

The Effect of Stem Cells Exosomes on Circumvallate Papillae of Albino-Rats Subjected to Cyclophosphamide Chemotherapy

Radwa S. Megahed*, Dina M. Abd Elkhalik, Medhat A. Elzainy

Oral Biology Department, Faculty of Dentistry, Ain Shams University, Egypt

*Corresponding author: Radwa S. Megahed, Email: Radwadent@gmail.com, Mobile: (+20) 01019044320

ABSTRACT

Background: There is an increasing attention towards stem cells exosomes and their regenerative potential, bone-marrow derived stem cells exosomes was shown to enhance tissue regeneration. **Objective:** To explore the effect of bone-marrow derived stem cells exosomes on circumvallate papillae subjected to cyclophosphamide chemotherapy.

Methods: Forty-four adult male albino-rats, weighing between 180 and 200grams were used. Two rats were used as a source of bone-marrow stem cells exosomes and the rest were divided equally into 3 groups; 14 rats each as follows: Group I: Control; Group II: Cyclophosphamide group, this group received a single dose (150mg/kg) of cyclophosphamide intraperitoneal; Group III: exosomes group, this group received a single dose (150mg/kg) of cyclophosphamide intraperitoneal then after two days, they received single dose of bone-marrow mesenchymal-stem cells (BM-MSCs)-derived exosomes (100µg) suspended in 1ml phosphate buffered saline intraperitoneal. All groups were sacrificed on day 9 of the experiment. Circumvallate papillae were examined histologically and ultra-structurally. The inflammatory cell counts and percentage of cytoplasmic vacuolation were statistically analyzed.

Results: A significant improvement in the shape and size of cells of circumvallate papillae was noticed in both epithelium and connective tissue compared to the samples subjected to cyclophosphamide only, the ultrastructural examination of the tissues showed significant improvement in all cellular organelles especially nuclei shapes, endoplasmic reticulum and mitochondria. A statistically significant decrease in inflammatory cells and vacuolation percentage was also revealed.

Conclusion: Bone-marrow derived stem cells exosomes could be considered an effective regenerative treatment for cyclophosphamide induced circumvallate papillae damage.

Keywords: Bone-marrow stem cells exosomes; Cyclophosphamide; Circumvallate papilla.

INTRODUCTION

Mesenchymal Stem cells (MSCs) are immature cells isolated from various tissues with apparent regeneration capacity which makes them useful in the treatment of several diseases in various parts of the body such as bone, blood and neurological diseases. Bone-marrow (BM) derived stem cells⁽¹⁾. BM-MSCs are a type of mesenchymal stem cells that are isolated from bone-marrow. Its abundance and relatively easy extraction make them included in many treatments to induce regeneration⁽²⁾.

Stem cell exosomes were first discovered in the early 1980s. they were defined as nano scale extracellular lipid bilayer vesicles that are secreted normally by many cells. They are intraluminal vesicles formed by the inward

budding of the endosomal membranes during the maturation of multivesicular endosome. They have role in a wide range of physiological and pathological processes. Because exosomes have the capability to carry molecular cargos and transfer bioactive components, more studies are published recently on exosome-based disease diagnosis and therapeutics⁽³⁾.

Mesenchymal stem cells or their derivative exosomes could increase Brain-Derived Neurotrophic Factor (BDNF) expression which is an important growth factor for the proliferation and differentiation of epithelial basal progenitor cells into taste bud cells⁽⁴⁾. Cyclophosphamide (CTX) is an important antineoplastic drug with immunosuppressive action used for treatment of many

types of cancers and immunologically mediated diseases. Its cytotoxic action, although effective on cancer cells, leads to side effects due to damage of other normally functioning body cells. The main oral side effects include glossitis, loss of taste, oral ulcers and bleeding tendency⁽⁵⁾.

Effects of exosomes on damaged tissues were studied in some papers, however, most of them focus on body parts other than oral tissues, with different damage induction agents such as diabetes or local wounds. In this study the effect of exosomes is experimented on the circumvallate papillae in tongues of albino-rats damaged by chemotherapeutic drug cyclophosphamide.

MATERIAL AND METHODS

This animal-study included 44 male albino-rats weighing between 150 and 200grams housed in the Medical Research Centre, Faculty of Medicine, Ain-Shams University, under controlled temperature and dark-light cycle, five animals per/cage. Control of pain was by using analgesics (Ketamine) 0.2ml/rat. They were fed standardized diet and tap water was available *ad libitum*. The animals were acclimatized for a week then 2 rats were sacrificed to obtain the BM-MSCs and 42 rats divided equally into 3 groups each included 14 rats:

- **Group I (Control group):** Served as control group and received no treatment.

- **Group II (Cyclophosphamide group):** Treated with Cyclophosphamide in the form of a single dose of intraperitoneal (IP) injection 150mg/kg, then left for 9 days before scarification⁽⁶⁾.

- **Group III (Cyclophosphamide/Exosomes group):** Received a single dose of IP injection of a dose of 150mg/kg of CTX, then after 48 hours, they received a single dose of BM-MSC-derived exosomes 100µg IP suspended in 1ml phosphate buffered saline, then left for 7 days⁽⁷⁾.

Drugs

BM-MSCs were harvested from the bone marrows of two male albino-rats 10 weeks old at the Medical Global laboratory, Cairo, Egypt. Exosomes were obtained from supernatants of the third passage of BM-MSCs (5×10⁶ cells/ml) supplemented with 0.5% of bovine serum

albumin. The total exosome was isolated from culture media using the total exosome isolation from cell culture media, cat no.: 4478359, Invitrogen, Life Technologies, USA. As a preliminary step, the cells were cultured in exosome depleted fetal bovine serum (FBS).

The cell was harvested from culture media, then the culture media was centrifuged at 2000xg to remove the cell debris, and the supernatant of the media was transferred to a new collection falcon tube without disturbing the cell pellet. In order to isolate the exosomes, a 5mL of cell free culture media was added to 5mL of total exosome reagent, mixed well by vortex until the mixture become a homogenous solution, and the mixture is at 2°C-8°C overnight. On the next day, the samples undergo centrifugation at 10,000 × g for 1 hour at 2°C-8°C, followed by aspiration and discard of the supernatant. At this step, the exosomes are present in a pellet at the bottom of the tube, which are not visible in most cases. Finally, the pellet containing the exosomes was resuspended in 1mL of 1X PBS. The isolated exosomes were stored at -20°C until used. Cyclophosphamide drug was purchased from “Sigma Aldrich” under the trade name of “Cytoxan” in the form of powder to be mixed with saline then injected IP.

Tissue-extraction preparation

At the end of the experimental period, the rats were sacrificed by overdose of anesthesia (Ketamine). After sacrificing the rats, the tongues were excised free. The circumvallate papilla (CVP) is found most posteriorly in the middle of the anterior two-thirds of the tongue, the location of the CVP in the tongue was identified, and then the tissue specimen was isolated by two oblique cuts posteriorly and a cut anterior to the papilla ⁽⁸⁾.

After separating the circumvallate papillae, half the specimens were fixed in 10% buffered formaldehyde for 48h. The other half of the specimens were fixed for 1h in buffered Glutaraldehyde (2.5%) at 4°C temperature followed by 2hs in osmium tetroxide (1%).

Specimens were then taken for subsequent processing for histological examination. The Oral Pathology Department at Ain Shams University produced paraffin blocks for the light microscope tissue samples,

collected various sections, stained slides with hematoxylin and eosin (H&E) stains, and examined the findings under a light microscope⁽⁹⁾. The Electron Microscopy Unit at Ain Shams University produced resin blocks for the tissue samples, collected various sections, stained slides, and examined the findings under a transmission electron microscope⁽¹⁰⁾.

Statistical analysis:

The data were statistically analyzed using statistical package for social sciences version-20 (SPSS, Inc., Chicago, USA). For quantitative data; Mean (M) \pm Standard deviation (SD) were calculated and one way analysis of variance (ANOVA) test was used to define the significance between ≥ 3 groups; significance was accepted at $P < 0.05$. Also, t-test was used to define the significance between each 2 groups.

Ethical approval:

This study was reviewed and approved by Institution Guidelines of Ain-Shams University Ethical Committee with authorization number "937".

RESULTS

Light Microscope (H&E)

Control group (Group I):

The examined (H&E) stained sections of the circumvallate papillae of control rats showed apparently normal epithelium and taste buds, the inverted cone shape of the circumvallate papillae was present, connective tissue was infiltrated by normal vasculature (**Fig. 1, a**).

Cyclophosphamide group (Group II):

The examined (H&E) stained sections of the circumvallate papillae of cyclophosphamide group showed apparently non uniform thickness of epithelium and ill-defined shrunken taste buds, connective tissue was infiltrated by inflammatory cells. The inverted cone shape of the circumvallate papillae was present. Size, number and shape of taste buds along the epithelium at the sides of the circumvallate papilla within the trough were apparently reduced (**Fig. 1, b**).

Cyclophosphamide/Exosomes group (Group III):

The examined (H&E) stained section of this group

showed apparently normal epithelium thickness and taste buds. The inverted cone shape of the circumvallate papillae was present. Less vacuolation in connective tissue and less inflammatory cell infiltrate (**Fig. 1, c**).

Transmission Electron Microscope

Control group (Group I):

Electron microscopic examination of taste buds in circumvallate papilla of control group showed apparently normal form and structure of most organelles. The cytoplasm seemed intact and apparently normal desomsomal attachments were present between cells. Cells in the taste buds showed central large rounded to oval nuclei with apparently normal chromatin. Endoplasmic reticulum (ER) was evident and well organized around the nuclei, appearing as intercommunicating parallel stacks of flattened cisternae. Mitochondria of apparently normal shapes appear scattered the cytoplasm. The cell membrane appears intact around the cell (**Fig. 2, a-c**).

Cyclophosphamide group (Group II):

Electron microscopic examination of a taste bud in circumvallate papilla of group II showed irregular form and structure of most organelles. The cytoplasmic membrane showed significant vacuolation and desmosomes were starting to detach between cells. Cells in the taste buds show pleomorphic nuclei with apparently abnormal chromatin arrangement. Pyknotic cells can be identified. Endoplasmic reticulum showed swollen stacks. Mitochondria appear either swollen, or dark and ill-defined. The cell membrane appears irregular and indefinite (**Fig. 3, a-c**).

Cyclophosphamide/Exosomes group (Group III):

Electron microscope examination of taste bud cells circumvallate papillae of group III showed evidence of improvement of cell form and structure. Cells in the taste buds showed elongated cells with central large rounded to oval nuclei with apparently normal chromatin. Endoplasmic Reticulum was better organized around the nuclei. Mitochondria showed apparently regular form. The cytoplasm showed less vacuolation and desmosomes were restored among cells (**Fig. 4, a-c**).

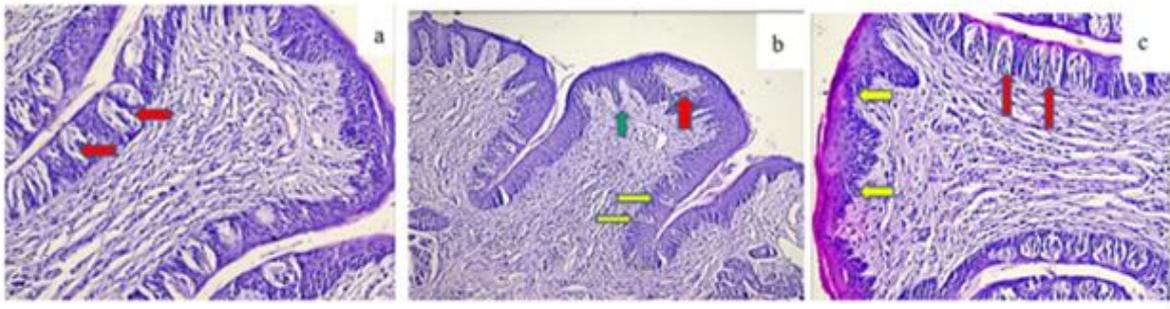


Fig. 1: **a)** A photomicrograph of circumvallate papilla of control group showing apparently normal epithelium and taste buds shapes (red arrows), connective tissue infiltrated by normal vasculature (H&E, org. magx, 200). **b)** A photomicrograph of circumvallate papilla of group II disorganized epithelium with irregular thickness (red arrow) and disturbed basal cell layer (green arrow), ill-defined taste buds (yellow arrow) (H&E, org.magx100). **c)** A photomicrograph of circumvallate papilla of group III showing apparently normal epithelium and intact basal cell layer (yellow arrows), many taste buds resembling normal shapes (red arrows) (H&E, org.magx200).

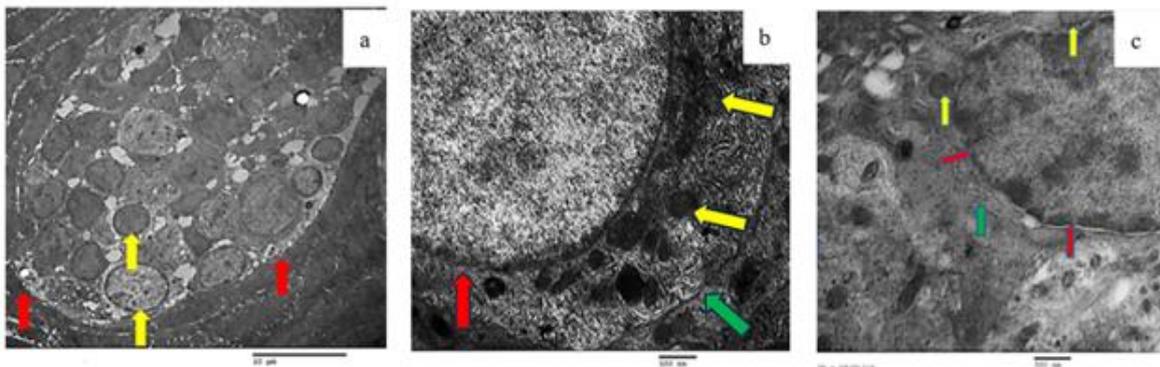


Fig.2: Electron micrograph of circumvallate papilla of control group showing **a)** section in a taste bud with definite outline (red arrows); nuclei of different taste cells look regular in shape with definite envelope (yellow arrows) (x750). **b)** Nucleus of a taste cell regular in shape with definite envelope (red arrow), mitochondria of apparently normal shapes appear scattered the cytoplasm (yellow arrows), the cell membrane is intact around the cell (green arrow) (x6000). **c)** Nucleus of a taste cell regular in shape with definite envelope and normal nuclear indentation and pores are shown (red arrows). Smooth and rER organized around the nuclear membrane (green arrow), mitochondria of normal shapes appear scattered the cytoplasm (yellow arrows) (x6000).

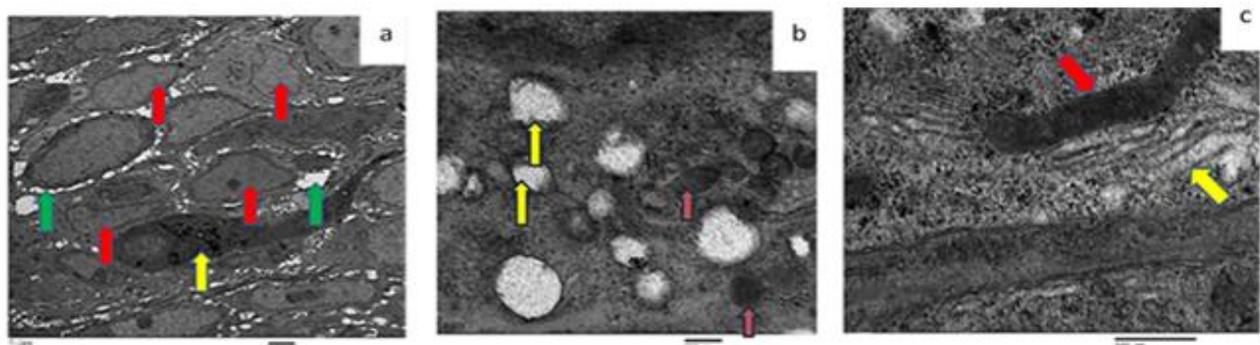


Fig. 3: Electron micrograph of circumvallate papilla of group II showing **a)** pleomorphic nuclei (red arrows), degeneration of cytoplasm and vacuolization (green arrows), and cell pyknosis (yellow arrow) (x1000). **b)** Vacuolated cytoplasm (yellow arrows) and apparently degenerating organelles (red arrows) (x3000). **c)** Swollen irregular ill-defined mitochondria (red arrows) and ill-defined, swollen endoplasmic reticulum (yellow arrows) (x3000).

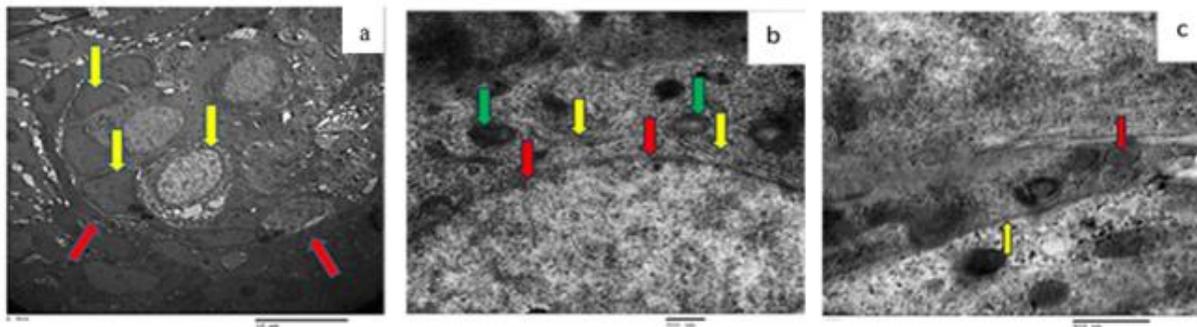


Fig. 4: Electron micrograph of circumvallate papilla of group III showing: **a)** normal taste bud outline (red arrows) and less vacuolation, apparent improvement of nuclei shapes and chromatin content are observed (yellow arrows) (x750). **b)** Apparently definite nuclear envelope and chromatin of a taste cell (red arrows), improved size and shape of mitochondria are observed (green arrows) along with better shapes of strands of Endoplasmic reticulum (yellow arrows) (x6000). **c)** Apparently intact cell membrane of an elongated taste cell (yellow arrow), apparent normal size and shape of mitochondria are observed (red arrow) (x12000).

Statistical Results:

Inflammatory cells count:

The greatest mean of inflammatory cell count was recorded in group II, whereas the lowest value was recorded in control group. One way analysis of variance (ANOVA) test revealed that the difference between all groups was statistically significant ($P < 0.0001$). Tukey’s post hoc revealed significant difference between each 2 groups (Table 1; Fig. 5, a).

Table 1: Counting of inflammatory cells in all groups and significance of the difference.

P.O.C	Control (Group I)	Group II	Group III
Mean	13 ^c	46.6 ^a	23 ^b
Std Dev	2.12	6.8	3.7
Max	16	53	26
Min	11	36	18
F-value	68.9		
P-value	<0.0001*		

*significant at $p < 0.05$

t-test: means sharing the same superscript letter are not

significantly different.

Area percent of stromal vacuolation

The greatest mean of stromal vacuolation was recorded in group II, whereas the lowest value was recorded in control group. ANOVA test show that the difference between all groups was statistically significant ($P < 0.0001$). Significant difference between each 2 groups (Table 2; Fig. 5, b) using t-test was significant.

Table 2: Counting of stromal vacuolation in all groups and significance of the difference.

P.O.C	Control	Group II	Group III
Mean	12.5 ^c	24 ^a	19.1 ^b
Std Dev	2.49	1.97	2.47
Max	16.646	26.438	22
Min	10.881	21.29	15.747
F-value	31.27		
P-value	<0.000017*		

*significant at $p < 0.05$, T-test: means sharing the same superscript letter are not significantly different.

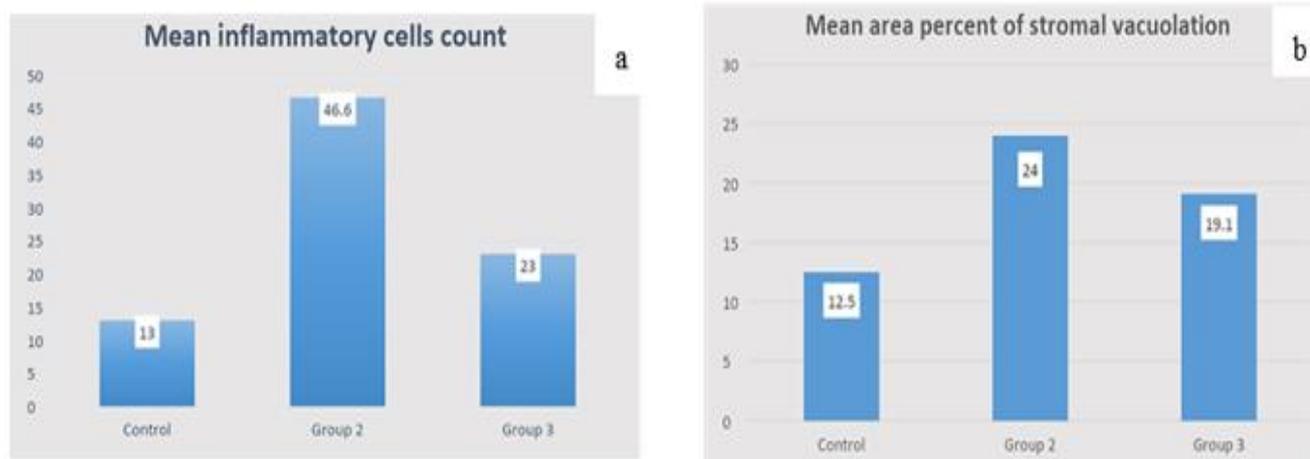


Fig. 5: a) Column chart showing mean count of inflammatory cells in the three groups. **b)** Column chart showing mean area of stromal vacuolation in the three groups.

DISCUSSION

The present study was performed to evaluate the effect of exosomes on circumvallate papillae in tongues of albino-rats affected by cyclophosphamide chemotherapy injection.

Cyclophosphamide apoptotic effect was assumed to be done 48 hours post injection so the treatment with exosomes was given after this interval to ensure the effect has taken place, this was interpreted from **Mukherjee et al.**⁽¹¹⁾ who used caspase-3 marker of apoptosis to track the apoptotic effect of cyclophosphamide. This marker was at its highest levels 12-36 hours after injection then decreased at 48 hours after injection.

BM-MSCs exosomes were used in this study to explore its effect on oral tissues specifically circumvallate papillae because of their emerging use in the dental field, and some researchers found promising regenerative effect on damaged tissues such as **Hue et al.**⁽⁷⁾ and **Hassan et al.**⁽¹²⁾.

In the present study, H&E examination of cyclophosphamide group (group II) showed areas of atrophied epithelium, areas of keratinocyte degeneration and thinning of the epithelial covering. This result agreed with **Al-Refai**⁽¹³⁾. In his study, he injected 40 albino-rats with a single dose of IP CTX (300mg/kg) each to induce mucositis and found marked pathological changes like decrease in epithelium and keratin thickness on the ventral

surface of the tongue mucosa of rats. This observation was also found by **Essawy et al.**⁽¹⁴⁾ who used the same dosage and route on 40 albino-rats which induced mucositis and atrophied and thin filiform papillae and areas with lost epithelial covering after 8 days of administration.

The H&E examination of connective tissue of cyclophosphamide group revealed high inflammatory cell infiltrate and dilated vasculature, this finding is congruent with **Saghir et al.**⁽¹⁵⁾ who observed significant increase in inflammatory cells in lung alveolar tissues after single IP dose of cyclophosphamide. The same finding was also stated in **Al-Refai**⁽¹³⁾ study where severe inflammatory cell infiltrate and dilated blood vessels were observed in the connective tissue of the ventral surface of the tongue in cyclophosphamide injected rats.

The degeneration of cells and vacuolation observed in the connective tissue of cyclo-phosphamide in the present study was also noted by **Ibrahim et al.**⁽¹⁶⁾ who injected 24 rats with daily doses of 20mg/kg of CTX IP, after 4 weeks the hippocampal neurocytes of these rats showed degeneration and necrosis, with significant decrease in cell layers.

In the present study H&E examination of cyclophosphamide group showed degeneration and loss of basal cell layer with areas of basement membrane discontinuity. This finding agreed with **Mukherjee et al.**⁽¹¹⁾ that used immuno-labeling for different cells to observe

the effect of CTX on them. In the mentioned study used IP CTX injection to induce damage on mice tongues, the study showed that pH3 which is a marker for mitotic activity was at its lowest levels at 4 days post injection, suggesting the anti-proliferative activity of CTX that mostly affects the basal cell layer due to its high proliferative rate.

This was proved by examination of the basal cell layer and observing the degeneration and apoptosis the followed CTX injection.

The H&E examination of cyclophosphamide group showed apparent decrease number of taste buds in circumvallate papillae, this can be also explained by **Mukherjee et al.**⁽¹¹⁾ that labeled different taste cells in the taste bud with suitable immuno-markers to track the effect of CTX on them. This study concluded that CTX causes decrease in proliferation of all cell types of taste buds with subsequent decrease in number of taste buds in tongue papillae.

In the present study, the electron microscope examination of CTX group showed pleomorphic nuclei of circumvallate papillae taste buds, this finding agrees with **Ibrahim et al.**⁽¹⁶⁾ which stated the same observation in hippocampal tissue of rats receiving cyclophosphamide in his experiment mentioned above.

The electron microscope examination also revealed evidence of degeneration of cells by spotting pyknotic cells with disintegrating nuclei and organelles with many electrons' dense vesicles within the cytoplasm. This finding was mentioned in **Saghir et al.**⁽¹⁵⁾ work on lung tissue in which rats were administered a single dose of (300mg/kg) IP of CTX to induce cytotoxicity of lung tissue, in the mentioned study it was stated that cyclophosphamide caused degeneration and pyknosis of alveolar tissue of the lung.

The mitochondria in group II of the current study were swollen, with lost cisternae and high electron density. The Endoplasmic reticulum was discontinuous and dispersed in the cytoplasm. This effect on cell organelles was also discussed by **Bashandy and Zidan**⁽¹⁷⁾ on cardiac cells were cyclophosphamide treated group showed

irregular mitochondria outline and swollen Endoplasmic reticulum.

The cytoplasmic vacuolation seen in group II by electron microscopic examination was also observed by **Ibrahim et al.**⁽¹⁶⁾ along with degeneration and lysis of other cell ultrastructure.

These findings of the damage caused by cyclophosphamide might be due to its cytotoxic effect by enhancing the release of chemical inflammatory mediators from neutrophils, and the release of interleukin-1 from lymphocytes and macrophages. These chemicals cause severe inflammatory response that attribute to the increased vascular permeability and tissue damage. The release of free radicals released by cyclophosphamide metabolism also contribute to the cell damage and degeneration observed in group II as observed by **Al-Refai**⁽¹³⁾.

Cell damage and degeneration mentioned earlier can be attributed to the cross linkage of adjacent DNA strands at the guanine N-7 position caused by cyclophosphamide trigger programmed cell death especially in highly proliferating cells such as basal cells of oral epithelium according to **Ogino and Tadi**⁽¹⁸⁾.

In the present study, light microscope examination of Cyclophosphamide/ Exosomes group (Group3) revealed overall evidence of normal epithelial cells and restoration of intact basement membrane and basal cell layer, this finding is in harmony with **Hassan et al.**⁽¹²⁾ were normal histological features were noted after exosomes treatment of rats with induced Alzheimer disease. This finding can be attributed to the regenerative function of mesenchymal stem cells exosomes mentioned in **Tang et al.**⁽¹⁹⁾ who reviewed the acquisition methods, characteristics, biological functions and clinical applications of different types of exosomes and concluded this promising regenerative effect of stem cells exosomes.

The significant improvement in the number and shape of taste buds in group III is probably due to exosomes contribution by releasing micro-RNA that enhances neural remodeling and inhibits apoptosis as discussed by **Hassan et al.**⁽¹²⁾ and **Cheng et al.**⁽²⁰⁾.

The inflammatory cell infiltrate in the connective tissue of circumvallate papillae of group III was significantly less than group II, this can be explained by anti-inflammatory function of Msc-exosomes stated in **Tang *et al.*⁽¹⁹⁾**.

Decreased vacuolation and increased integrity of connective tissue in group III can be attributed to the enhancement of tissue regeneration and cell proliferation by microRNA from Exosomes as discussed by **Wan *et al.*⁽²¹⁾**. In the mentioned review, the recent advances of exosomes in soft tissue recovery were critically reviewed, and exosomes were introduced as an alternative for cell-based therapy.

The electron microscope examination of circumvallate papillae in cyclophosphamide/Exosome group (group III) revealed restoration of normal form and shape of cells and their nuclei. No evidence of pyknosis or apoptosis was seen, with significantly less vacuolation within the cytoplasm and around cells than the group receiving cyclophosphamide only. Cell organelles, especially mitochondria and Endoplasmic reticulum that were greatly affected by cyclo-phosphamide in group II were closer to the control shape and form in group III receiving a single IP dose of exosomes. This finding is in harmony with **Hassan *et al.*⁽¹²⁾** which stated that the group receiving exosomes had almost similar structure to control.

The overall evidence of regeneration both in tissue structure and intracellular structure can be attributed to the healing promoting potential of Exosomes due to their ability to suppress pro-inflammatory reactions and suppress oxidative stress and damage by free radicals. This function helps create a more regeneration friendly environment giving a chance for endogenous stem cells to repair injured tissues successfully. This potential is due to the synergic effect of regulatory proteins and microRNAs secreted by Exosomes as mentioned in **Börger *et al.*⁽²²⁾** review that included major outcomes of published MSC extracellular vesicles in vivo studies.

CONCLUSION

Cyclophosphamide administration in the utilized

dose was associated with clear degenerative changes in the circumvallate papillae of albino-rats and BMSCs derived exosomes IP injection resulted in significant evidence of tissue regeneration rendering a possible treatment for cyclophosphamide induced circumvallate papillae damage.

- **Conflicts of Interest:** The authors declare no conflicts of interest regarding the publication of this paper.
- **Funding:** No fund.

REFERENCES

1. **Kuroda Y, Kitada M, Wakao S, Dezawa M (2011):** Bone marrow mesenchymal cells: how do they contribute to tissue repair and are they really stem cells? *Archivum Immunologiae Therapiae Experimentalis*, 59:369-78.
2. **Ding D, Shyu W, Lin S (2011):** Mesenchymal stem cells. *Cell Transplant.*, 20(1):5-14.
3. **Xing X, Han S, Li Z, Li Z (2020):** Emerging role of exosomes in craniofacial and dental applications. *Theranostics*, 10(19):8648.
4. **Sarkar A, Allyn D, Delay R *et al.* (2021):** Cyclophosphamide-induced inflammation of taste buds and cytoprotection by amifostine. *Chem Sci.*, 46:bjab031.
5. **Zhang Y, Liu Y, Liu H *et al.* (2019):** Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci.*, 9(1):1-8.
6. **Gado A, Adam A, Aldahmash B (2013):** Cardiotoxicity induced by cyclophosphamide in rats: protective effect of curcumin. *J Res Environ Sci Toxicol.*, 2(4):87-95.
7. **Hu L, Wang J, Zhou X *et al.* (2016):** Exosomes derived from human adipose mesenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci Rep.*, 6(1):32993.
8. **Mostafa S, Hakam H, El-Motayam A (2019):** Gustatory dysfunction in relation to circumvallate papilla's taste buds' structure upon unilateral maxillary molar extraction in Wistar rats: an in vivo study. *F1000Research*, 8:1667.
9. **Feldman A, Wolfe D (2014):** Tissue processing and hematoxylin and eosin staining in Histopathology: methods and protocols. Editor: Christina E. Day; Humana

- Press, New Jersey, USA.
10. **Hayat M (1981)** Principles and techniques of electron microscopy. Biological applications. Edward Arnold; 4th Ed. Cambridge University Press, Edinburgh, UK.
 11. **Mukherjee N, Pal Choudhuri S, Delay R *et al.* (2017):** Cellular mechanisms of cyclophosphamide-induced taste loss in mice. *PloS One*, 12(9):e0185473.
 12. **Hassan R, Rabea A, Ragaie A *et al.* (2020):** The prospective role of mesenchymal stem cells exosomes on circumvallates taste buds in induced Alzheimer's disease of ovariectomized albino rats: (Light and transmission electron microscopic study). *Arch Oral Biol.*, 110:104596.
 13. **Al-Refai A (2017):** Chemoprotective effect of ascorbic acid on cyclophosphamide induced oral toxicity. *Zanco J Med Sci.*, 21(1):1540-51.
 14. **Essawy A, Issa N, Tayel S (2022):** Glycyrrhiza glabra root extract alleviates cyclophosphamide induced mucositis of the tongue in adult male albino rats. *Egypt J Histol.*, 45(4):1222-34.
 15. **Saghir S, Alharbi S, Al-Garadi M *et al.* (2020):** Curcumin prevents cyclophosphamide-induced lung injury in rats by suppressing oxidative stress and apoptosis. *Processes*, 8(2):127
 16. **Ibrahim H, Zommara M, Elnaggar M (2021):** Ameliorating effect of selenium nanoparticles on cyclophosphamide-induced hippocampal neurotoxicity in male rats: light, electron microscopic and immunohistochemical study. *Folia Morphologica*, 80(4):806-19.
 17. **Bashandy M, Zedan O (2019):** Role of alpha lipoic acid on cyclophosphamide induced cardiotoxicity in adult male albino rat: Histological study. *Egypt J Histol.*, 42(4):888-99.
 18. **Ogino M, Tadi P (2023)** Cyclophosphamide. [Updated 2023 Jul 3]. In: *Stat Pearls*. Treasure Island (FL). Available at: <https://www.ncbi.nlm.nih.gov/books/NBK553087>
 19. **Tang Y, Zhou Y, Li H (2021):** Advances in mesenchymal stem cell exosomes: a review. *Stem Cell Res Ther.*, 12(1):1-2.
 20. **Cheng X, Zhang G, Zhang L *et al.* (2018):** Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration. *J Cellular Molecular Med.*, 22(1):261-76.
 21. **Wan R, Hussain A, Behfar A *et al.* (2022):** The therapeutic potential of exosomes in soft tissue repair and regeneration. *Inter J Mol Sci.*, 23(7):3869.
 22. **Börger V, Bremer M, Ferrer-Tur R *et al.* (2017):** Mesenchymal stem/stromal cell-derived extracellular vesicles and their potential as novel immunomodulatory therapeutic agents. *Inter J Mol Sci.*, 18(7):1450.