Histological changes in the parotid gland in ovariectomized rats and the possible protective role of estrogen and vitamin E: histomorphometric and ultrastructural study.

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Abstract: Many women undergo hormonal replacement therapy to relieve menopausal symptoms. Oral discomfort is a common symptom in these women and may be documented as one of the systemic manifestations of menopause. This work aimed to study the histological changes in the parotid gland in ovariectomized adult female albino rats and the possible protective role of estrogen and vitamin E against such changes. Forty five adult sexually mature female albino rats were used in the present study. The rats were divided into four groups as follows: group I (normal and sham control), group II (overiectomized group), Group III (Estrogen treated group) treated with 17β estradiol valerate; 1 mg/kg/daily for 12 weeks and Group IV (Estrogen and vitamin E treated group); treated with estrogen (1 mg/kg/day orally) +vitamin E (400 mg/kg orally) daily for 12 weeks. The animals were sacrificed, parotid glands were extracted from all rats and stained with H & E, Masson's trichrome and PAS stains. Electron microscopic examination of the parotid specimens was done. The percentage area of collagen fibers, PAS optical density and the percentage area of damaged acini were measured. Morphometric results were statistically analyzed. Examination of parotid in group II showed different forms of degenerative changes. The acini appeared irregular and lined with some apoptotic cells. Their cytoplasm contained extensive vacuolations. Homogenous acidophilic material, cellular infiltration within thick connective tissue septa, dilated ducts with stratification in their lining and congestion of blood vessels were also seen. Ultrastructurally, rarified dilated RER, electron dense and lucent granules were also seen. Estrogen replacement improved the histological changes in group III and vitamin E supplementation with Estrogen in group IV produced marked improvement in the degenerative changes occurred in the parotid gland in group II. Results obtained in this study concluded that estrogen deficiency in led to variable degenerative changes in parotid salivary glands of adult female albino rats with relative limitation of these changes in estrogen supplemented group and marked improvement in these changes with supplementation of vitamin E and Estrogen.

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Introduction

Approximately, about one-third of the average women lives beyond menopause and many of them undergo hormonal replacement therapy to relieve menopausal symptoms. Oral discomfort is a commonsymptom in these women and may be documented as one of the systemic aspects of menopause ***(Eliasson et al.,2003)***.

In postmenopausal period, the endogenic estrogen level decreases, which is responsible for many alterations in almost all the body systems. Estrogen acts through two intracellular receptor proteins called estrogen receptors (ER). It was reported that ER were present in oral buccal mucosa, parotid and submandibular glands ***(Rahnama et al., 2004)***.

The most important and annoying oral affections in women in the postmenopausal period are alteration in taste and burning mouth syndrome (BMS). BMS which refers to a chronic or facial pain disorder. Hormonal changes may also affect the composition and the rate of saliva ***(Maltsman-Tseikhin et al., 2007)***.

There was reduction in the salivary flow of the parotid gland with increasing age and reduction of estrogen production. Estrogen acts on the oral mucosa by specific receptors, which recognize, and bind the hormone to the cell cytoplasm or nucleus. The deficiency of this hormone causes changes in the oral mucosa at the tissue level, as it influences the proliferation, differentiation, and keratinization of the gingival epithelium and also stimulates the proliferation of fibroblasts ***(Seko et al., 2005)***.

Estrogenis known to regulate cell growth, differentiation and function in reproductive as well as in nonreproductive tissues. Also, this hormone appears to playa significant role in the physiology of the human oral cavity ***(Valimaa et al., 2004)***.

Vitamin E is a natural component of cell membranes; it is considered the main defense against

membrane lipid peroxidation. It also reacts quickly with peroxyl free radicals interrupting the free radical chain reaction and consequently protecting cells from damage ***(Carolina et al., 2013)***.

Vitamin E supplementation protected the salivary glands against radioiodine exposure which generated reactive oxygen species and induced damage to critical macromolecules such as DNA ***(Uma et al., 2010)***.

2. Material and methods

Forty five adult sexually mature (4 months age) female albino rats of Wister strain weighing 150-200 g., were used. The rats were housed in separate cages and maintained under standard laboratory and environmental conditions. Diet and water were given ad libitum. The rats were divided into four groups, **Group I (Normal control and sham control group):** Normal control: Formed of 5 rats, they were not subjected to any manipulation or medication, Sham control: Formed of 10 rats. Five rats were subjected to lower abdominal incision and the other five received normal saline and olive oil orally for 12 weeks.

Group II (Ovariectomized group): Formed of 10 rats. They were submitted to bilateral ovariectomy. **Group III (Ovariectomized + estrogen treated group):** Formed of 10 rats. They were submitted to bilateral ovariectomy and then received estrogen orally (17β estradiol valerate) 1 mg/kg/daily for 12 weeks. **Group IV (Ovariectomized+estrogen+vitamin E treated group):** Formed of 10 rats. They were submitted to bilateral ovariectomy and then received estrogen (1 mg/kg/day orally) +vitamin E (400 mg/kg orally) daily for 12 weeks.

The animals of groups II, III and IV were anesthetized with intra peritoneal injection of ketamine (75 mg/kg body weight) for the surgical procedure. Small incisions were made on each flank. The ovaries and their surrounding fat were externalized and after ligation of the ovarian arteries the ovaries were removed. The distal ends of each uterine horn were then returned to the peritoneal cavity and the incisions were closed with 4-0 nylon sutures.

The rats in the sham control subgroup were subjected to lower abdominal incision in each flank and then the wound was closed. At the end of the experiment; the animals were sacrificed by cervical dislocation and the parotid gland of each animal was extracted fixed in 10 % neutral formol saline for 24 hours and were processed to prepare 5 µm thick paraffin sections for haematoxylin and Eosin stain, Masson's trichrome and PAS stains Also, parts of the same specimens were processed for electron microscopic examination.

Histomorphometric study

Serial sections stained with H & E, Masson's trichrome and PAS stains were morphometrically analyzed for detection of ara % of damaged acini; area % of collagen fibers in thesepta around ducts and blood vessels and the mean optical density using Leica Qwin 500image analyzer computer system (Cambridge, England) at Pathology Department, Faculty of Dentistry, Cairo University. The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the Olympus microscope (CX 41) and controlled by Leica Qwin 500software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The measuring frame of a standard area is equal to 7286, 78µm². For each parameter ten different non overlapping fields from ten different specimens were examined in each group.

Statistical analysis

The obtained data from morphometric analysis were subjected to one way analysis of variance (ANOVA) test using Statistical Package for the Social Sciences (SPSS) version11. The P value <0.05 was considered significant, <0.001 were considered highly significant.

3. Results

Histological results:

Examination of the parotid gland sections in the control and sham control groups showed the same structure, the classic architecture of the parotid appeared in the form of acini lined by cuboidal cells with basal rounded basophilic nuclei with narrow lumina, the striated ducts were lined by columnar cells with central rounded nuclei. Thin layer of collagen fibers present around the serous acini and the ducts. PAS stained sections revealed a strong positive reaction. Electron microscopic examination of the parotid gland sections of the control group showed serous acini composed of pyramidal cells with basal euchromatic nuclei. Their cytoplasm presented many serous secretory granules. The acinar cells were arranged around a narrow lumen. The striated ducts were composed of cuboidal cells with indented nuclei; their cytoplasm was seen rich in mitochondria and their lumens were characterized by presence of many microvilli. The nuclei of the acinar cells were surrounded by extensive rough endoplasmic reticulum and the mitochondria had intact mitochondrial membrane and cristae.

Examination of the parotid gland sections in group II showed marked degenerative changes in the form of pyknotic nuclei of the serous acini. The striated ducts were disrupted with hemorrhagic

contents in their lumina. Blood vessels were seen markedly congested. The nuclei of the cells were exfoliated in the duct lumina. The acini showed irregular walls and multiple cytoplasmic vacuoles, the interstitial spaces were wide, edematous and filled with acidophilic exudate. Mononuclear cellular infiltration was noticed in the interstitial spaces. Adipose tissue accumulation was seen especially around the ducts. Increased collagen fibers deposition around the blood vessels was noticed. PAS stained sections showed weak positive reaction.

Electron microscopic examination of the parotid gland sections of group II showed condensed heterochromatic nuclei of the acinar cells, dilated rough endoplasmic reticulum and multiple cytoplasmic vaculations. Accumulation of lipid droplets was seen between the acini. The mitochondria were seen with degenerated cristae, dilated lumina of the ducts were seen with exfoliated microvilli inside.

Examination of parotid sections in the treated group III showed apparent normal appearance of serous acini and ducts. The striated and the interlobular ducts appeared with regular walls and narrow lumina. Mild collagen fibers deposition around blood vessels and striated ducts and moderate PAS reaction was noticed. Electron microscopic study of the parotid specimens showed preserved parenchymal architecture of the ducts and the serous acini.

Histological sections of the rat parotid gland of group IV showed marked improvement in the parenchymal architecture of the parotid gland. The serous acini appeared with normal outline and arranged in groups, the ducts preserved their regular walls and narrow lumina. Minimal deposition of collagen fibers around striated and interlobular ducts, PAS stained sections revealed strong positive PAS reaction. Electron microscopic examination of the parotid gland sections of group IV showed normal architecture of the serous acini and ducts.

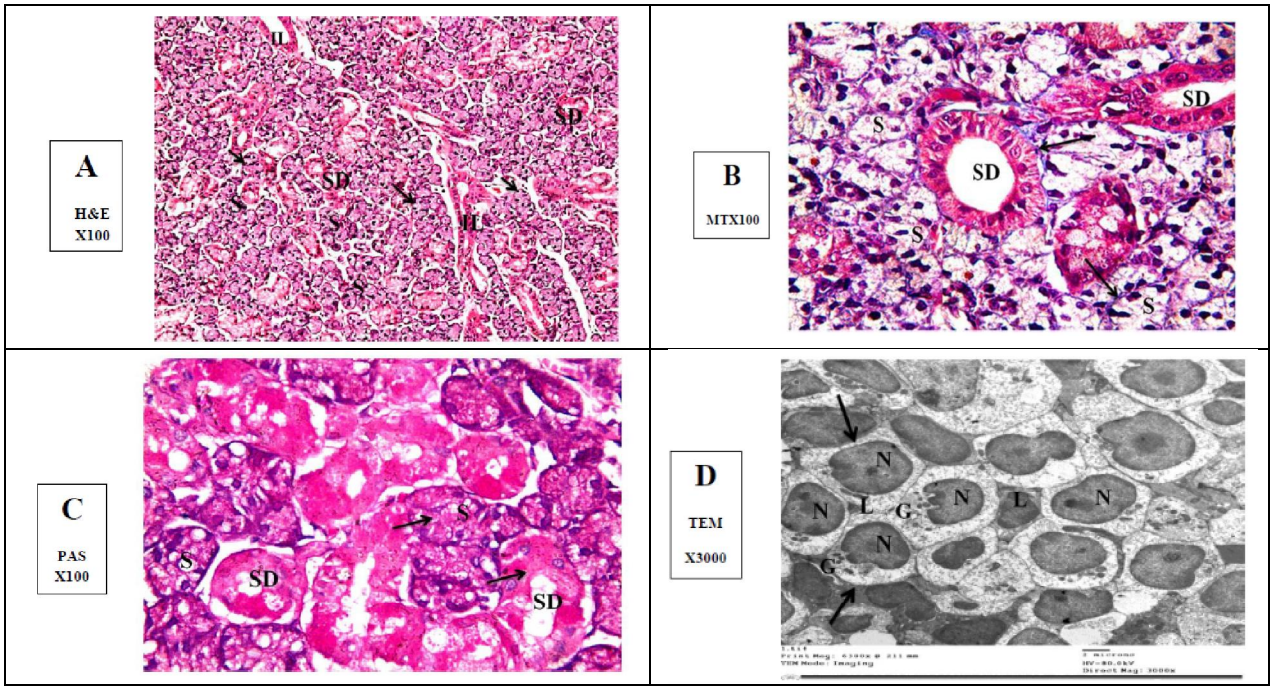

Fig.1. Control group. A. Parotid gland sections showed serous acini (S) with narrow lumina and lined by cuboidal cells with basal rounded basophilic nuclei (arrows). Striated ducts (SD) and interlobular ducts (IL) are seen between the acini. **B.** Minimal collagen fibers deposition (arrows) was seen around the serous acini (S) and the ducts (SD). **C.** The serous acini and the striated ducts exhibiting strong positive PAS reaction. **D.** The serous acini composed of pyramidal cells (arrows) with basal euchromatic nuclei (N). The acinar cells are arranged around a narrow lumen (L). The rest of the cytoplasm contains many serous secretory granules (G).

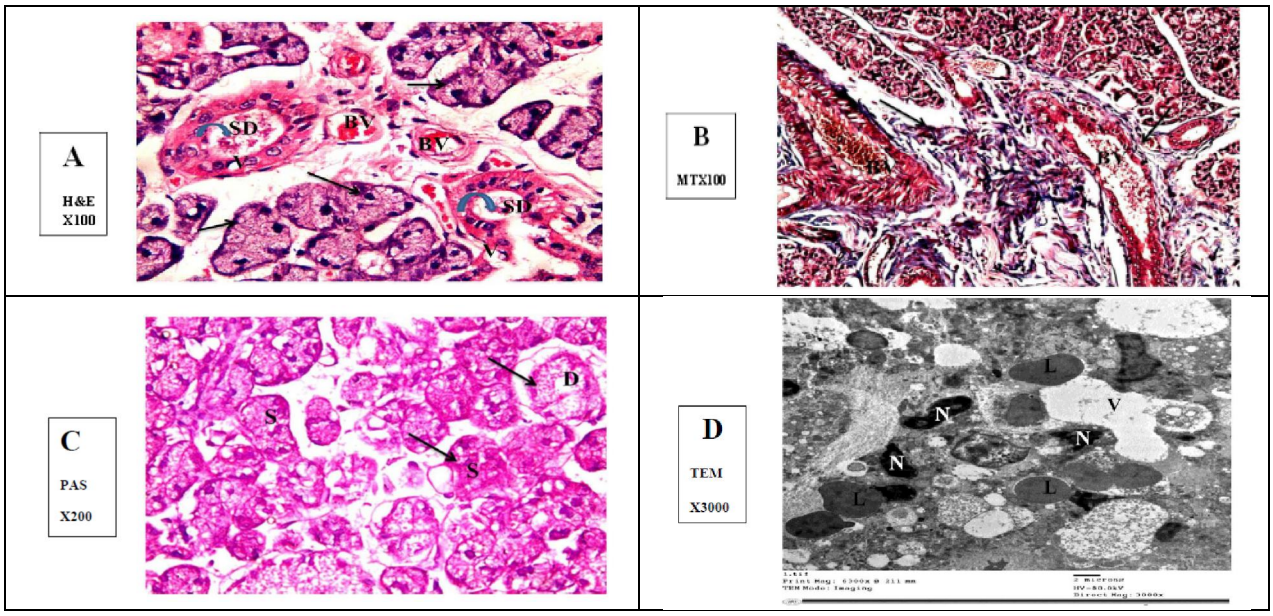

Fig.2. Overiectomized group. A. Parotid gland sections showed distortion of the acini, degeneration of cells and pyknotic nuclei (arrows), vacuolation of cytoplasm (V). The striated ducts (SD) are seen dilated with hemorrhagic contents (curved arrows) in their lumina; blood vessels (BV) are dilated and congested. **B.** Marked increase in collagen fibers (arrows) in the interstitial tissue and around blood vessels (BV). **C.** The serous acini (S) and the ducts (D) exhibiting faint positive PAS reaction (arrows) in the cytoplasm of the acinar and duct cells. **D.** Theacinar cells showed dense irregular and shrunken nuclei (N), accumulation of lipid (L) droplets and extensive vacuolations (V) in the cytoplasm are present.

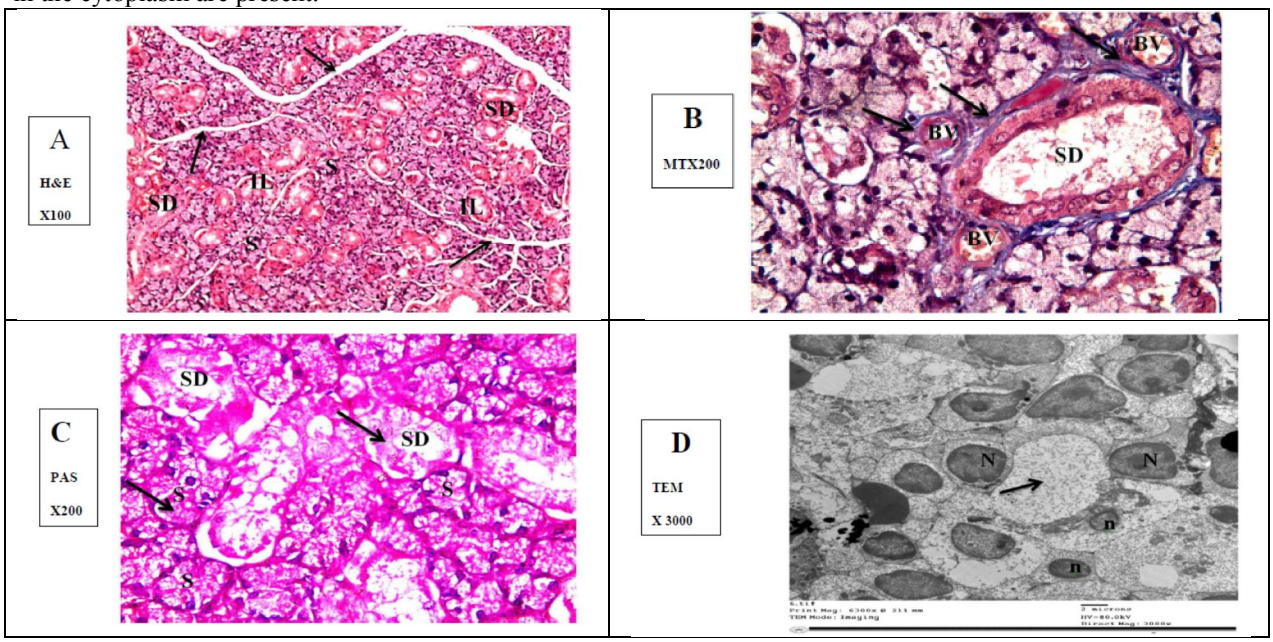

Fig.3. Estrogen treated group. A. Parotid gland sections showed restoration of the normal architecture, the serous acini (S) with regular walls arranged in groups in the parotid lobules. These lobules are separated from each other by septa (arrows). The striated ducts (SD) appear regular with narrow lumina. The interlobular ducts (IL) are regular with relatively wide lumina. **B.** Marked decrease in collagen fibers (arrows) which are present mainly around blood vessels (BV) and striated duct (SD). **C.** the serous acini (S) and the striated ducts (SD) exhibiting moderate PAS reaction (arrows). **D.** the striated duct appeared normal and composed of cells containing rounded euchromatic nuclei (N), other cells still have small dense nuclei (n). The lumen of the duct is wide and contains exfoliated villi (arrow).

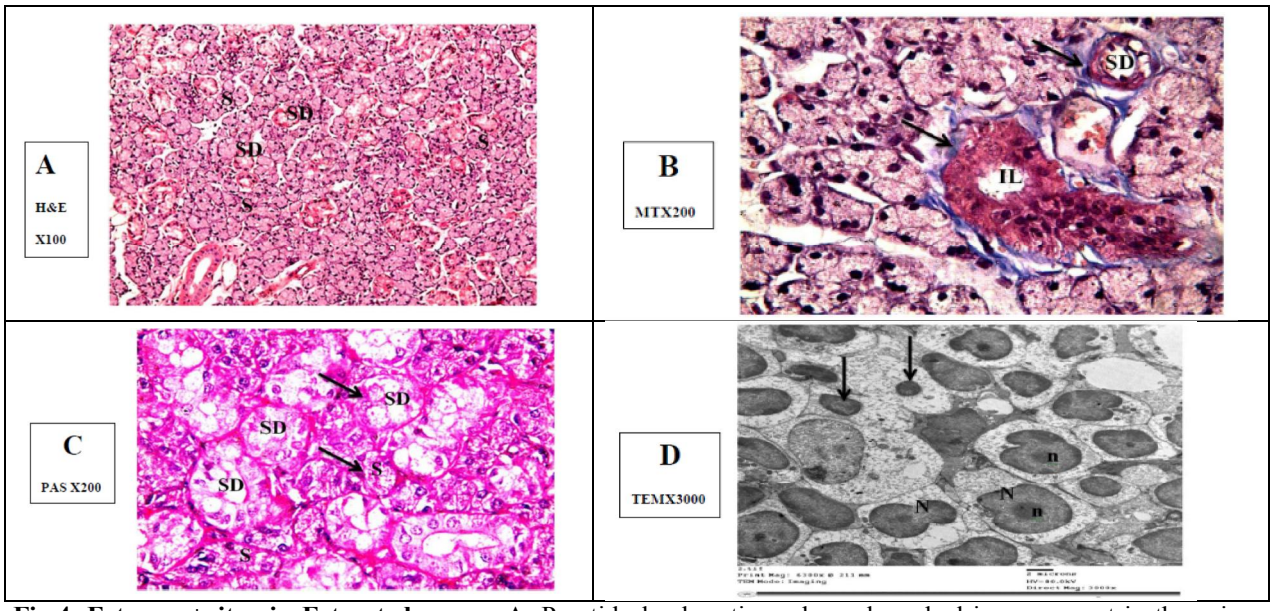

Fig.4. Estrogen+vitamin E treated group. A. Parotid gland sections showed marked improvement in the acinar architecture of the parotid lobules including the acini (S) which are arranged in groups, separated by narrow interlobular septa. The striated ducts (SD) appear rounded with regular walls and narrow lumina. **B.** Minimal amount of collagen fibers (arrows) seen around the striated (SD) and the interlobular ducts (IL). **C.** The serous acini (S) and the striated ducts (SD) exhibiting strong positive PAS reaction (arrows) in the cytoplasm of acinar and duct cells. **D.** The acini restore their normal architecture. The nuclei (N) of the cells appear normal with prominent nucleoli (n). Few cells contain condensed and pyknotic nuclei (arrows).

Histomorphometric results:

The mean optical density, area % of collagen fiber and area % of damaged aciniforall groups were presented in (Tables 1, 2, 3). There was a significant increase (P<0.05) in collagen % and area% of damages acini in group II compared with control group I. While, there was a significant decrease in collagen fibers % and area percentage of damaged acini in treated group (group III) and high significant decrease in these values in group IV as compared with group II. The mean values of the PAS optical density in different experimental groups showed statistically high significant difference in group II compared to group I (p≤ 0.01). The difference in group III was statistically highly significant compared to the control group (p≤ 0.01), while the mean values in group IV showed no significant difference compared to group I.

Table (1): showing the mean optical density of PAS stained sections in different experimental groups.

Group	Mean ± SD	P-value	F-ratio
Group I	72.03 ± 1.31	0.706	122.672
Group II	53.23 ± 3.31	0.0001**	
Group III	64.69 ± 2.15	0.0001**	
Group IV	71.60 ± 1.67	0.706	

* p- Value is significant ≤ 0.05
** P-value is highly significant ≤ 0.01

Table (2): showing the mean Percentage area of collagen fibers deposition in parotid sections of different experimental groups

Group	Mean ± SD%	P-value	F-ratio
Group I	0.86 ± 0.32%	0.794	92.381
Group II	18.93 ± 4.79%	0.0001**	
Group III	1.91 ± 0.62%	0.428	
Group IV	1.20 ± 0.31%	0.593	

* p- Value is significant ≤ 0.05
** P-value is highly significant ≤ 0.01

Table (3): showing the mean area Percentage of damaged acini in parotid sections of different experimental groups.

Group	Mean ± SD	P-value	F-ratio
I	2.26 ± 0.61	0.140	199.239
II	13.09 ± 1.51	<0.0001**	
III	4.51 ± 0.78	<0.0001**	
IV	3.02 ± 0.51	0.140	

* p- Value is significant ≤ 0.05
** P-value is highly significant ≤ 0.01

Discussion

Menopause is characterized by decrease in estrogen production that reduces the quality of life. With the increase in the life span of people in the last

century; people have to maintain the standard of life. It can lead to many problems despite the pathophysiology is not fully understood. The metabolic disorders and oxidative stress accompanying menopause cause disturbance in many body systems including salivary glands ***(Secil et al., 2014)***.

In the present study, manifestations of pathological impact of ovariectomy on the parotid gland of rats were recorded. Light microscopic examination of the parotid gland sections revealed disturbed parotid architecture, degeneration of the acinar cells with pyknosis of their nuclei, dilatation and congestion of blood vessels, inflammatory cellular infiltration, dilatation and distortion of the striated and the interlobular ducts.

These finding were in agreement with ***Secil et al. (2014)*** who reported that the parotid gland of the ovareictomized rats after 12 weeks showed esinophilic stained cytoplasm of the acinar cells, polymorph nuclear cellular infilteration in the connective tissue and lipid accumulation.

These results were compatible with those reported by ***Dalia et al. (2015)*** who reported that most of the acini of the parotid gland of rats after ovariectomy for two weeks appeared irregular with darkly stained nuclei and many vacuoles were present in their cytoplasm. They were separated by thickened septa containing cellular infiltration. Homogenous acidophilic material was also seen. The interlobular ducts were dilated and lined by stratified epithelium, Congested blood vessels were also found. ***Leimola et al. (2000)*** attributed these pathological changes to the deficiency of female sex steroids which have a beneficial effect on the salivary glands and demonstrated increased saliva flow rate after hormonal treatment.

The results of the present study was also supported by ***Basak et al. (2015)*** who noticed degeneration of the striated duct cells and the serous acini accompanied with pyknotic nuclei and acidophilic cytoplasm, few polymorph nuclear cellular infiltration were noticed in the connective tissue of the parotid glands of rats 12 weeks after ovariectomy. They explained these pathological changes by deficiency of estrogen receptors in the salivary glands and suggested that estrogen has an antioxidant action in these tissues.

Increased collagen fibers deposition in the parotid gland was observed in the present study in the overiectomized rats. ***Deconte et al. (2011)*** were in agreement with the present study and reported that collagen fibers appeared in extensive amount in between the lobules and around blood vessels in the parotid gland of the ovariectomized rats. They mentioned that estrogen is antioxidant hormone and acts as radical scavenger and inhibits lipid peroxidation so, low level of this hormone could lead to oxidative stress which increased collagen fibers deposition in the parotid gland.

Ultrastructurally, vacuolation of the cell cytoplasm, degeneration of the microvilli lining the ducts and dilated rough endoplasmic reticulum were noticed. These pathological changes were confirmed by ***Younis et al. (2013)*** who clarified the presence of apoptotic acinar cells with irregular heterochromatic nuclei, dilated rarified rough endoplasmic reticulum, electron lucent and electron dense secretory granules. Extensive cytoplasmic vacuoles and apoptotic cells lining their ducts were also found. ***Kiray et al. (2007)*** explained these ultrastructural changes in the acinar cells of the parotid gland by generation of large number of free radicals which could damage cellular proteins.

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