

Original Article**Comparative Study between the Protective Role of Rosmarinic Acid and The Effect of Mesenchymal Stem Cells on Ameliorating the Toxic Effect of Duloxetine on the Parotid Gland of Male Albino Rats.**

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ABSTRACT:

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Background: Duloxetine is an antidepressant drug utilized for treatment of anxiety disorders and depressive disorders. This study aimed to compare the potential protective effect of Rosmarinic acid (RA) as natural antioxidant with the potential therapeutic effects of mesenchymal stem cells on treating the toxic effect of Duloxetine on the parotid gland.

Material & methods: 60 male albino rats were classified into 4 groups, 15 rats in each group. The first group1 (control): negative control (group 1a) formed from 7 rats received no treatment, positive control (group 1b) formed from 8 rats received 1ml distilled water using oro-pharyngeal tube for 12 weeks, group 2 (Duloxetine): received 10 mg/kg/d duloxetine dissolved in distilled water using oro-pharyngeal tube for 12 weeks, group3 (Duloxetine+RA): received Duloxetine the same as Group2 and received at the same time 120 mg/kg/d Rosmarinic acid dissolved in distilled water for 12 weeks using oro-pharyngeal tube, group4 (Duloxetine+MSCs): received Duloxetine the same as Group2 then a single injection of MSCs just after stopping of Duloxetine, In all groups animals were sacrificed after 16 weeks for histological evaluation, the parotid gland was stained with hematoxylin and eosin, and Mallory's trichrome stain. Also, Transmission electron microscope and immunohistochemistry using caspas-3 were used to examine the parotid gland. Also the level of MDA and GSH-Px level in the parotid tissue were done.

Results: Examination of parotid sections in Group2 revealed inflammatory cells infiltration and massive distortion and vacuolation of serous cells, however Group3 and Group4 showed marked improvement in the histological structure of the parotid gland.

Conclusion: MSCs and RA have good effect in decreasing the toxic effect of Duloxetine administration on the parotid gland with insignificant difference between the two methods.

Keywords: Duloxetine, Rosmarinic acid, MSCs, parotid toxicity, GSH-Px, MDA.

INTRODUCTION:

Depression is one of the most common causes of health retardation, decreased productivity and loss of interest in work. The disease leads to a severe decrease in quality of life for those afflicted and their nearest ones. Mental Disorders' diagnostic and Statistical Manual provides a description of depression's emotional symptoms including the loss of interest, sadness, suicidal attempt, and guiltiness (Fountoulakis et al., 2003).

There are many methods to treat depression and anxiety. Medicines are an alternative; psychotherapy such as cognitive behavioral therapy (CBT) is another. In drug therapy there are many classes of medicines to choose between. The first discovered class of drugs for treatment of depression is tricyclic antidepressants (TCAs). But TCA has many side effects which may lead discontinuation of treatment. These side effects are due to the non-selective binding to various receptors (Clayton et al., 2002).

Also, the overdose may lead to severe arrhythmias. So, TCAs use is restricted. Another class of antidepressant is introduction of selective serotonin reuptake inhibitors (SSRIs). They are described as being selective due to their major effect on serotonin (5-hydroxytryptamine; 5-HT), not on other neurotransmitters. They are able to mitigate moderate to severe depression symptoms. They are relatively safest and has typical result in less side effects compared to TCAs.

Moreover, through increasing serotonin levels in the brain by inhibiting serotonin reuptake, leading to higher availability of serotonin, the SSRIs are able to mitigate depression. However, they can have severe side effects including delayed ejaculation, anorgasmia, and sexual dysfunction and there could be a decrease in libido in about 60% of patients (Clayton et al., 2002), sleep disturbances, anorexia, constipation and dry mouth (Richelson, 2003).

A new class of SSRI antidepressants are the drug of choice for the treatment of depression because they are more effective, have fewer side effects, and are well tolerated compared to other antidepressants classes and resulting in more effective control of depression emotional and physical symptoms. This class of antidepressants is used for major depressive disorder (MDD) and possibly for anxiety disorders treatment. According to Cashman and Ghirmai (Cashman and Ghirmai, 2009), dually has several advantages compared to other antidepressant drugs as it treats a wide range of symptoms. Duloxetine is a part of this class, which was proven in August 2004 (Iyengar et al., 2004) to treat depression, MDD, neuropathic pain, fibromyalgia (Russell et al., 2008), generalized anxiety disorder (GAD) (Wernicke et al., 2006) and diabetic neuropathy. And also, enormous success in chronic musculoskeletal pain treatment, which involves chronic pain of low back and osteoarthritis, has been recently reported by duloxetine

(Skljarevski et al., 2010). There is also a putative relationship between its analgesic efficacy in the center of the brain and its effect on descending inhibitory pain pathways. Duloxetine is also recommended in severe dental and parodontal pain. Duloxetine is used as the first-option medication as in some reports in dentistry for burning mouth syndrome (BMS) (Kim et al., 2014).

Duloxetine can significantly mitigate chronic nonorganic oro-facial pain as confirmed by Nagashima et al., (2012). Its effect of reducing the pain began to appear 2 weeks after starting treatment. Duloxetine effects of pain mitigation have appeared regardless presence or absence of the baseline depressive symptoms. Most prescribed antidepressant medications and the number of significant oral reactions is related, which include dysgeusia, tongue edema with discoloration, xerostomia, sialoadenitis, glossitis, and stomatitis. These oral reactions appear because of dysfunction of salivary gland. Burning sensation and taste alteration are caused by reduction of saliva production (Kumar et al., 2014).

A range of cell types may be developed from bone marrow-derived stem cells. Immediately after bone marrow-derived mesenchymal cells injection, several reactions are possible including the stem cells homing to the bone marrow and being directed to the injured sites, where they are driven to produce a significant number of growth factors and cytokines. In ischemic areas BM-MSCs may promote vascularization

and angiogenesis through their paracrine activities. Consequently, they may initiate the healing process by stimulating the development of endogenous stem cells. (Nakano-Doi et al., 2010)

Rosmarinic acid (RA) is a natural phenolic chemical which is water-soluble, is one of the *S. glabra* active principal constituents and is extensively diffused in several plant species. Multiple pharmacological effects of RA include anti-inflammatory, antiangiogenic (Jiang et al., 2018) and anticancer (González-Vallinas et al., 2015). Its antioxidant function may interfere with unsaturated fatty acids for lipid peroxyl group binding, therefore interfering with the lipid peroxidation chain reaction and slowing it down. RA inhibits free radical-induced cell damage and has a greater protective effect on the parotid gland than other several natural antioxidants, as chlorogenic acid, folic acid and caffeic acid. RA has a protective effect against the damages caused by ionizing radiation (Xu et al., 2016).

The aim of the work: This study aimed to compare the potential protective effect of Rosmarinic acid (RA) as natural antioxidant with the potential therapeutic effects of mesenchymal stem cells on treating the toxic effect of Duloxetine on the parotid gland.

Materials and methods:

Animals:

Sixty male Albino rats with about 200 ± 30 g weight, obtained from Benha

University's Faculty of Veterinary Medicine animal house; they were housed at 12 hr light/dark cycle and an appropriate temperature. Prior to the experimental investigation, the animals were kept for 10 days and fed a basic diet.

Drugs:

DULOXETIN: The antidepressant drug duloxetine (Duloxetine), was purchased from (UTOPIA) medical Company in Egypt in the form of capsules with 30mg /cap concentration. These capsules were evacuated and dissolved in a distilled water to a concentration of 10 mg/ml. rats in Group2 (Duloxetine), group3 (Duloxetine+RA) and group4 (Duloxetine+MSCs) received 10 mg/kg body weight/daily duloxetine using oro-pharyngeal tube (Patel et al., 2015).

ROSMARINIC ACID: Rosmarinic acid (RA) was purchased from (Sigma-Aldrich Chemie GmbH, Munich, Germany) in the form of water-soluble powder and was given to group3 (Duloxetine + RA) with intragastric tube daily after dissolving it in a distilled water in a dose of 120 mg/kg body weight for 12 weeks (Sotnikova et al., 2013).

Kits: The bio-diagnostic kits were purchased from Diagnostic and Research Reagents (Giza, Egypt) for estimation of Malondialdehyde (MDA) and Glutathione (GSH) levels.

Experimental protocol:

Sixty male albino rats were equally classified into four groups of 15 rats in each group. group1 (control): negative control (group 1a) formed from 7 rats

received no treatment, positive control (group 1b) formed from 8 rats received 1ml distilled water using oro-pharyngeal tube for 12 weeks. group 2 (Duloxetine): received by Oro-pharyngeal tube 10 mg/kg/d Duloxetine dissolved in distilled water for 12 week, group 3 (Duloxetine+RA): received duloxetine the same as group 2 and received at the same time 120 mg/kg body weight RA in the form of powder dissolved in distilled water by oro-pharyngeal tube for 12 week, group 4 (Duloxetine +MSCs): received duloxetine the same as group 2 for 12 week and then single injection of (BMMSCs) 1×10^6 cells by intravenous injection at tail vein. In group 2, group 3 and group 4 animals were sacrificed sixteen weeks following the beginning of the study.

Histological study:

By intraperitoneal injection of anesthesia in a dose of 0.86 mg/kg sodium pentobarbital, animals were euthanized, then sacrificed by cervical dislocation and their parotid glands were excised, preserved in formalin 10%, and solidified into paraffin blocks. Using a rotary microtome (LEICA RM 2125; UK), these blocks were sliced to a 5 m thickness. Hematoxylin and eosin (H&E), the conventional stain of histological examination were used for staining the blocks, and Mallory's (MT), a specialized stain for assessing collagen fibers (Bancroft and Layton, 2013). Using a light microscope (Olympus-Bx; 4500) equipped with a digital camera (Nikon-Coolpix; 4500) and objective lens magnifications of $\times 200$ and $\times 400$,

slides were examined and histomorphometric evaluation were performed.

Immunohistochemical analysis:

Using the avidin-biotin peroxidase complex method, then by diaminobenzidine (DAB) staining, Caspase-3 immunohistochemical was performed (Jackson and Blythe, 2013). For blood elimination, 10 ml of 0.9% saline solution was used for cleaning the right parotid which then frozen for biochemical analysis. For the histopathological examination, left parotid gland was prepared.

Biochemical analysis:

Right parotid gland samples were perfused to remove RBCs and clots, homogenized in 0.9% saline solution, centrifuged for 15 min at 3000 rpm, and the supernatant was stored at -20°C until detection of parotid MDA, the breakdown product of lipid peroxidation (Ohkawa et al., 1979). Commercial kit (Biodiagnostic, Egypt) was used for detection of the amount of glutathione peroxidase (GSH-Px) in the parotid gland (Beutler et al., 1963).

Morphometric study:

In 10 randomly chosen microscopic fields at a magnification of X 200 for each specimen, by using image analysis software (Image j. version 1.46), Caspase-3 immune-positive staining and area percent of deposition of collagen fibers was determined.

Preparation of BM- MSCs:

Extract of BMMSCs was acquired from the Benha University Histology Department, Faculty of Medicine, Stem

Cell Research Unit.

Labeling Of Stem Cells with PKH26 Dye

PKH26 fluorescent linker dye was used for labeling the MSCs after isolation during the 4th passage. PKH26 is a red fluorochrome with 567 nm emission and 551 nm excitation. Both proliferative and biological activities are preserved in the labeled cells. Therefore, the linker is suitable for long-term in vivo cell tracking, in vitro cell labeling, and in vitro cell proliferation study. The dye is stable and will proportionally divide as cells divide. Detection of injected cells homing in the rat's tongue: 1wk. after MSCs transplantation into the rat, rat parotid was inspected with a fluorescent microscope to detect the cells dyed with PKH26 dye to confirm the injected cells engraftment into the parotid gland of Gr4, cells tagged with PKH 26 exhibit substantial red autofluorescence (Figure1).

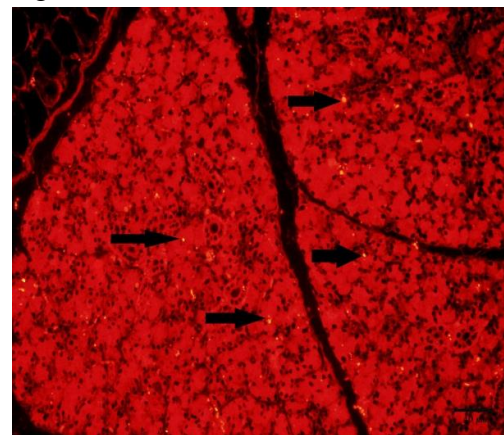


Figure (1): A Photomicrographs of PKH26 fluorescent-stained sections revealing many differentiated red fluorescent BMSC distributed in acinar cells (black arrows) and ducts all over the parotid section (PKH26 \times 200).

Electron microscopic studies:

The left parotid glands were extracted and putted rapidly for 4 h in 2.5 % phosphate-buffered glutaraldehyde, and then fixed for 2 h in in 0.1 mol/L 1 % osmium tetroxide. In ascending grades of alcohol, the specimens were dehydrated, to select the suitable sites for ultrathin sections (50-60 nm thick) semithin sections were stained with toluidine blue, then by ultramicrotome they were double stained with lead citrate and 2% uranyl acetate, and then a JEM-1200XXII, Transmission Electron Microscope UK transmission electron microscope at the Faculty of Medicine, Benha University was used for examination and photographing (Wu et al., 2010).

Ethical approval:

This experimental research was evaluated and approved by the Research Ethical Committee of Faculty of Medicine, Banha University, Rc.14.10.2022.

Statistical analysis:

The data was statistically analyzed using SPSS version 20 .The data was presented in mean \pm SD. The t-test was used to check normality with the assumption that $P > 0.05$ indicates normality. One-way ANOVA for normally distributed variables tested differences between groups, followed by post hoc multiple comparisons using Bonferroni and Games-Howell tests to identify statistically significant pairs in biochemical result and with Tukey's tests to identify statistically significant

pairs in Mallory's and caspas-3 results, P value of less than 0.05 is regarded to be statistically significant.

RESULTS**Biochemical analysis**

The biochemical analysis of both control negative & positive (1a&1b) groups revealed similar results. Thus, we choose control negative (group 1a) as a representative group in the biochemical results.

In group 2 (Duloxetine), the MDA level was significantly higher compared to the control group ($P \leq 0.01$), whereas it was significantly lower in group3 (Duloxetine+RA) and group 4 (Duloxetine+MSCs) compared to group2 (duloxetine) ($P \leq 0.01$) (**Table 1**).

GSH-Px activity was significantly lower in group 2(duloxetine) compared to the control group ($P \leq 0.01$), but it was significantly higher significantly in group 3(Duloxetine+RA) and group 4 (Duloxetine+MSCs) compared to group 2(Duloxetine) ($P \leq 0.01$) (**Table 1**).

Table (1): Comparisons between mean values of the MDA and the GSH in different studied groups using Bonferroni and Games-Howell tests

Groups	MDA (nmol/g tissue) \pm SD	GSH (nmol/g tissue) \pm SD
Control group	8.03 \pm 1.51	23.26 \pm 1.99
(Duloxetine) group	18.00 \pm 0.33**	17.81 \pm 1.33**
(RA+ Duloxetine) group	9.63 \pm 0.51*	22.03 \pm 1.64*
(MSC +Duloxetine) group	8.93 \pm 0.54 *	22.54 \pm 1.11*

The data are shown as the mean \pm standard deviation SD (in each group). ** = significant difference relative to the control group, and * = significant difference compared to the Duloxetine group. (MDA: malondialdehyde & GSH: reduced glutathione)

Histological result:

The light microscopic examinations of both control negative & positive (1a&1b) groups revealed similar results. Thus, we choose control negative (group 1a) as a representative group for the control groups in the histopathological & immuno-histochemical images.

Hematoxylin and Eosin staining:

The parotid sections stained with H&E from group 1(control) revealed nearly normal acini composed of serous cells, striated excretory duct lined by cuboidal epithelium and striated duct lumen can be observed (Figure 1), in group 2(Duloxetine): parotid sections revealed massive collagen infiltration around ducts, mononuclear inflammatory cells infiltration can be observed in between serous acinar cells with pyknosis of some nuclei of serous cells can be seen, blood vessels were congested, abnormal division in cuboidal epithelium which lined striated excretory duct (Figure 2). Also, we found in same group interlobular connective tissue degeneration and serous cells duct distortion and vacuolation (Figure 3).

While in group 3 (Duloxetine+RA), parotid sections showed small vacuoles in serous cells and focal area of collagen fibers between the serous acini can be observed with slight fibrosis around the

ducts and nearly normal serous acinar cells (Figure 4), In group 4 (Duloxetine+MSCs) parotid sections showed most probably normal architecture of parotid tissue with normal serous acinar cells and normal striated excretory duct and the lumen of excretory duct can be seen, also the excretory duct lined by normal cuboidal epithelium (Figure 5)

Mallory's trichrome stain:

Mallory's stained parotid section of rat from group 1 (control) revealing no collagen fibers in between the serous acini and minimal collagen fibers around the ducts, but in group 2 (Duloxetine) showed extensive accumulation of collagen fibers in between acinar cells and around ducts. In group3 (Duloxetine+RA) showed that the ducts were surrounded with a modest number of collagen fibers. Moreover in group 4 (Duloxetine+MSCs), showing minimal collagen fibers in between the serous acini and around the ducts. (Figure 6)

Caspase-3 Immuno-histochemical staining:

In group1 (control), parotid sections showing negative Caspase-3 Immuno-histochemical staining is observed within the serous acini and ducts, and in group 2 (Duloxetin) showing strong

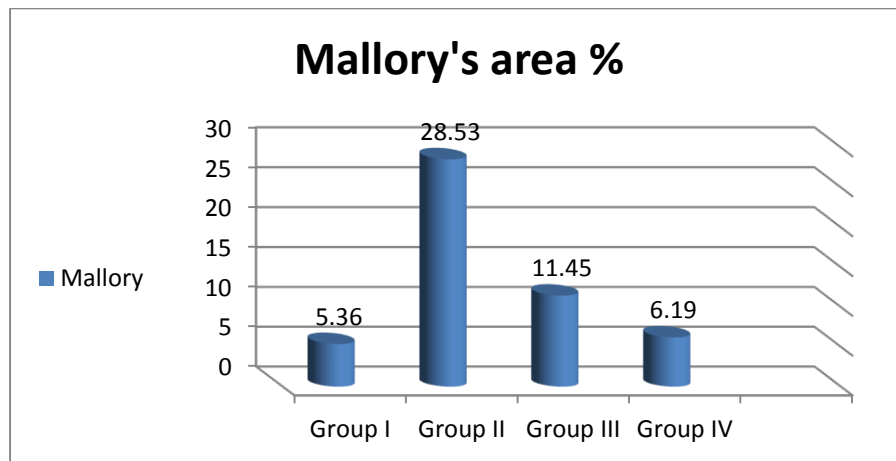
positive Caspase-3 Immuno-histochemical staining is observed within the acinar cells and ducts. Moreover, Group 3 (Duloxetine+RA) showing weak Caspase-3 Immuno-histochemical staining can be seen within the acinar cells. While in Group 4 (Duloxetine+MSCs) showing negative Caspase-3 Immuno-histochemical staining is observed within the acinar cells and ducts. (Figure 7)

Morphometric results:

The morphometric results of both control negative & positive (1a&1b) groups revealed similar results. Thus, we choose control negative (group 1a) as a representative group for the control groups in the morphometric results.

Table (2) showing mean values of area percent of Mallory's trichrom stain \pm SD in the 4 groups using one way anova with Tukey's tests.

Mean % \pm SD	Group 1 (control)	Group 2 (Duloxetine)	Group 3 (Duloxetine+RA)	Group 4 (Duloxetine+MSCs)
Mallory %	5.36 \pm 1	28.53 \pm 4.9	11.45 \pm 3.1	6.19 \pm 0.95
Significance P \leq 0.05	With group 2	With groups 1, 3 & 4	With group 2	With group 2



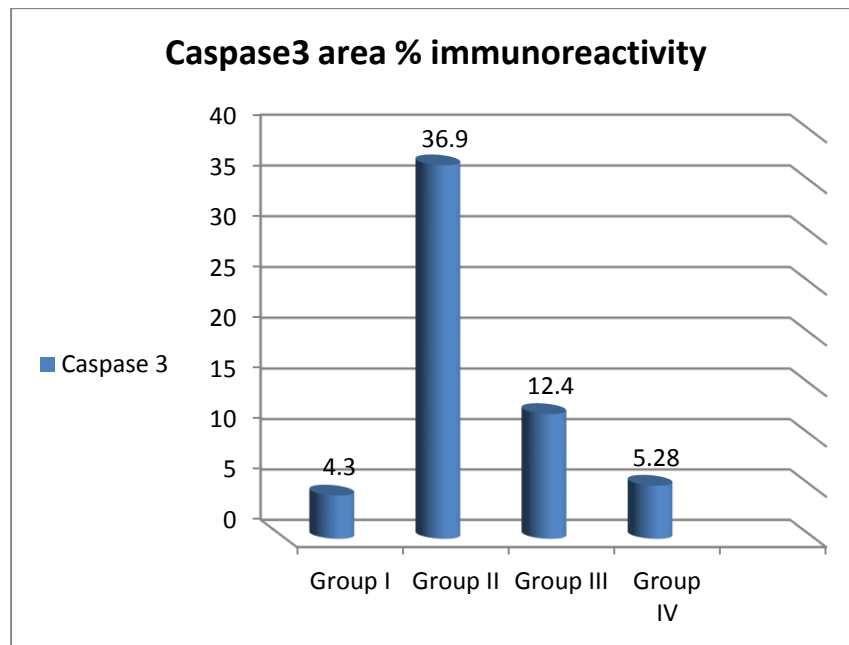
Histogram (I) showing mean values of area percent of Mallory's trichrome stain in the four groups.

Table (3) and histogram (II) Showed the mean area % of caspase-3 expression in parotid gland sections demonstrated in Table (3) and histogram (II). Group 2 (Duloxetine) demonstrated a significant increase in caspase-3 immuno-positive expression and collagen fiber deposition compared to group1 (control). Compared to group2 (Duloxetine) caspase-3

immunopositive expression and the % of deposition of collagen fiber decreased significantly in group 3 (Duloxetine+RA) and group 4 (duloxetine+MSCs). While there was a non-significant decrease in caspase-3 immuno-positive expression and main area percent of collagen fiber deposition in group 4 (Duloxetine+RA) compared to group 3 (Duloxetine+MSCs)

Table (3) showing mean values of area percent of Caspase3 immunoreactivity \pm SD in the four groups using one way anova with Tukey's tests.

Mean % \pm SD	Group 1 (control)	Group 2 (Duloxetine)	Group 3 (Duloxetine+R A)	Group 4 (Duloxetine+MSC s)
Caspase 3 %	4.3 \pm 1.8	36.9 \pm 10.8	12.4 \pm 1.1	5.28 \pm 1.4
Significance P \leq 0.05	With group 2	With groups 1, 3 & 4	With group 2	With group 2



Histogram (II) showing mean values of area percent of Caspase3 Immunoreactivity in the 4 groups.

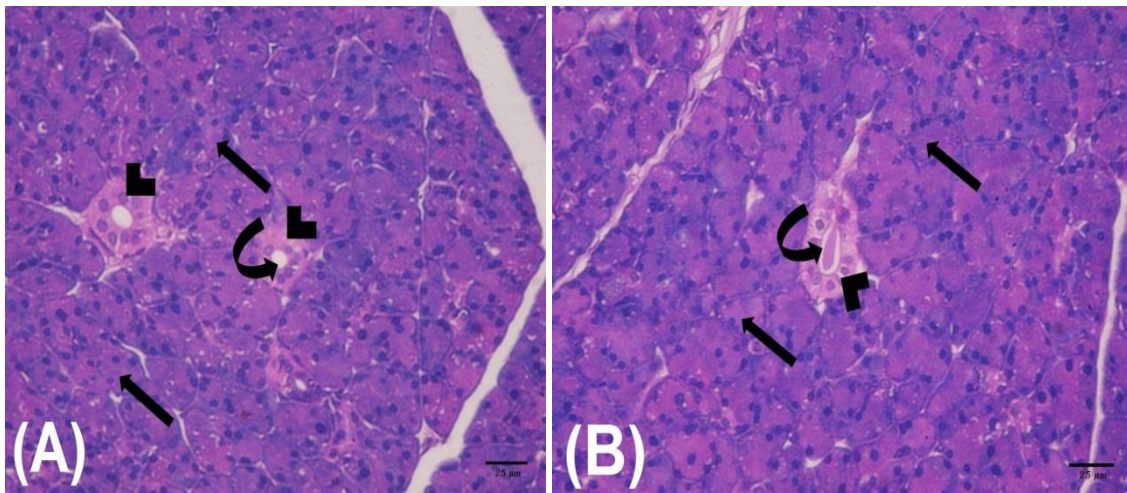
Electron microscopic results:

Ultrastructure of parotid gland in Group1(control) revealed pyramidal shape acinar cells, their nuclei present at basal side, normal shaped mitochondria, rough endoplasmic reticulum (RER) and the secretory granules show homogeneous high electron dense, with intact junctional complex between the cells can be observed. (Figure 8)

Ultrastructure of parotid gland group2 (Duloxetine) showed irregular and indentation of nuclei of serous acinar cells, decrease of secretory granules in distorted cells with dilated RER dilated smooth endoplasmic reticulum, less electron dense nucleus, partial lysis of cristae of mitochondria, rarified cytoplasm can be observed (Figure 9)

Ultrastructure of parotid gland in Group3 (Duloxetine+RA) showing serous acinar cells have mild irregular euchromatic nuclei, swollen degenerated mitochondria, normal RER, and numerous electron dense secretory granules can be notice (Figure 10).

Ultrastructure of parotid gland group4 (Duloxetine+MSCs) showing serous acinar cells. The serous acinar cells have large regular euchromatic nuclei, extensive regular parallel cisternae of RER, nearly normal mitochondria and numerous electron dense secretory granules can be seen. The lateral borders of acinar cells are closely interdigitated, junctional complex is observed between the adjacent cells (Figure 11)



Figure(2): A photomicrograph of a section in parotid gland of Group1 (control): (A) Showing nearly normal acini composed of serous cells (arrow), striated excretory duct lined by cuboidal epithelium (arrowhead) and striated duct lumen (curved arrow) can be observed. (B) Showing more or less normal acini composed of serous cells (arrow), striated excretory duct lined by cuboidal epithelium (arrowhead) and striated duct lumen (curved arrow) can be observed (H&E $\times 400$).

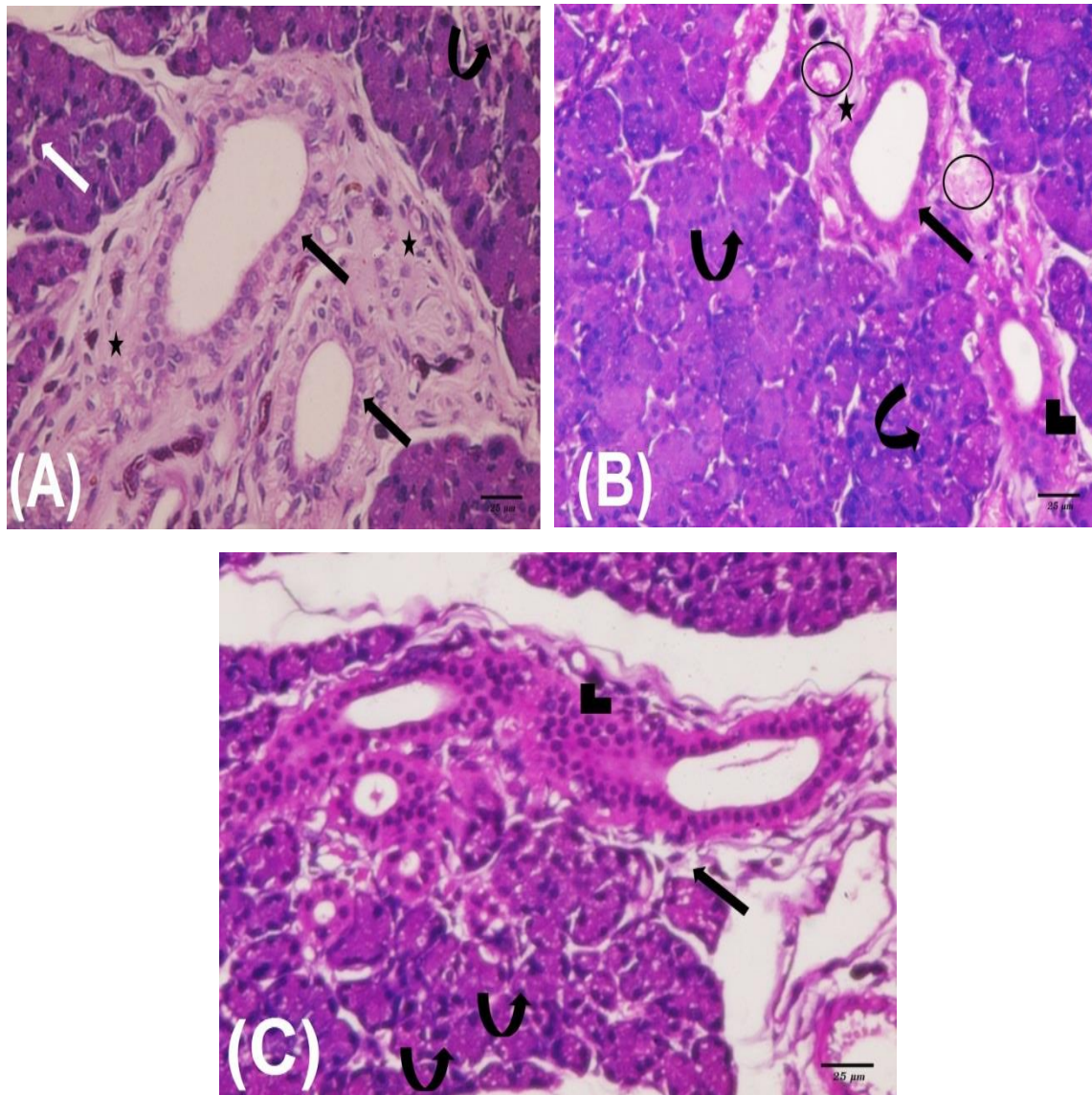


Figure (3): A photomicrograph of a section in parotid gland of Group2 (duloxetine group) : (A) Showing massive infiltration of collagen (astric) around ducts (black arrow), mononuclear inflammatory cells infiltration (curved arrow) can be observed in between serous acinar cells. Note, pyknosis of some nucleus of serous cells (white arrow) can be seen. (B) Showing infiltration with collagen (astric) around ducts (arrow), blood vessels were congested (circle), abnormal division in cuboidal epithelium which lined striated excretory duct (arrowhead). Note, pyknosis of some nucleus of serous cells (curved arrow) can be seen. (C) Showing massive abnormal division in cuboidal epithelium which lined striated excretory duct (arrowhead), degeneration of interlobular connective tissue (arrow), distortion and vacuolation of serous cells (curved arrow). (A, B, C are H&E $\times 400$)

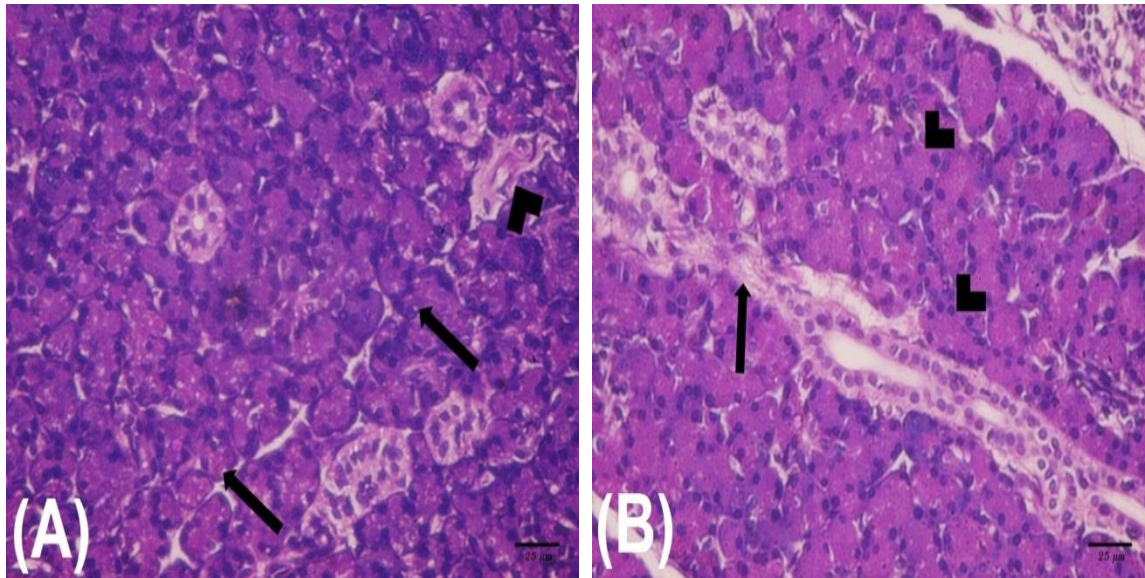


Figure (4): A photomicrograph of a section in parotid gland of Group3 (Duloxetine +RA) :(A) showing small vacuoles in serous cells (arrow), focal area of collagen fibers (arrowhead) between the serous acini can be observed. (B) Showing slightly fibrosis around the ducts (arrow), nearly normal serous acinar cells (arrowhead). (H&E $\times 400$)

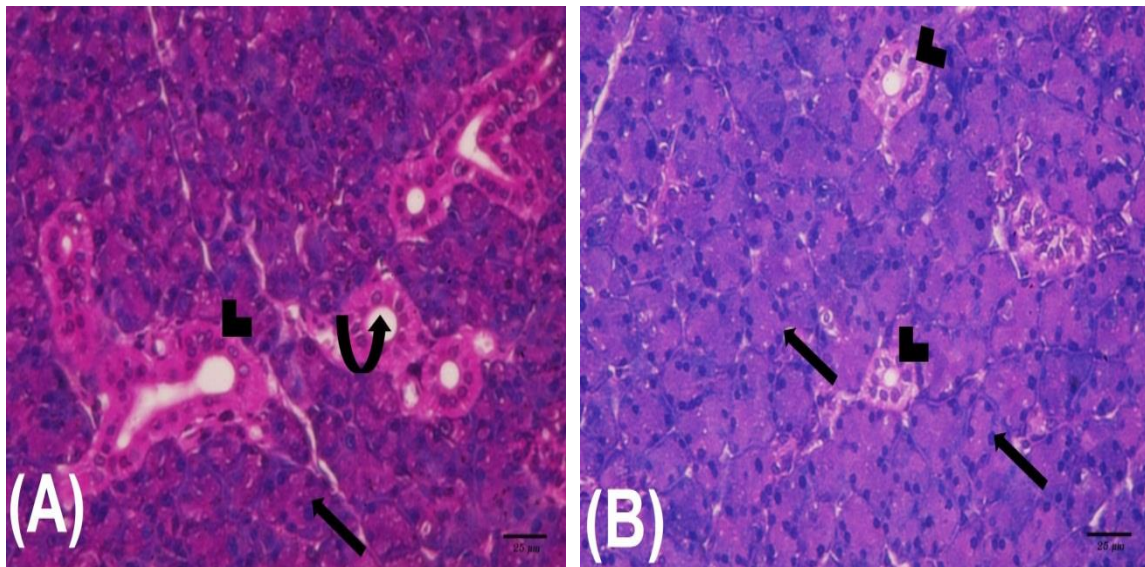


Figure 5: A photomicrograph of a section in parotid gland of Group4 (duloxetine + MSCs): (A) Showing most probably normal architecture of parotid tissue with normal serous acinar cells (arrow) and normal striated excretory duct (arrowhead), lumen of excretory duct (curved arrow) can be seen. (B) Showing most probably normal serous acinar cells (arrow) and nearly normal striated excretory duct lined by cuboidal epithelium (arrowhead) can be observed (H&E $\times 400$).

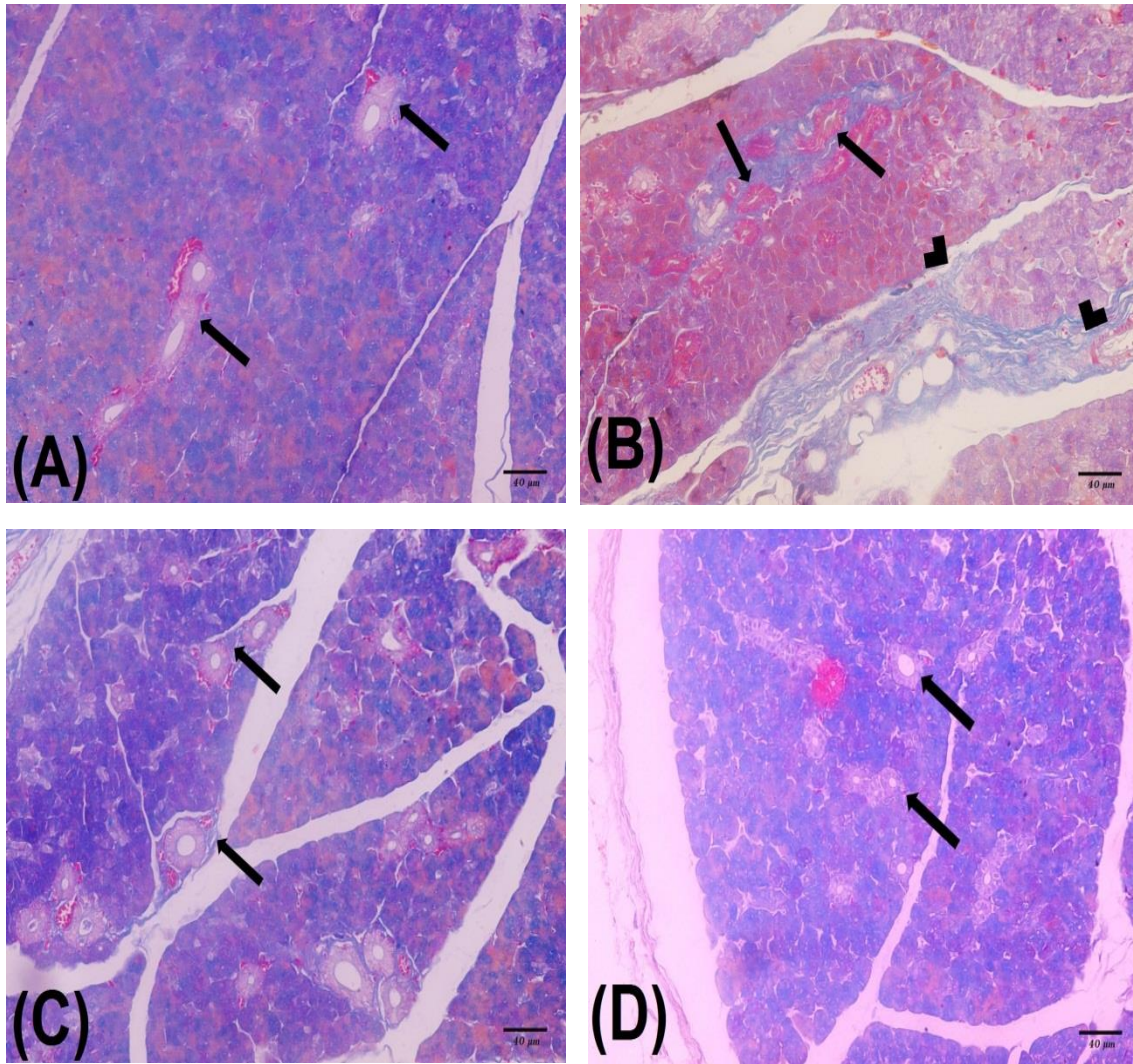


Figure 6: A Photomicrograph of Mallory's Trichrome stained parotid sections: Figure 6 (A) Section from Group1 (control) showing minimal collagen fibers (arrow) around the ducts and no collagen fibers in-between the serous acini, Figure 6 (B) section from duloxetine group2 (Duloxetine) showing extensive accumulation of collagen fibers (arrow) around ducts and in between acinar cells (arrowhead). Figure 6 (C) section from Group3 (Duloxetine +RA) showing moderate amount of collagen fibers (arrow) around the ducts. And Figure 6(D) section from group4 Group4 (duloxetine + MSCs) showing minimal collagen fibers (arrow) around the ducts and in between the serous acini. (A, B, C and D are Mallory's Trichrome x 200)

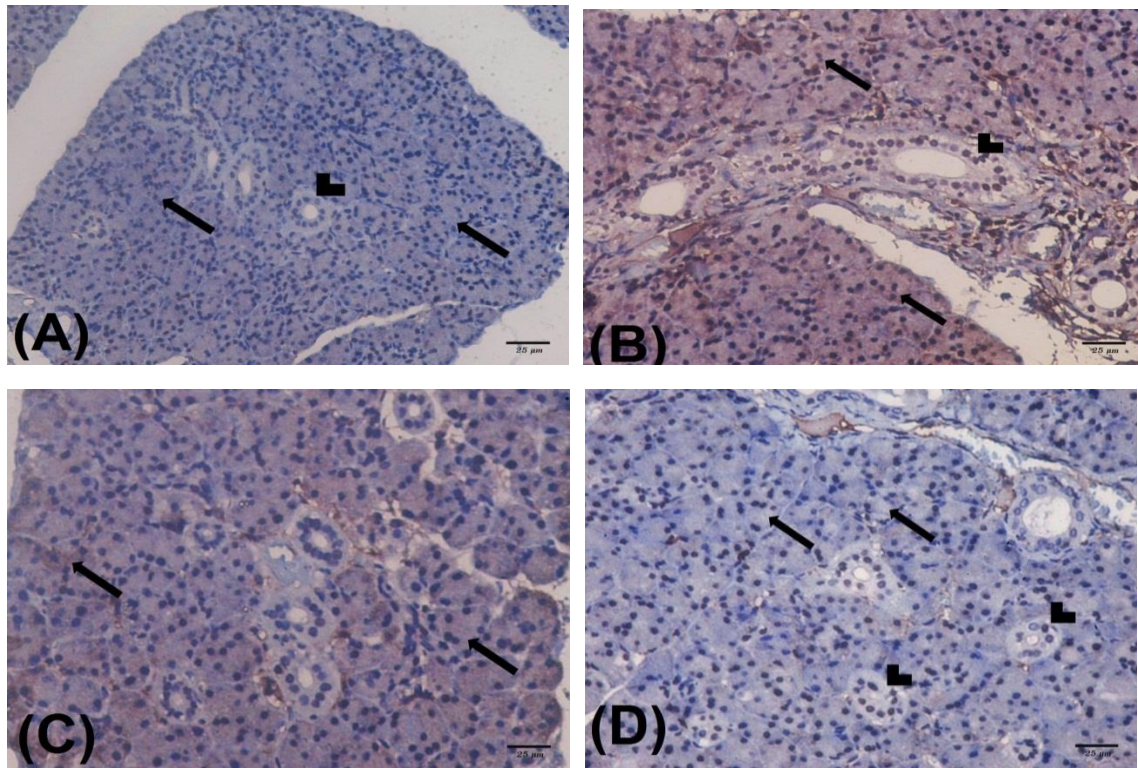


Figure (7): A Photomicrograph of: Figure 7 (A) a section in the parotide of Group1(control) control group showing negative Caspase-3 Immuno-histochemical staining is observed within the serous acini (arrow) and ducts (arrowhead). Figure 7 (B) a section in the parotid of duloxetine group, group2 (Duloxetine) rat showing strong positive Caspase-3 Immuno-histochemical staining is observed within the acinar cells (arrow) and ducts (arrowhead). Figure 7 (C) a section in the parotid of group 3 (Duloxetine +RA) rat showing weak Caspase-3 Immuno-histochemical staining can be seen within the acinar cells (arrow) and ducts. Figure 7 (D) a section in the parotid of group4 , (duloxetine + MSCs) rat showing negative Caspase-3 Immuno-histochemical staining is observed within the acinar cells (arrow) and ducts (arrowhead). (A, B, C and D Caspase-3 x400).

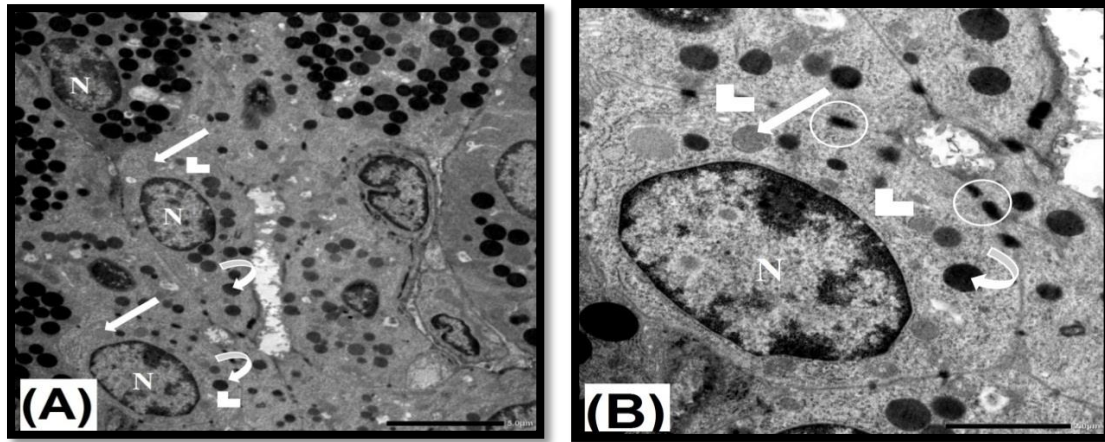
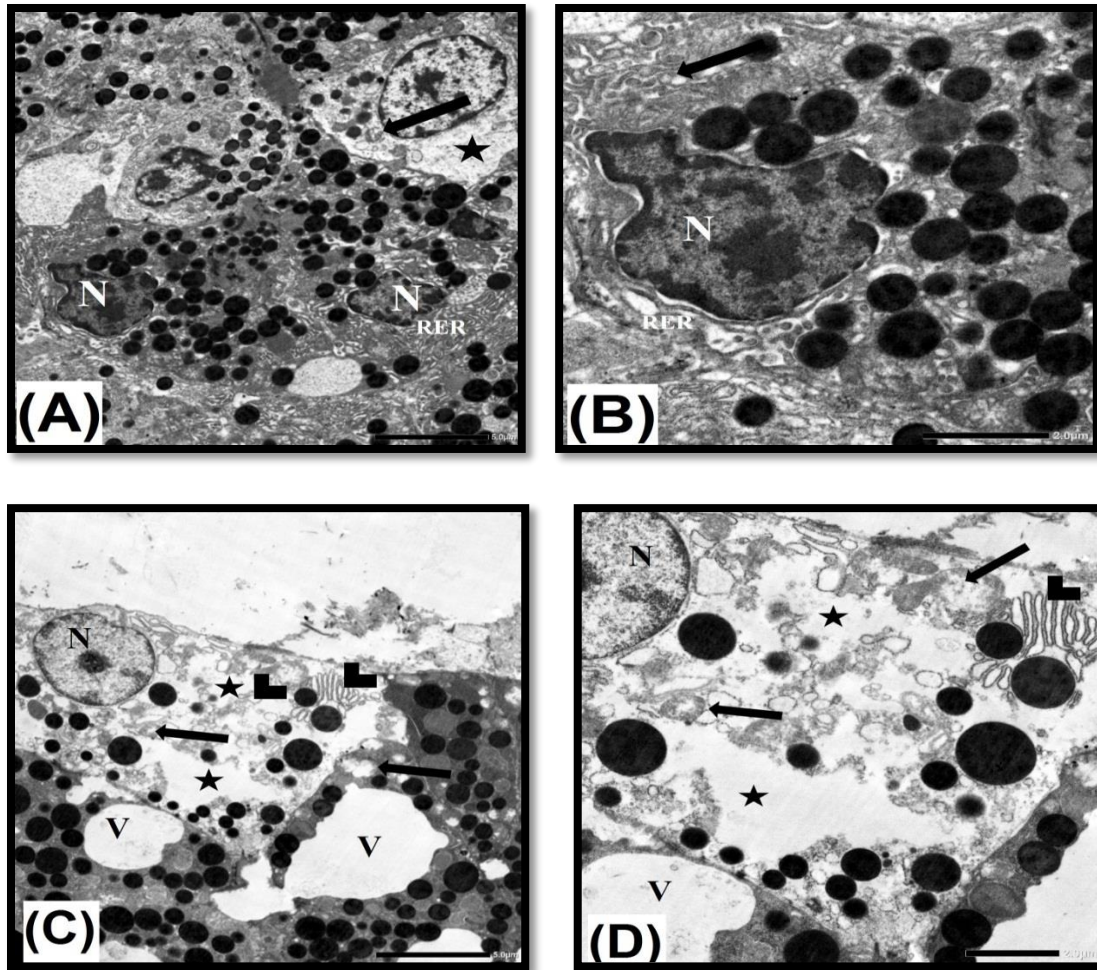


Figure 8: Ultrastructure of parotid gland in Group1 (control): Figure 8 (A) Showing pyramidal shape acinar cells, their nuclei exist at basal side (N), mitochondria (arrow), RER (arrowhead). The secretory granules show homogeneous high electron dense (curved arrow). (TEM x 8000). Figure 8 (B) showing nearly normal acinar cell, their nucleus exists at basal side (N), mitochondria (arrow), rough endoplasmic reticulum (arrowhead). The secretory granules show homogeneous high electron dense (curved arrow). Intact junctional complex between the cells can be observed (circle). (TEM x 20000).



Figure(9): Ultrastructure of parotid gland in Duloxetine group, Group2: Figure 9 (A) Showing irregular and indented of nuclei of serous acinar cells with dilated RER, less electron dense nucleus (N), partial lysis of cristae of mitochondria (arrow), rarified cytoplasm (astric) can be observed. (TEM x 8000). Figure 9 (B) Showing irregular and indented of nuclei of serous acinar cells with dilated rough endoplasmic reticulum (RER), less electron dense nucleus (N), dilated smooth endoplasmic reticulum (arrow) can be observed. (TEM x 20000). Figure 9 (C) Showing distortion of serous acinar cells with dilated and damaged rough endoplasmic reticulum (arrowhead), less electron dense nucleus (N), swollen degenerated mitochondria (arrow), decrease of secretory granules in distorted cell and rarified cytoplasm (astric) can be noticed. Notice, large vacuoles (V) can be observed in serous cells. (TEM x 8000). Figure 9 (D) showing distortion of serous acinar cells with dilated and damaged rough endoplasmic reticulum (arrowhead), less electron dense nucleus (N), swollen degenerated mitochondria (arrow), decrease of secretory granules in distorted cell and rarified cytoplasm (astric) can be noticed. (TEM x 20000).

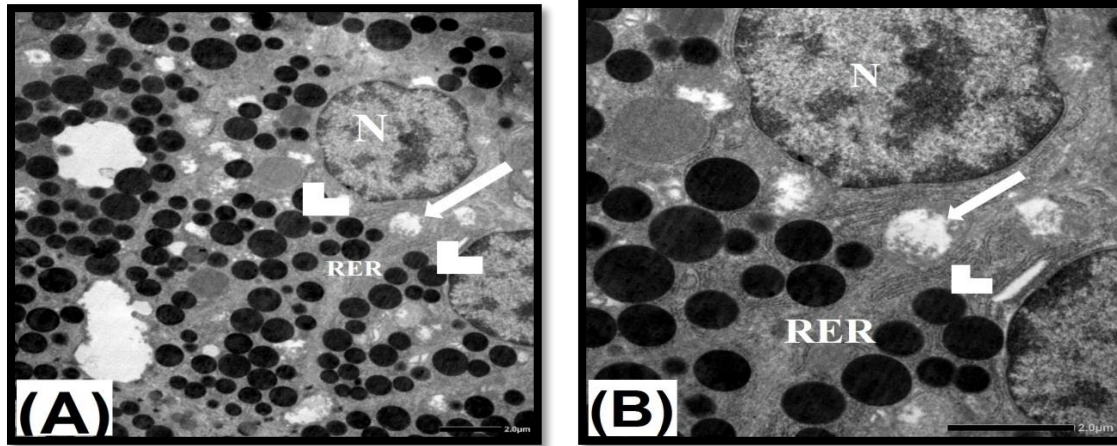


Figure (10): Ultrastructure of parotid gland in Group 3(Duloxetine +RA): Figure 10 (A) showing serous acinar cells have mild irregular euchromatic nuclei (N), swollen degenerated mitochondria (arrow), normal RER, and numerous electron dense secretory granules (arrowhead) can be noticed. (TEM x 8000). Figure 10 (B) showing serous acinar cells have mild irregular euchromatic nuclei (N), swollen degenerated mitochondria (arrow), normal rough endoplasmic reticulum (RER), and numerous electron dense secretory granules (arrowhead) can be noticed. (TEM x 20000).

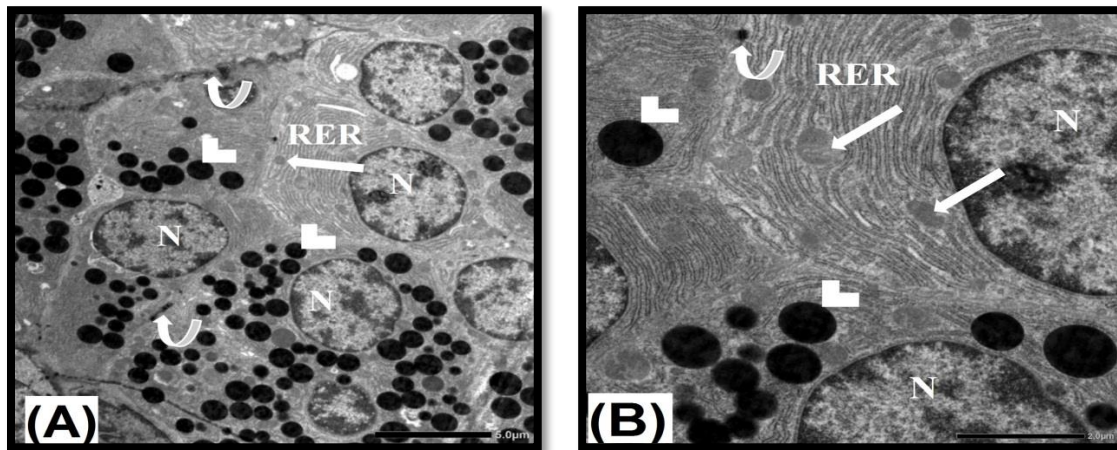


Figure (11): Ultrastructure of parotid gland in Group4 (duloxetine + MSCs) : Figure 11 (A) showing serous acinar cells. The serous acinar cells have large regular euchromatic nuclei (N), extensive regular parallel cisternae of RER, mitochondria (arrow) and numerous electron dense secretory granules (arrowhead) can be seen. The lateral borders of acinar cells are closely interdigitated, junctional complex is observed between the adjacent cells (curved arrow). (TEM x 8000). Figure 11 (B) showing serous acinar cells. The serous acinar cells have large regular euchromatic nuclei (N), extensive regular parallel cisternae of rough endoplasmic reticulum (RER), mitochondria (arrow) and numerous electron dense secretory granules (arrowhead) can be seen. The lateral borders of acinar cells are closely interdigitated, junctional complex is observed between the

adjacent cells (curved arrow). (TEM x 20000).

DISCUSSION:

Many studies have reported that drugs acting on central nervous system (CNS) have pharmacological actions on the salivary glands. The efforts to interpret the xerostomic effects of these medications have been of great scientific value, revealing their physio-pharmacological effects and providing additional benefits to patients receiving these treatments. Described as being SNRI antidepressants, the duloxetine is used in pain, depression, and anxiety treatment. That is why it is one of the most commonly used medications in adults (Kumar et al., 2014).

The histological results of the current research revealed that long term intake of duloxetine resulted in marked atrophic and degenerative changes in the parotid gland of adult albino rat. Light microscopic examination showed disorganized acini with disappearance of their lumens. Most nuclei of the acinar cells at the peripheral and pyknotic or darkly stained are observed. These acini are widely separated with connective tissue fibers and hemorrhage, dilated ducts with retention of secretion are also observed. Our finding in agreement with (Youssef et al., 2020) who described a similar picture of marked atrophic and degenerative changes in the lingual salivary glands in the adult albino rats.

Mitochondria are the house of power in eukaryotic cells, and they also regulator of cell function by their role in oxidative stress and apoptosis. The results of the current study were

consistent with studies done by Youssef, (2017), Souza et al.(1994) and Elmorsy et al. (2017) who demonstrated that fluoxetine has harmful effect on the histological structure of the pancreatic and hepatic cells. They come backed this toxic effect to the mitochondria and stated that mitochondrial damage result in reduction of ATP with successive biosynthesis failure, maintenance of the cytoskeleton, signaling and ion transport, membrane pumps, oxidative stress and induction of apoptosis. Consequently, in our opinion the marked degenerative changes, cystic transformations and collection of stagnant secretions in the acini and ducts of the lingual glands are caused by ATP reduction and depletion of cellular energy used for transport of secretions.

The balanced production of ROS (reactive oxygen species) and its neutralization in healthy humans was achieved by the production of antioxidant defense, which was primarily caused by the defense mechanisms of the cells via the formation of antioxidants and antioxidant enzymes, which provide protection against oxidative damage to cells and tissues. In long term administration of duloxetine there is deviations in the antioxidant controlling process in many body tissues (Townsend et al., 2003), RA is a known natural antioxidant that protects against duloxetine's harmful side effects.

In the present study we found that RA group had nearly normal serous acinar cells and slightly fibrosis around the ducts, this was in agreement with Zhang et al. (2020) who found that RA reduced the radiation induced toxic damage on the parotid gland of adult

albino rat, and resulted in a marked decrease acinar atrophy, less luminal dilation, less vacuolization, increase in acinar number, less interstitial fibrosis, and decrease capillary congestion. The glandular lobule structure got close to be normal.

A substantial number of cytoplasmic vacuoles with shrunken nuclei and loss of acinar outlines were found in the parotid salivary glands in the current study as a result of Duloxetine therapy. After I.V injection of BMSCs, the parotid gland showed a more regular glandular architecture, In accordance with the findings of Khalili et al.,(2012) Stem cells have anti-inflammatory and immunostimulatory properties, according to their research on the treatment of NOD (non-obese diabetic) mice with Sjogren's-like disease employing multipotent MSCs as a therapy for autoimmune diseases (Khalili et al., 2012). BMSCs can be collected by a needle aspiration and divided into a large number of multipotent MSCs. Numerous studies were undertaken to examine the MSCs efficacy as a novel treatment for autoimmune diseases, as rheumatoid arthritis, systemic lupus erythematosus and type I diabetes (Fotino et al., 2010).

The Current explanations demonstrated an appropriate homeostatic non-inflammatory translocation of BMSCs into the parotid glands, hence reducing parotid gland morphological alterations. This experimental transfer occurred rather quickly and was observable within the 1st week. MSCs recruited from bone marrow appeared to be primarily responsible for the improved performance of salivary gland tissues via cytokine-mediated interactions with tissue stem cells

(Lombaert et al., 2006).

In the present study we found that In Group 2(Duloxetine), the MDA level was significantly higher compared to the control group , whereas it was significantly lower in Group3 (Duloxetine+RA) and Group 4 (Duloxetine+MSCs) compared to Group2 (duloxetine) .GSH-Px activity was significantly lower in Group 2(duloxetine) compared to the control group , but it was significantly higher in Group 3(Duloxetine+RA) and Group 4 (Duloxetine+MSCs) compared to Group 2(Duloxetine) .This was in agreement with Zhang et al. (2020) who found that Rosmarinic acid treatment significantly attenuated ROS through a direct and the indirect activation of PGC1- α /NOX4 signaling. Moreover, RA reduced cell apoptosis and suppressed fibrosis by downregulating inflammatory factor levels in the parotid glands.

Conclusion: BMSCs were an effective therapy for harmful effects of Duloxetine on salivary glands. Significant non-inflammatory and homeostatic MSCs migration from the bone marrow to the salivary glands increases saliva output and maintains glandular architecture.

In contrast, the current study reveals that the duloxetine-induced increase in ROS generation plays a crucial role in the rat parotid glands pathophysiology. ROS was greatly reduced by RA's direct antioxidative action. Moreover, RA reduced cell apoptosis and suppressed fibrosis in the parotid glands. Therefore, RA has a greater potential for parotid gland injuries treatment than BMSCs, although not to the same degree.

Conflicts of Interest: The authors declare no conflict interest.

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دراسه مقارنه بين الدور الوقائي لحمض الروزمارينيك وتأثير الخلايا الجذعيه الوسيطة في تقليل التأثير السام لعقار الدوليكستين علي الغده النكفيه في ذكور الفئران البيضاء

أمل محمد الصفي الشاذلي 1 ، بدور بيومي 1 ، نعمه محمود طه 2 ، أسماء يوسف حسين 3
وياسمين محمد اسماعيل السيد 4 واسامة فؤاد

1 قسم التشريح والأجنة بكلية الطب جامعة بنها. مصر. 2 قسم علم وظائف الأعضاء ، جامعة أم القرى ، المملكة العربية السعودية. 3- قسم الطب الشرعي والسموم السريرية بكلية الطب جامعة بنها. مصر. 4 قسم الصيدلة بكلية الطب جامعة بنها. مصر

الخلفية: ديلوكسيتين هو دواء مضاد للاكتئاب يستخدم لعلاج اضطرابات القلق واضطرابات الاكتئاب. هدفت هذه الدراسة إلى مقارنة تأثير حمض الروزمارينيك (RA) على الغدة النكفية كمضاد طبيعي للأكسدة مع التأثيرات العلاجية المحتملة للخلايا الجذعية الوسيطة في علاج التأثير السام لـ عقار الديلوكسيتين على الغدة النكفية. **المواد والطرق:** تم تصنيف ستون جرذاً من الذكور إلى أربع مجموعات ، خمسة عشر جرذاً في كل مجموعة. المجموعة الأولى: المجموعة الضابطة لا تتلقى أي علاج ، المجموعة الثانية: تم تلقيها 10 مجم / كجم / يوم دولوكستين لمدة اثني عشر أسبوعاً ، المجموعة الثالثة: تم تلقي عقار الديلوكسيتين مثل المجموعة الثانية وتلقيها في نفس الوقت مرة واحدة 120 مجم / كجم / يوم من حمض الروزمارينيك لمدة اثني عشر أسبوعاً ، المجموعة الرابعة: تم تلقي عقار الديلوكسيتين مثل المجموعة الثانية ثم حقنة واحدة من الخلايا الجذعية الوسيطة مباشرة بعد إيقاف الديلوكسيتين ، في جميع المجموعات تم التضحية بالحيوانات بعد ستة عشر أسبوعاً من بداية الدراسة. للتقييم النسيجي ، تم صباغه مقاطع الغدة النكفية بالهيموتكسيلين والأيوسين ، وتم استخدام صبغة مالوري ثلاثية الكروم لإظهار ألياف الكولاجين. أيضاً ، تم استخدام مجهر الإرسال الإلكتروني والكيمياء المناعية باستخدام كاسباس 3 لفحص الغدة النكفية.

النتائج: كشف فحص المقاطع النكفية في المجموعة الثانية عن تسلل للخلايا الالتهابية وتشوه كبير وتفريغ للخلايا المصلية ، بينما أظهرت المجموعة الثالثة و المجموعة الرابعة تحسناً ملحوظاً في التركيب النسيجي للغدة النكفية. **الخلاصة:** الخلايا الجذعية الوسيطة المستخلصة من نخاع العظام و حمض الروزمارينيك لهما تأثير جيد في تقليل التأثير السام لإعطاء عقار الديلوكسيتين على الغدة النكفية مع اختلاف طفيف بين الطريقتين.