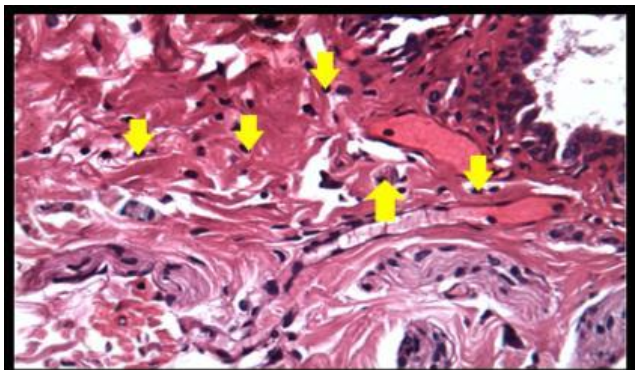
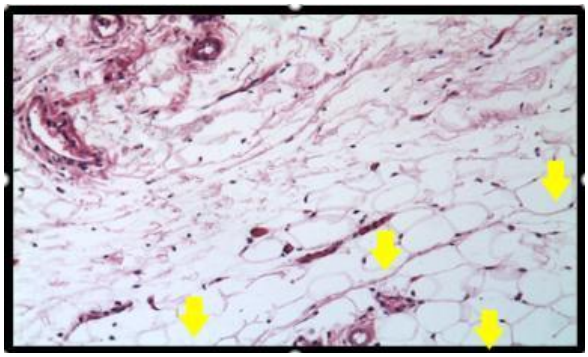


Fig 3a): Higher magnification of the previous figure 2 showing extensive fibrosis (a) with numerous chronic inflammatory cells spread in the wide area of connective tissue and surrounded the blood capillaries (Hx &E.X400)



(Fig 4a): A Light microscopic picture of the submaxillary gland of aged mice (first group) displaying extensive fatty cells in connective tissue stroma (b) (Hx &E.X200)

***B)* Second group (geriatric mice received antioxidant vitamin C for one month):**

Old rats treated with **antioxidant vitamin C** 100mg once daily by syringe, showed an improvement in the structural architecture of the submandibular salivary glands, e.g. noticeable decrease of the intracytoplasmic vacuolization and less degeneration of the secretory portions in comparison to the control group **(Fig1b).** Histopathological results of the first group revealed the feature of the parenchyma was approximatelyintact. Acinar cells were intact with well-defined boundaries and regularly arranged around a central narrow lumen **(Fig1b&2b).** Also, they had round or oval basally situated nuclei with homogenous **(Fig1b &2b).** Moreover, samples of the experimental group showed the approximately normal volume of the tubular cells with basically situated nuclei & eosinophilic cytoplasm**(Figs.1b&2b).** Few vacuolizations with minor distortion appeared in the tubular cells in comparison to the first group**(Figs. 1b &2b).** There were numerous striated ducts nearly natural with obvious lower striations in their cells. **(Fig.3b).** Moreover, the normal width of the glandular stroma was showed with many fibroblasts and collagen fibers **(Figs.3b &4b).** The fibrosis and inflammation markers consequently reduced **(Figs.3b &4b).** A small number of adipose cells was observed in the stroma **(Figs.3b &4b).**

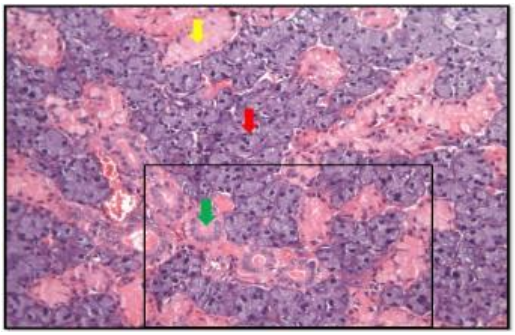


Fig (1b): A Light microscopic picture of the submaxillary gland of aged mice (second group) displaying nearly intact serous acini (red arrow) &granular convoluted tubules (yellow arrow) together with normally appearing striated ducts (green arrow). (Hx. & E.X 200)

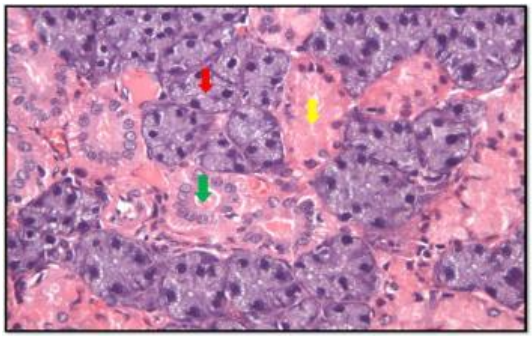


Fig (2b): Higher magnification of the quadrant area in fig1b showing intact numerous striated ducts (green arrow) with columnar cells with serous acini with centrally placed rounded nuclei and basal striations associated with well-defined cell boundaries (red arrow). Granular convoluted tubules were also seen (yellow arrow). (Hx. & E.X 400)

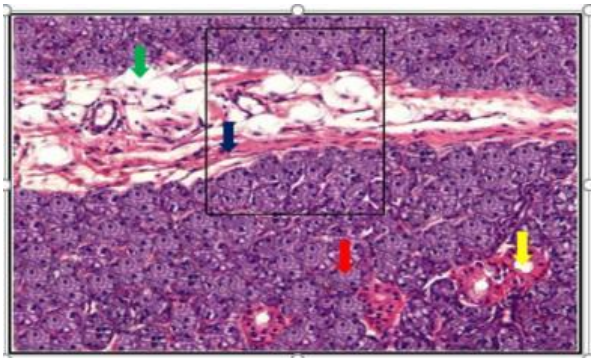


Fig (3b): A Light microscopic picture of the submaxillary gland of aged mice (second group) displaying intact features of acini (red arrow) striated ducts (yellow arrow) & the stromal network (blue arrow) with few fat cells .(green arrow). (Hx. & E.X 200)

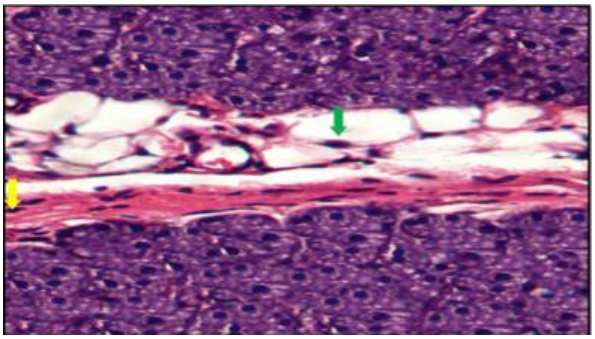


Fig (4b): Higher magnification of the quadrant area in fig 3b showing stromal connective tissue with a small amount of inflammatory (yellow arrow) and fatty cells (green arrow) (Hx. & E.x 400)

II) Ultra-structural results:

A) First group:

Investigation of the submandibular salivary gland of elderly mice by transmission electron microscope showed degenerative changes in the ultrastructure of the parenchymal and stromal elements. The cells of glandular acini revealed contracted dead nuclei with irregularly arranged chromosomes. The cytoplasm of the serous cells showed degenerated and coalescent secretory granules **(Fig.5a).** A large number of secretory granules of acinar and tubular cells of different sizes, shapes and degrees of electron densities were also seen **(Fig.5a).** Moreover, disfigured mitochondria and noticeable vacuoles. Moreover, smooth endoplasmic reticulum in some of the acinar and tubular cells with slightly degenerated cisternae. Also, desmosomal junctions between tubular cells were seen **(Fig.6a).** The maximum impact of organelles in the secretory part was the mitochondria. They showed damage with the abnormal feature of their cristae **(Fig.6a).** Ultra structurally, other signs of degeneration in lower parts of the striated duct cells **(Fig.7a).** Nuclei of cells of duct system as the granular convoluted tubule striated and intercalated ducts were electron-lucent with deficient chromatin**(Fig.7a).** In other specimens, striated and intercalated ductal cells showed nearly normal Golgi complex and nuclei with various **(Fig.7a).**The increased thickness of glandular septa in many specimenswas respected**(Fig.6a).** The most characteristic marker in the connective tissue of the submaxillary gland of geriatric rats was inflammatory cells. In other specimens, the glandular stroma had congested and thick-walled blood capillary with electron condensed red blood cells **(Fig.8a).**

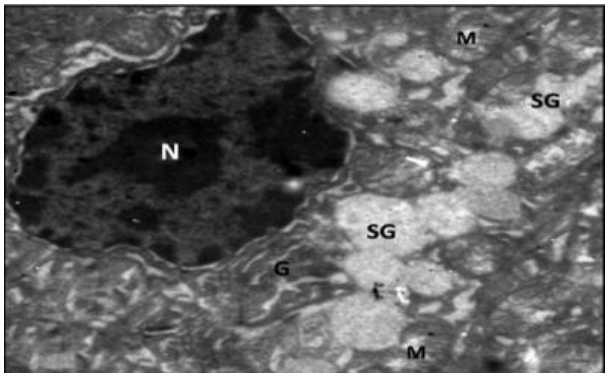


Fig.(5a): An electron microscopic picture of the submaxillary gland of aged mice (first group) displaying acinar cell with folded nucleus((N), coalescent secretory granules(SG), disfigured Golgi apparatus(G) & partially degenerated mitochondria with incomplete cristae (M). (X 2000)

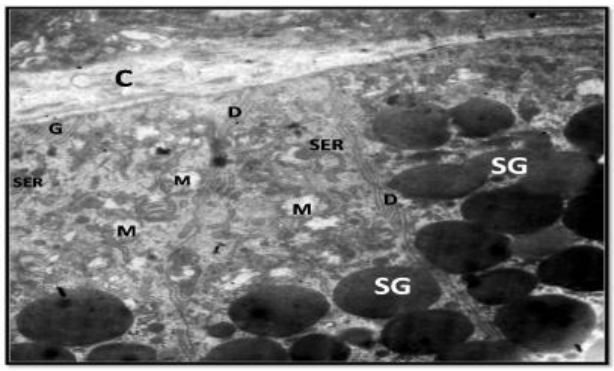


Fig.(6b): An electron microscopic picture of the submaxillary gland of aged mice (first group) displaying granular convoluted tubular cells with different degrees of electronlucency of secretory granules (SG), degenerated mitochondria(M), slightly distorted smooth endoplasmic reticulum (SER). Widening of connective tissue septa between lobules(C) & desmosomal junction (D) between tubular cells was shown. (X.2000)

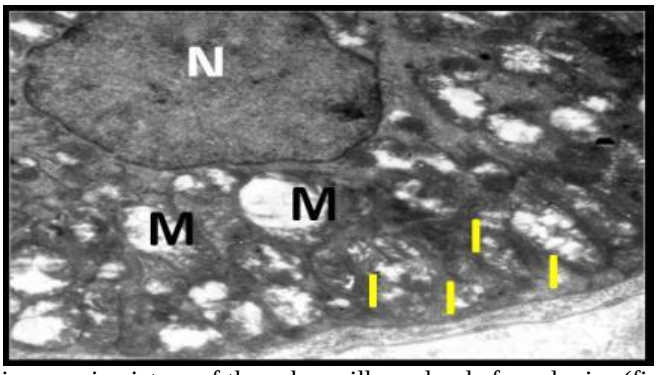


Fig. (7a): An electron microscopic picture of the submaxillary gland of aged mice (first group) displaying cell of the striated duct with hyperchromatic nucleus (N), numerous damaged mitochondria (M) between lowerinholdings (I). (X.2000)

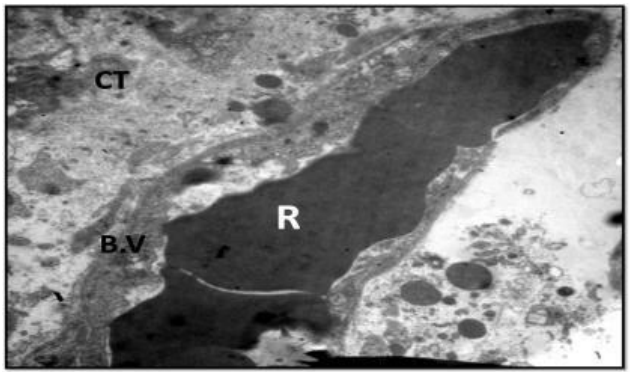


Fig. (8a): An electron microscopic picture of the submaxillary gland of aged mice (first group) displaying congested and dilated blood capillary with a thickened wall (B.V) and full of stagnant RBCs (R) in the connective tissue stroma (CT). (X.3000)

II) Ultra-structural results:

B) Second group (Geriatric mice with vitamin C receiving once daily for 1month):

As compared to the control group, microscopic examination of semi-thin sections of serous secretory acini of the submandibular salivary gland of old rats displayed noticeable advancement in the parenchymal tissue of the gland. The organelles of many acinar cells were showed approximately intact. There wasa huge number of rough endoplasmic reticulum backed basally and laterally to the nucleus in the most secretory and ductal cells.

Rough endoplasmic reticulum appeared closely packed membrane-bound flattened cisternae studded with ribosomes on their outer surfaces **(Fig.5b)**. The cells of the acini showed nearly intact nuclei, having electron-dense clear nucleoli**(Fig.5b)**. There were numerous secretory granules of variable sizes and densities in the cytoplasm of acinar and tubular cells with less degeneration and coalescence**(Fig.6b)**. Mitochondria of granular convoluted tubulesappeared nearly normal (rod-shaped) with a smooth outer surface and rough inner surface (discernible cristae) in the basal and lateral parts of these cells associated with rough endoplasmic reticulum and secretory granules **(Fig.6b)**.

The basal of part striated duct cell manifested as an increase of the obvious basal inholdings of the plasma membrane. Many large and radially arranged mitochondria in the cytoplasm of ductal cells. **(Fig.7b)**.The normal shape and structure of the fibroblasts in the connective tissue septa were observed **(Fig.8b)**. The lining of the blood capillaries is nearly normal. Additionally, there were a large amount of red blood cells inside these vessels **(Fig.8b)**.

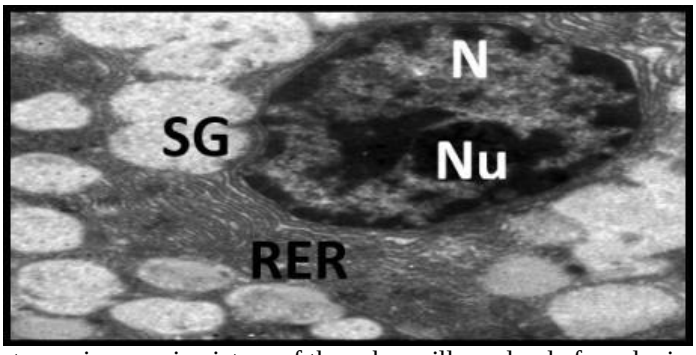


Fig. (5b): An electron microscopic picture of the submaxillary gland of aged mice (second group) displayingserious cells with closely packed cisternae of rough endoplasmic reticulum (RER) around the large hyper-chromatic nucleus (N) with obvious nucleolus (Nu). Many granules of various sizes (SG). (X.3000).

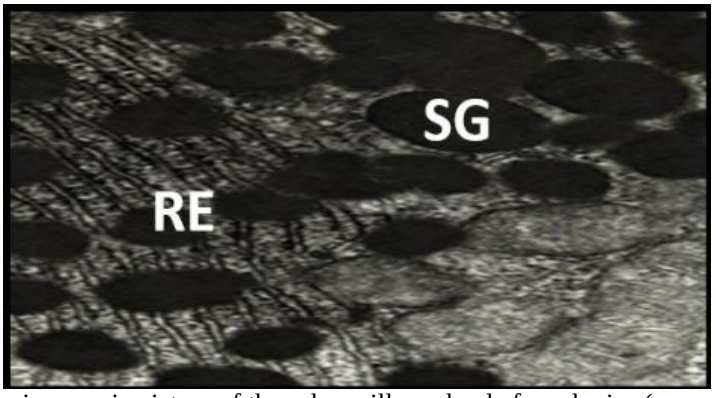


Fig.(6b): An electron microscopic picture of the submaxillary gland of aged mice (second group) displaying a cell of granular convoluted tubules containing numerous secretory granules of different sizes(SG), parallel arrays of rough endoplasmic reticulum cisternae studded with ribosomes on their outer surface (RER) & rod-shaped mitochondria with discernible cristae (M). (X.3000).

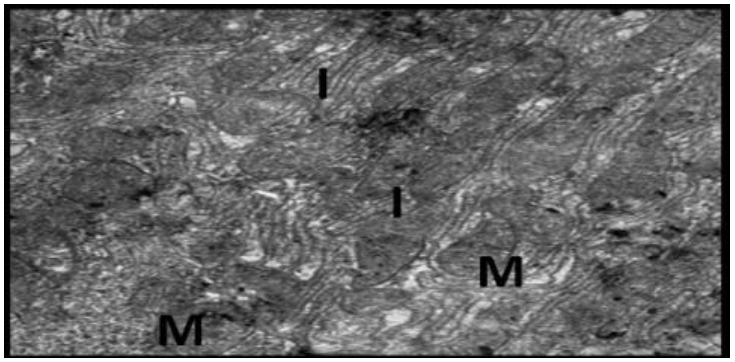


Fig.(7b): An electron microscopic picture of the sub-maxillary gland of aged mice (second group) displaying characteristic striated duct cell with lower inholdings (I) and normal architecture of the mitochondria (M). (X.3000).

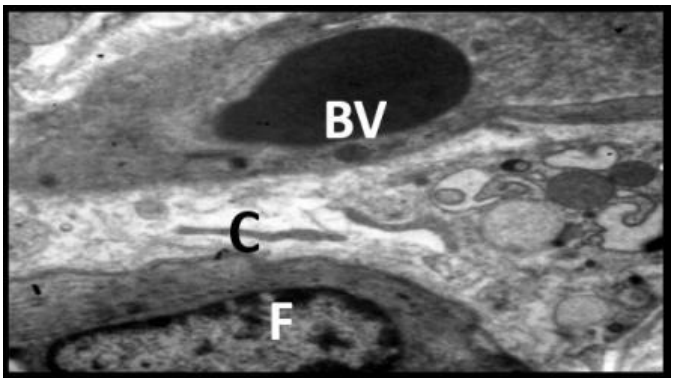


Fig.(8b): An electron microscopic picture of the submaxillary gland of aged mice (second group) displaying large fibroblast(F) with spindle-shaped nucleus & collagen fibers (C) in stromal connective tissue adjacent to a large blood vessel with a small amount of electron-dense blood corpuscles (BV). (X3000)

IV. DISCUSSION

For many years, numerous studies were considered around the anti-aging medicines principally, antioxidants. They play an important role to decrease disorders and senescence marks in all parts of the human body (Joshi *et al.*,2013). The maximum antioxidants practice is vitamin C drug because it had strong action toreduce thedamage related to aging, many inflammatory and tumor diseases(Padyatty et al., 2003). In the current research, the secretory portion of the salivary gland of group1 revealed the volume change. These results were in agreement with the investigations ofVered *et al.,* (2000**)**who observed that the aging process in mucous salivary glands of the palate in geriatric patients as compared to younger ones detected that the size of the acini reduced.

Histopathological investigation of slides of the first group showed a raise amount of fibrosis and adipose tissues of the submandibular gland.Larsson *et al.*, (2001**)** supported this result as they studied the aging transformations in the connective tissue of labial glands as presence large amount of inflammation and fibrosis. Furthermore, in the present work, the primary groups revealed noticeable lymphocytes and macrophages in the stroma of the salivary glands. These results could be expected to the generation of reactive oxygen species in the labial gland along increase life leadingto inflammation of the lip (Azevedo *et al.,* 2005). In this finding, the blood capillaries showed dilated walls in the aged mice. In agreement with this study,Sa et al., (2013)demonstrated that dilation of the blood capillaries in the stroma of the lingual gland in geriatric people was due to the presence of leukocyte spread. In the secondary group, the present research showedadvancement of some aging transformations in the submandibular gland, where the parenchymal part revealed approximately intact and the inflammatory cells reduced. In agreement with this finding,Miresmaeili et al., (2015**)** observed that dietary vitamin C had a great role to decrease inflammatory disorders as periodontitis related to orthodontic tooth movement in the rat. Whereas, ascorbic acid acts to constant and improve defected periodontal ligaments of teeth exposed to orthodontic force by decrease the number of inflammatory mediators. Biological observation of samples of the second group revealed a clear drop in fibrosis and fatty tissues of the submandibular gland, these results were supported by those of AlShamsi,(2006)who explained that administration of ascorbic acid to diabetic mice acted to decrease lipid disorders of liver and kidney through rising action of antioxidant enzymes against accumulation free radicals inside the hepatocytes and nephrons. In addition, Kalender*et al.,* (2007)reported theprotective action of vitamins C onarthrofibrosis and nephrotoxicity in male ratsbyreducing the presence of oxidative oxygen species in kidney cells.

Electron microscopic inspection of the first group slides showed remarkabledistortion of cellular organelles of salivary acini. This finding was also in agreement with the observation ofDel- Monte, 2005confirmed that defected liver of many geriatric patients was attributed to the presence of oxidative radicals in the hepatocytes which acted to damage cellular organelles. In the present work, the glandular cells of the aged mice (group1) showed dead and contracted nuclei with abnormal chromosomes(Elbaz and Salem,2013). Confirmed this finding as they demonstrated abnormal ultra-structural features in acinar cells of elderly mice like condensed chromosomes. Furthermore, the damage was observed in some secretory portions of the first group, this study could be attributed to the rising amount of inflammatory cytokines in the salivary gland by increase life leading to disruption of parenchymal cells construction and structure(Aljayer et al., 2015).

Moreover, the present research revealed an obvious reduction inthe amount of damaged parenchyma of the submandibular gland of the secondary group. These results were in agreement with the observations of Rice., (2000)who explained that the neuroprotective effects of ascorbate against reactive oxygen species in the brain leading to the preservation of organelles of neurons from distortions.Padayatty et al., (2003)reported that vit C played a necessary role in cellular structure and function as the formation of phospholipids of the plasma

membrane. They also said that ascorbic acid was used in the treatment of many inflammatory diseases because of its activity to decrease inflammatory mediators inside the tissues of the body. In the current research the maximum electron microscopic results in the submandibular gland of geriatric mice advanced by supplement of vitamin C. These events were confirmed by the study of **Ceriello, (2003)**who explained that antioxidant therapy as ascorbic acid managed the blood problems and also advanced the cellular metabolism of diabetic models. Additionally, vit C acted to conservation the cells from injury by suppressing the discharge of arachidonic acid from the plasma membrane.

V. CONCLUSION

The current finding showed that antioxidant vit C could perform a particular healthful supplement that has strong potential to reduce histological& functional alterations associated with salivary gland aging as xerostomia is becoming a major issue in dental and medical clinics.The biological effects of vit C have experienced huge researches in various specialties. Consequently, further work is mandatory to diagnose the antioxidant effect on age-related changes.

REFERENCES

[1]. Aljayer,A., El-Baz,D & Bashir ,M (2015). Impacts of Omega- 3 Fatty Acids on the Age Related Changes in the Submandibular Salivary Glands of Albino Old Rats. Nature and Science 2015;13(6)

[2]. Alshamsi., M, Amin., A &Adequate., E (2006) Effect of Vitamin C on Liver and Kidney Functions in Normal and Diabetic Rats. Ann. N.Y. Acad. Sci. 1084: 371–390

[3]. Azevedo, L ., Damante, J., Lara, V., & Lauris, J. (2005). Age-related changes in human sublingual glands: a post mortem study. Arch. Oral. Biol,50, 565-74.

[4]. Ceriello, A. 2003. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 26: 1589–1596.

[5]. Choi, J., Park.I., Kim, S., &et al. (2013). Analysis of age-related changes in the functional morphologies of salivary glands in mice.Archives of Oral Biology, 58, 1635–1642**.**

[6]. Dayan, D., Vered ., Paz, T. and Buchner A. (2000).Aging of human palatal salivary glands: A histomorphometric study. Exp. Gerodontol. 35, 85-93.

[7]. Del- Monte, U.(2005). Swelling of hepatocytes injured by oxidative stress suggests pathological changes related to macromolecular crowding. Medical hypothesis, 64,818-825.

[8]. El-baz ,D., and Salem, Z. (2013).The Effect of Omega- 3 Fatty Acids on the Age Related Changes in Submandibular Salivary Glands ofAlbino Rats. Journal of American Science, 9,149–154.

[9]. Haftek, M.,Mac-Mary, S &., Le. Bitoux. M.(2008). Clinical, biometricand structuralevaluation of the long-term effects of a topical treatmentwith ascorbic acid and madecassoside in photoaged kl; human skin. Exp. Dermatol, 17,946-52.

[10]. Iwaki, F., Damante, J., Consolaro, A.,& et al.(2006). Mouth floor enlargements related to the sublingual glands in edentulous or partially edentulous patients: a microscopic study.Appl. Oral. Sci, 14,264-9.

[11]. Joshi,B.,Agrawal, P., Sahu ,D., & Dashora ,A.(2013). Antiaging Cosmetics.J .Drug. Del. Ther,3,158-162.

[12]. Kalender S, Kalender Y, Durak D, Ogutcu A, Uzunhisarcikli M, Sitki Cevrimli B, Yildirim M. Methyl 41 parathion induced nephrotoxicity in male rats and protective role of vitamins C and E. Pes Biochem 42 Physiol, 2007; 88(2): 213-218.

[13]. Kerscher, M., and Buntrock, H. (2011). Anti-aging creams. What really helps? Hautarzt, 62,607-13.

[14]. Larsson, A., Henriksson, G., Manthor, p., & et al. (2001). Ku protein and DNA strand breaks in lip glands of normal and primary Sjorgen's syndrome subjects: lack of correlation with apoptosis. Sc .J. Immunol, 54, 328-334.

[15]. Michael, J. (2006). Orthomolecular medicine: the therapeutic use of dietary supplements for anti-aging. Clinical Interventions in Aging. 1,261–265.

[16]. Miresmaeili.,A,Mollaei.,N,Azar., R& et al (2015). Effect of dietary vitamin C on orthodontic tooth movement in rats. Journal of Dentistry, Tehran University of Medical Sciences. 12, No. 6.

[17]. Naidu, K. (2003). Vitamin C in human health and disease is still a mystery? An overview. Nutrition Journal, 2, 1-10.

[18]. Padayatty, S., Yaohui, A., Eck, W., & et al. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. Journal of the American College of Nutrition, 22, 18-35.

[19]. Pham-Huy, L., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. International Journal of Biomedical Science: I.J.B.S, 4, 89–96.

[20]. Rice., M.E. 2000. Ascorbate regulation and its neuroprotective role in the brain. Trends Neurosci. 23: 209–216.

[21]. Sa, J., Tolentino, E., Azevedo-Alanis, L., &et al. (2013). Morphology and morphometry of the human sublingual glands in mouth floor enlargement of edentulous patients. J. Appl. Oral. Sci, 21,540-546.

[22]. Signer, R., and Morrison, S. (2013). Mechanisms that regulate stem cell aging and life span. Stem Cell, 12,152–65.

[23]. Vered, M., Buchner, A., Boldon, P., & Dayan, D. (2000). Age related histomorphometric changes in labial salivary glands with special references to acinar components. Exp. Gerodontol. 35, 1075- 1084.