Impacts of Omega- 3 Fatty Acids on the Age Related Changes in the Submandibular Salivary Glands of Albino Old Rats

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Abstract: Objective: The present study has been designed to evaluate the histological effects of the daily consumption of omega-3 on submandibular salivary gland of old rats. **Material & methods:** Forty old white male rats (12-15 month age old), weighing 300 to 350gs, were used in this study. The rats were divided into two groups (20 rats/each). Control group where rats did not receive any dietary supplement. Experimental group where rats received omega-3 (60 mg/Kg) twice daily for three months through intra- gastric intubation. All rats were sacrificed after three months. Soft tissue specimens were obtained from submandibular salivary gland of the rats in all the studied groups. The sections were examined histologically and ultra-structurally. **Results:** Histopathological changes observed in control group included; distortion and decrease in the overall size of the acini and granular convoluted tubules increase in the amount of fatty tissue, fibrous tissue and inflammatory cell infiltration. In experimental group, the acini and ducts appeared nearly normal and there was marked decrease in the inflammatory cell infiltration. Electron microscopic examination of the control group revealed cytoplasmic vacuolization in acini and ducts, degenerated organelles and pyknotic nuclei. In experimental group, the acini and ducts appeared nearly normal in size, shape and structure. **Conclusion:** Omega-3 showed significant improvement in many age related changes of submandibular salivary gland.

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Key words: Omega-3, Aging, Submandibular salivary gland.

1. Introduction

Aging is a gradual biological impairment resulting in the incorporation of intrinsic and extrinsic factors like genetic, metabolic and radiation, which correlate to decrease activity and function of systems and organs of the body **(Mehta and Joshi, 2012).**

Many theories have been proposed to explain the mechanism of the aging; however, neither of them appears to be fully satisfactory **(Trubitsyn, 2013).** Generally, these theories fall into two main categories: programmed and non-programmed **(Trinadade *et al.*, 2013).** Indeed, these theories have three sub-categories: programmed longevity, endocrine and immunological theories **(Okusaga, 2014).** In general, the non programmed theories included many sub-theories such as wear and tear, living, cross-linking, somatic DNA damage, telomere and free radical theories **(Rattan and Clark, 2005).**

Omega -3 fatty acids are fats commonly found in fish oils, algal oil and some plant oils such as flaxseed oil.Omega3 Polyunsaturated fatty acids are considered essential fatty acids, which mean that they cannot be synthesized by the human body, but they are vital for normal metabolism **(Weaver *et al.*, 2008).**

Polyunsaturated fatty acids (PUFAs) have strong immunomodulatory activities, among the omega-3 PUFAs, those from fish oil EPA and DHA, which are more biologically potent than ALA, since they reduce oxidative stress, caused by excessive free radicals and inhibit the formation of fatty acids-derived pro-inflammatory eicosanoids including tumor necrosis factor and interleukins **(Kang and Weylandt, 2008).**

In humans and animals, cellular aging has been attributed to the shortening of telomeres, when telomeres(strings of DNA located at the chromosomes) become too short, the cells die.Omega-3 has a direct effect on biological aging by slowing down the rate at which protective caps on the ends of chromosomes (telomeres) shorten **(Ramin, 2010).**

Submandibular salivary glands, like many other organs, undergo changes in structure and function with age. It has been reported that by aging reduction occurs in the amylase content of saliva and the rate of secretory protein synthesis declines with decrease in the amount of saliva in the mouth (xerostomia). Moreover, the number of the acini decreases and the amount of fatty and fibrous tissues increases. Furthermore, acinar atrophy, ductal dilatation and inflammatory infiltration, increase with aging **(Dayan *et al.*, 2000 & Vered *et al.*, 2000).**

Although, there are many researches concerning the beneficial effects of omega-3, no interest had been directed toward its anti-aging effect on the oral tissues. Hence, the present study has been designed to evaluate the histological effects of the daily consumption of omega-3 on submandibular salivary gland of old rats.

2. Material and Methods

Forty old white male rats (12-15 month age old), weighing 300 to 350gs, were used in this study. The rats were obtained from the animal house, Faculty of Medicine, Cairo University. The rats were housed in specially designed cages and were maintained under good ventilation. They had free access to both sterile water and standard rodent soft chow diet. The experiment was conducted according to the recommendations of the ethics committee of the Faculty of Oral and Dental Medicine, Cairo University. The rats were divided into three groups (20 rats/each): Control group where rats did not receive any dietary supplement. Experimental group where rats received omega-3 EPA+DHA (60 mg/Kg) twice daily for three months through intra- gastric intubation **(Marjan *et al.,* 2012)**.

All rats were sacrificed by carbon dioxide inhalation, after three months. Soft tissue specimens were obtained from the submandibular salivary gland at the floor of the mouth of the rats from both studied groups (control and experimental).Some of the specimens were fixed in 10% neutral formalin solution and prepared for embedding in paraffin wax. Four to five microns thick sections were subjected to routine Haematoxylin and Eosin (H&E) staining for interpretation of any histological changes. Regarding the other specimens, about one mm^3 were cut and fixed in gluteraldhyde to be prepared for ultra-structural examination by the transmission electron microscope (TEM).

3. Results:

Light microscopic results:

A) Control group:

The acini, granular convoluted tubules and striated ducts of the submandibular salivary gland of this group showed partial degeneration, vacuolization and shrinkage in the overall size. Ill-defined boundaries and coalescence of some serous acini were also seen **(Fig.1a).** In addition, some specimens showed discontinuity and thinning of the epithelial lining of the excretory ducts and their lumen filled by retained secretion. Also, some excretory ducts were surrounded by excessive fibrous connective tissue **(Fig.2a).** Dilated and thin walled blood vessels were observed **(Fig.3a).** Chronic inflammatory and fatty cells in all parts of stroma, especially, around the blood vessels were seen **(Fig.3a).**

B) Experimental group:

Histological sections of the experimental group showed numerous acini, which seemed nearly normal in shape and size **(Fig1b).**

Furthermore, the thickness and density of the connective tissue stroma appeared nearly normal **(Fig.1b).** The striated ducts were almost normal lined by columnar cells with rounded, central and darkly stained nuclei. Eosinophilic cytoplasm with apparent basal striations was observed **(Fig.1b).** The excretory duct exhibited nearly normal architecture with pseudo stratified columnar cells with rounded or oval deeply stained nuclei appearing at different levels and regularly arranged around empty lumen **(Fig.2b).** The blood vessels appeared nearly normal lined by endothelial cells and filled with RBCs **(Fig.3b).** The inflammatory cell infiltration and fibrosis were significantly decreased **(Fig.3b).** Furthermore, the thickness and density of the connective tissue stroma appeared nearly normal **(Fig.3b).**Minimal amount of fat cells was detected in the connective tissue stroma **(Fig.3b).**

Electron microscopic results:

A) Control group:

Acinar cells showed shrunken, pyknotic nuclei and condensed chromatin. The cytoplasm of the serous cells showed secretory granules of different size, shape and electron lucency. There were some distortions and dilatation in curved saccules of Golgi apparatus of acinar cells in other specimens. Widening of intercellular junction between acinar cells in some specimen was also seen **(Fig.4 a).** Moreover, noticeable vacuoles with huge size were observed in the cytoplasm of acinar cell **(Fig.5 a).** The mitochondria were the most affected cellular organelles in the duct system. They appeared degenerated with loss of the normal architecture of their cristae. Widening of connective tissue septa between lobules in some specimens was noticed **(Fig.6 a)**. The striated duct cells showed decrease of their basal cytoplasmic infoldings associated with mitochondria **(Fig.6 a).** Chronic inflammatory cell infiltration was an outstanding feature in the connective tissue stroma of submandibular salivary gland of the old rats of the control group. In other specimens, the connective tissue had dilated and congested blood vessel full of electron dense blood corpuscles **(Fig.7 a).**

B) Experimental group

Experimental group showed marked improvement in the ultrastructure of the secretory portions and duct system. The most of parenchymal cells appeared with nearly normal architecture of their organelles.

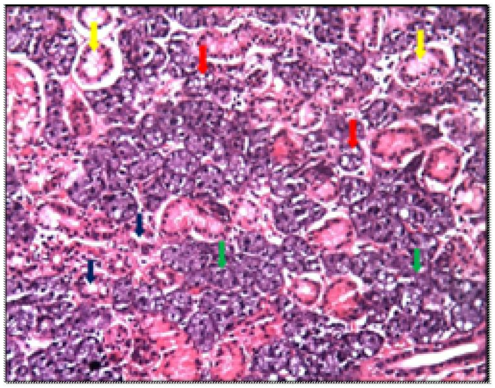

Fig. (1 a): A photomicrograph of submandibular salivary gland of old rat (control group) showing partial degeneration, vacuolization and shrinkage in granular convoluted tubules (yellow arrows), striated ducts (blue arrows) & serous acini (red arrows). Ill-defined boundaries and coalescence of the serous acini were also seen (green arrows). (H & E. X200)

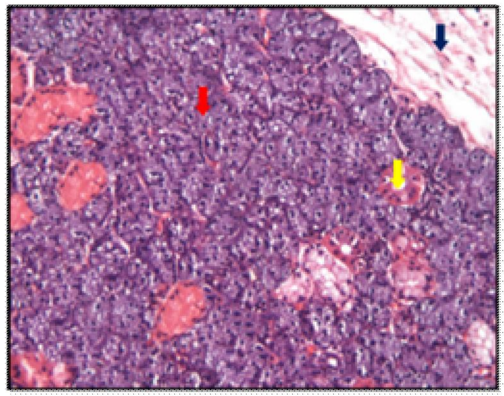

Fig (1 b): A photomicrograph of submandibular salivary gland of old rats (experimental group) showing approximately normal secretory portion (red arrow) and duct system (yellow arrow) with loose connective tissue (blue arrow). (H. & E. X 200)

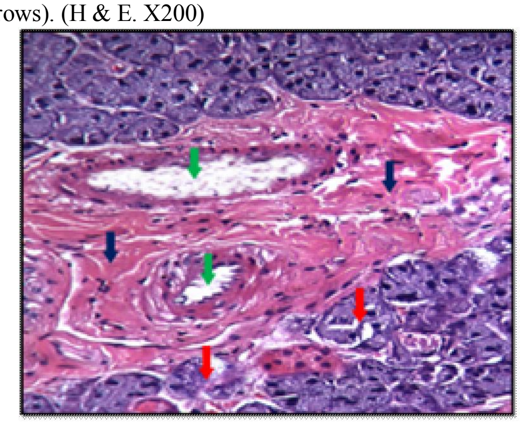

Fig. (2 a): A photomicrograph of submandibular salivary gland of old rat (control group) showing degenerated serous acini (red arrows), excretory ducts with thinning walls (green arrows) & massive fibrosis (blue arrows) (H & E. X200).

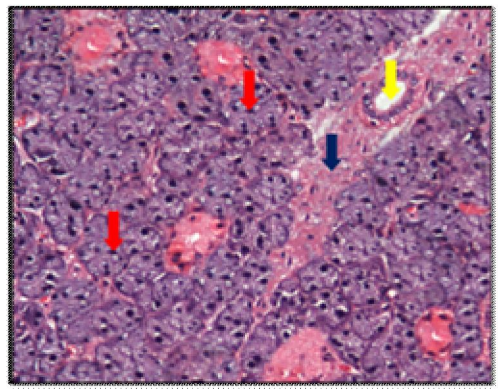

Fig (2 b): A photomicrograph of submandibular salivary gland of old rats (experimental group) showing parenchymal elements (red arrow) and connective tissue septa (blue arrow) with nearly normal excretory duct (yellow arrow). (H. & E.X 200)

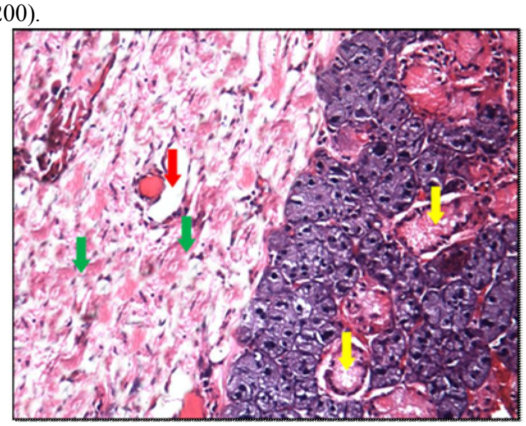

Fig (3 a): A photomicrograph showing changes in the normal architecture of submandibular salivary gland of old rats (control group) manifested as fibrosis (green arrows), dilated and thinned walled blood vessel (red arrow) & shrunken and vacuolated granular convoluted tubules (yellow arrows) (H. & E.X 200)

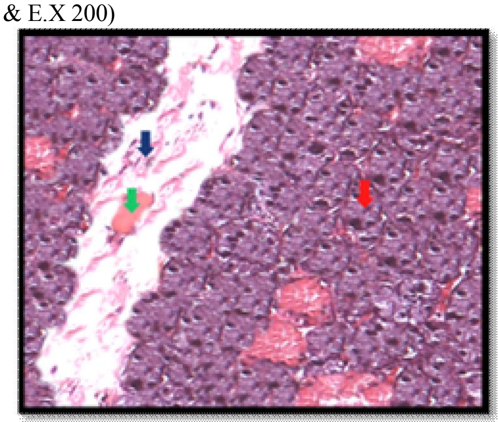

Fig (3 b): A photomicrograph of submandibular salivary gland of old rats (experimental group) showing normal architecture of parenchymal elements (red arrow) & loose connective tissue (blue arrow) and slightly dilated blood vessel filled with blood (green arrow). (H. & E. X 200)

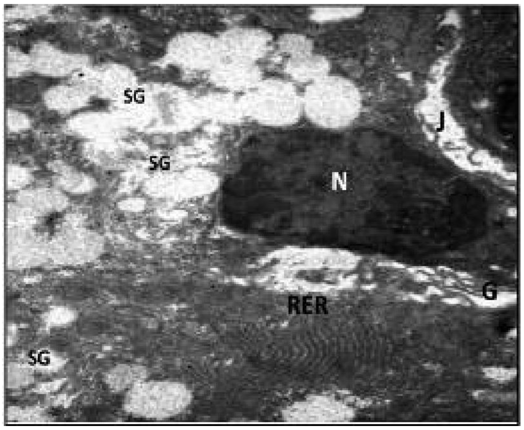

Fig. (4 a): An electron micrograph of submandibular salivary gland of old rats (control group) showing serous cell with shrunken and pyknotic nucleus (N),degenerated and dilated of Golgi apparatus (G) & Pooled and coalesced secretory granules (SG).Widening of intercellular junction(J) and nearly normal rough endoplasmic reticulum (RER) were also noticed.(X 2000)

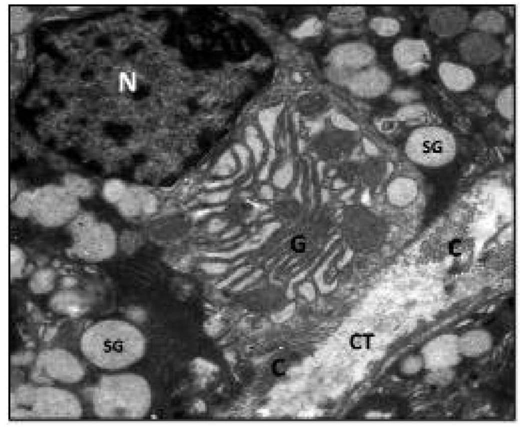

Fig. (4 b): An electron micrograph of submandibular salivary gland of old rats (experimental group) showing basal part of a serous acinar cell with hyperchromatic nucleus (N) & nearly normal (slightly curved) saccules of Golgi apparatus with flat centers and dilated ends (G) adjacent connective tissue (CT) with collagen fibers (C). Secretory granules of variable size and shapes were also seen (SG) (X.2000).

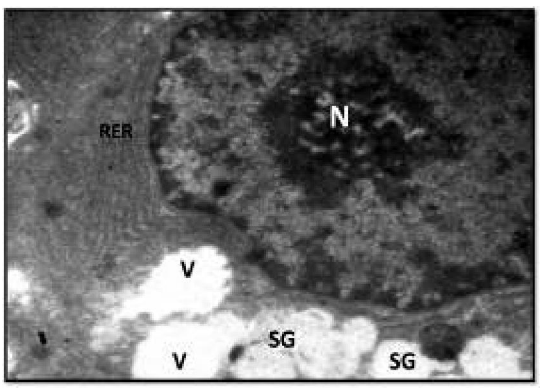

Fig. (6 a): An electron micrograph of submandibular salivary gland of old rats(control group) showing striated duct cell with prominent nucleus (N), multiple degenerated mitochondria (M) arranged between short basal infoldings (I). (X. 2000)

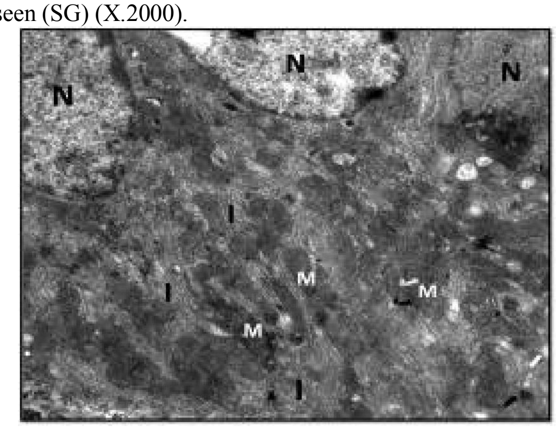

Fig. (6 b): An electron micrograph of submandibular salivary gland of old rats (experimental group) showing basal part of striated duct cells with numerous rod shaped mitochondria (M) arranged radially among long and characteristic basal cytoplasmic infoldings (I). Large nuclei were also seen in apical part of striated ductal cells (N) (X.2000)

The cytoplasm of serous and tubular cells contained large amount of rough endoplasmic reticulum and well developed Golgi apparatus as backed basally and laterally to the nucleus. The acinar cells appeared with approximately normal large nuclei, with defined nuclear membrane and electron dense prominent nucleoli **(Figs.4b & 5b).** The basal part of striated duct cell showed obvious basal infoldings of the plasma membrane. Many large mitochondria arranged radially in between the basal folds **(Fig.6 b).** The connective tissue septa with collagen fibers and large flat fibroblasts with spindle shaped and euchromatic nucleus were clearly seen. The blood vessels lined by endothelial cells and filled with electron dense blood corpuscles were also seen **(Fig.7b).**

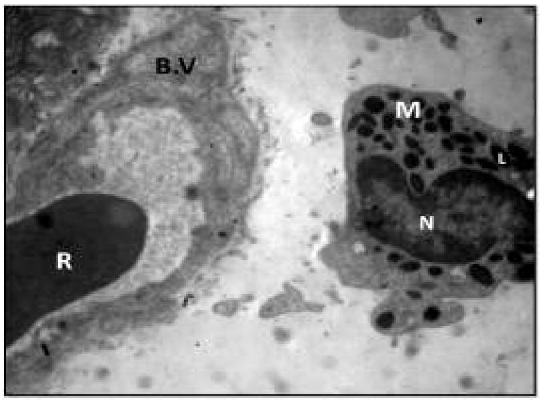

Fig.(7 a) : An electron micrograph of submandibular salivary gland of old rats (control group) showing interlobular connective tissue septa with thinned walled and congested blood vessel (B.V) studded with electron dense red blood corpuscle (R) & macrophage(M) with large nucleus (N) and numerous lysosomes (L).(X.3000)

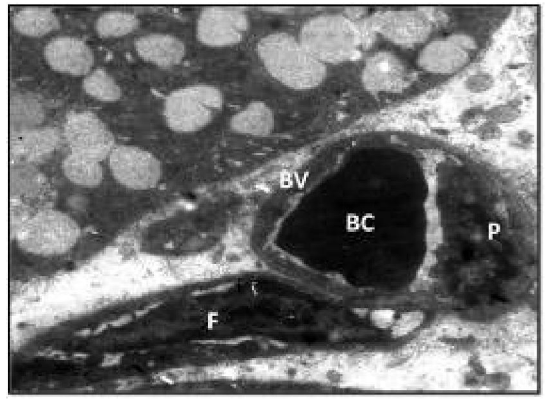

Fig.(7 b): An electron micrograph of submandibular salivary gland of old rats(experimental group)showing blood vessel(BV) lined with endothelial cells surrounded by pericyte (P) contained electron dense blood corpuscle (BC) adjacent to large fibroblast with spindle shaped nucleus (F) in interacinar space.(X3000)

4. Discussion

For centuries, many researches were concerned about the anti-aging compounds; especially; antioxidants that had beneficial effects on the delay of aging procedure, improvement of the body health and prevention of degenerative diseases **(Joshi *et al.*, 2013).** One of the most important antioxidants is omega3 supplements, a unique nutritional intervention that has great potential to decrease the deterioration and concomitant functional changes associated with aging, many inflammatory and autoimmune disorders **(EL-Baz and Salem, 2013).**

In the present study, the acini and granular convoluted tubules of submandibular gland of control group showed shrinkage in the overall size. **Dayane *et al.*, 2000** supported this result as they studied the age related changes of palatal salivary glands in older individuals as compared to younger ones and found that the mean volume of the acini decreased**.**

Histological examination of sections of the control group revealed marked increase in the fibrous; as well as, the fatty tissues of the submandibular salivary gland capsule, these results were in agreement with the observations of **Vered *et al.*, 2000** who demonstrated that the volume of stromal components, fat cells and inflammatory cell infiltrations of labial salivary glands, increased with age. Moreover, in the current study the control group showed marked chronic inflammatory infiltration in the connective tissue septa. These results could be attributed to an increase in the production of oxygen free radicals and metabolites in labial gland by age, with the ultimate results of inducing chronic inflammatory conditions **(Larsson *et al.*, 2001).**

In this study, the blood vessels appeared dilated and congested with flattened wall in the control rats. In agreement with this finding, **Azevedo *et al.*, 2005 & Sa *et al.*, 2013** observed that congestion of the blood vessels in connective tissue septa of lingual gland in edentulous patients was due to presence of mononuclear cell infiltration.

In the experimental group, the current study demonstrated improvement of some age related changes in the submandibular salivary gland, where the acini and granular convoluted tubules appeared nearly normal and the inflammatory cell infiltrate markedly decreased. These results were supported by those of **Serhan *et al.*, 2008,** who reported that omega3 fatty acids played an important role in inflammatory diseases as periodontitis by conversion of omega-3 to some derived mediators, resolvins and protectins inside the body. These mediators had a high ability to decrease the inflammation through promotion of T-cell apoptosis, reduction of tumor necrosis factor-α (TNF-α) expression and neutrophil activity.

Histological examination of specimens of experimental group showed a decrease in fibrous and adipose tissues of the submandibular salivary gland, these results were in agreement with the investigations of **Carpentier *et al.*,2008 & De-Assis *et al.*, 2012** who reported that omega3 had a direct effect on hepatic steatosis (fatty liver disease) of diabetic rats by slowing down the lipid formation and oxidation that were caused by oxidative stress in the hepatocytes, through increase activity of antioxidant enzymes. In addition, **Shaaban *et al.*, 2013** demonstrated that hepatoprotective and anti-fibrotic activities of omega-3and Olmesartan (anti-hypertensive drug) played an important role in decreasing liver injury and fibrosis in rat model, which were caused by carbon tetrachloride as a free radical.

Ultra structural evaluation of the control group sections revealed noticeable dilatation and degeneration of the cell organelles as endoplasmic reticulum, Golgi apparatus and mitochondria of acinar cell. These results were in agreement with those of **Del- Monte, 2005** demonstrated that injured liver of some old people was attributed to presence of free radicals inside the hepatocytes. The free radicals caused degeneration of hepatocyte organelles through leakage of hydrolytic enzymes of the lysosomes.

In the current study the acinar cells of the old rats (control group) revealed pyknotic and shrunken nuclei with condensed chromatin. **Papadimitriou, *et al.*, 1992** supported this result as they reported chromatin condensation of acinar cells in old rats by electron microscopic examination.

Moreover, degeneration was reported in some acini and granular convoluted tubules of control group, this finding could be attributed to the increased levels of inflammatory cell infiltration and their pro-inflammatory cytokines, which may cause degradation of the basement membrane and disruption of acinar and ductal cell architecture by age, through matrix metalloproteinase-2 (MMP-2) modulation **(Azuma *et al.*, 1997).**

In addition, the current study showed apparent decrease in number of degenerated acini and granular convoluted tubules of the submandibular salivary gland of the experimental group. These results were in agreement with the observations of **Simopoulos, 2008 & Hussein *et al.*, 2011** who reported that omega-3 fatty acids was rapidly incorporated into the membrane phospholipids of human cells, suggesting that they have an effect on several aspects of cell function. He also added that omega-3 PUFA have anti-inflammatory properties and, therefore, might be useful in the management of many inflammatory disorders. **Naqshbandi *et al.*, 2012** who explained that the hepatoprotective effects of fish oil (omega3) against ROS in case *of hepatotoxicity* could be attributed to its natural antioxidant properties. Omega3, protected the plasma membrane, mitochondria and other organelles of hepatocytes from damage by oxidative stress.

In the present study the most ultra-structural age related changes in the submandibular salivary gland of old rats improved by administration of omega3 fatty acids. These results were supported by the findings of **Katsumata, 1998,** who observed that omega-3 fatty acids improved the blood flow and consequently metabolism in the cells. Also, omega-3 fatty acids stabilized the cells and protect them from damage by inhibiting the release of arachidonic acid from the cell membrane (**Xiao and Li, 1999).**

5. Conclusion

The current study revealed that omega-3 fatty acids could represent a unique nutritional supplement that has great potential to decrease the deterioration and also the functional changes associated with salivary gland aging.

6. Recommendation

The biological effects of omega3 fatty acids (ALA, EPA and DHA) have undergone considerable study in different fields. Hence, further work is required to identify the differential effects of ALA, EPA and DHA on age related changes.

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