Original Article

The Effect of Adipose Derived Stem Cells versus Platelet Rich Plasma in Ameliorating Cisplatin-Induced Injury on The Submandibular Salivary Gland. "A Comparative Histological Study"

Shaymaa Mamdouh Dessoukey, Ahmed Mahmoud Halawa, Iman Ahmed Fathy and Dina Mohamed Abd Elkhalik

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

ABSTRACT

Background: Cisplatin is a potent anti-carcinogenic agent; however, it has many dose-limiting side effects. The aim of our study was to determine and compare the effectiveness of adipose-derived stem cells (ADSCs) and platelet rich plasma (PRP) as a treatment modality against cisplatin-induced cytotoxicity in the submandibular glands of Albino rats.

Methods: Thirty-eight adult male albino rats, were used in the present study. Ten rats were used as a source of PRP, and the rest were included in the experimental design as follows: negative control group, cisplatin group (positive control) received (5mg/kg/week) cisplatin intraperitoneal on day 1 and 8. ADSCs group received ADSCs (2 × 10^6 cells/rat) once intravenous via tail vein + cisplatin. PRP group received PRP (0.5 mL/kg by intraperitoneal injection 3times/week) + cisplatin.

Results: Both ADSCs and PRP enhanced the regeneration of submandibular salivary gland tissue after the cisplatin induced cytotoxicity; represented by significant increase in area fraction of acini, percentage of hemolysis as well as significant decrease in area fraction of apoptotic cells in both groups compared to cisplatin group respectively. There was no significant difference between both groups regarding all comparative parameters used in this study. In addition, there was a non-significant difference in the area fraction of apoptotic cells between PRP group and the control group, but we detected a significant difference between ADSCs group and the control group regarding the same parameter.

Conclusions: Both adipose-derived stem cells and Platelet rich plasma are considered as effective treatment for cisplatin-induced cytotoxicity in submandibular salivary glands. However, PRP is recommended over ADSCs as a potent and effective treatment due to its safety, easy accessibility and proved treatment effect.

Received: 06 June 2020, **Accepted:** 23 July 2020

Key Words: Apoptosis, regeneration, treatment effect, transmission electron microscope.

Corresponding Author: Shaymaa Mamdouh Dessoukey, PhD, Department of Oral Biology, Faculty of Dentistry, Ain Shams University, Egypt, **Tel.**: +20 147667161, **E-mail:** shaymaamamdouh23@gmail.com

ISSN: 1110-0559, Vol. 44, No.2

INTRODUCTION

Cisplatin (as a chemotherapeutic agent) has been indicated for the treatment of various tumors or prior to radiotherapy and surgery but unfortunately, chemotherapeutic agents can lead to morphologic change in salivary gland tissue. This complication also significantly affects the duration of hospitalization and survival rates[1].

Many studies have handled salivary gland damage-regeneration through adipose derived stem cells (ADSCs) application, by means of systemic and local administration. It was demonstrated that ADSCs may migrate engrafting to an injured location via the blood stream systemic use[2]. Many experiments have proved that ADSCs could be considered as a safe treatment strategy for salivary gland damage[3].

Platelet-rich plasma (PRP) has been the subject in different studies as a new approach in regenerative medicine and attracted considerable attention over the last two decades[4]. However, to our knowledge, few studies were concerned with the effect of PRP on submandibular salivary gland (SMG) after chemotherapy. The aim of our present study was to compare the effect of ADSCs and PRP on treatment of the structural changes that occurred in the cisplatin treated submandibular salivary glands and find out which is more effective and profound. To our knowledge, no statistically significant relationship as a post chemotherapeutic treatment modality is presented between the use of ADSCs and PRP.

METHODS

Study Design

Thirty-eight adult male albino rats weighing between (300-350 grams) were housed in wire mesh cages, in the Animal Research Centre, Faculty of Medicine, Ain Shams University, under controlled temperature and dark-light cycle. They were fed standardized diet (ad libtium) and tap water was available. All procedures involving animals were reviewed and approved by the institution guidelines of Ain Shams University Ethical Committee with authorization number (688).

Ten rats were used as source of PRP, and the remaining 28 rats were used in the experimental design.

 DOI: 10.21608/ejh.2020.32113.1309

Experimental design

Twenty-eight rats were divided into 4 main groups

1-(Control group)

Seven rats received intraperitoneal injection of saline (5mg/kg) on day 1 and 8 similar to group II hence, considered as a negative control. After exclusion of the first 3 days, rats were sacrificed after 15 days from administration of cisplatin in the corresponding experimental groups[5].

2-(Cisplatin group)

Seven rats received intraperitoneal injection of cisplatin at a dose of (5mg/kg) on day 1 and 8 and were considered as positive control[6]. Rats were sacrificed after 15 days from administration of cisplatin.

3-(ADSCs group)

Seven rats received intraperitoneal injection of cisplatin at a dose of (5mg/kg) on day 1 and 8 then ADSCs (2 × 106 cells/rat) were injected via tail vein once after 72h from cisplatin injection[5]. Rats were sacrificed after 15 days from administration of cisplatin.

4-(PRP group)

Seven rats received intraperitoneal injection of cisplatin at a dose of (5mg/kg) on day 1 and 8, then PRP in a dose of (0.5 mL/kg) by intraperitoneal injection 3times/week, starting from 72 h after cisplatin injection. Rats were sacrificed after 15 days from administration of cisplatin.

Platelet rich plasma preparation

PRP was prepared using the double centrifugation tube method[7]. Ten male Albino rats were used as blood donors and anesthetized with ether, and 3mL blood was collected from the retro-orbital plexus into tubes containing 0.3 mL (3.2% sodium citrate) anticoagulant. Blood was centrifuged twice. First centrifugation was at 1600 rpm for 10 min. The portion just above the buffy coat was obtained without disturbing the buffy coat. The plasma was centrifuged again at 2000 rpm for 10 min to yield two portions: the top consisting of platelet-poor plasma (PPP) and the bottom consisting of the platelet button. After discarding the PPP, the platelet button was resuspended in phosphate-buffered saline (PBS) (1: 1). A hematology analyzer (MICROS abc LC-152; Horiba Ltd., Kyoto, Japan; at least 1, 000, 000 cells/μL) was used for platelets counting and then PRP was frozen at –80°C until use[8]. PRP was activated immediately before use with CaCl2 (0.8mL PRP + 0.2mL 10% CaCl2)[9].

Samples from the right SMG were freely excised, processed in paraffin and were examined for routine hematoxylin and eosin (H&E)[10] and (Anti-active Caspase 3) by light microscopic examination. Rabbit polyclonal antibody to caspase 3 (CPP32, H-277) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Immuno-histochemical Technique

The immunohistochemical staining technique was as follows; after de-paraffinization and rehydration of sections, endogenous peroxidase activity was blocked by incubating the specimen in 3% H_2O_2 in methanol at 23ºC for 15 min. To facilitate antigen retrieval, sections were then microwaved in citrate buffer (PH 6) for 9 min., followed by the application of polyclonal primary rabbit anti-active caspase 3 (1:100 in PBS), for 60 min. at 23ºC. The antibody is characterized with species reactivity to human and rats. Sections were then exposed to secondary biotinylated antibody for 30 min. at room temperature. Slides were then incubated for 5 min. at room temperature with Biocare's diamino-benzidine plus chromogen. The sections were counterstained with hematoxylin for 2 min. and then rinsed in distilled water, dehydrated in graded alcohol and cleared in xylene. Finally, specimens were mounted on positive slides using aqueous mounting medium, then cover slips were put in place and examined under light microscope. The positive reaction was detected as brown color; the cellular localization of this antibody is nuclear and cytoplasmic. The negative reaction was detected as blue color[11]. The primary antibody was replaced with PBS as a negative control. Spleen tissue as a positive control and a negative control of normal tissue expression was also stained along with the procedure.

On the other hand, left SMG samples were taken, processed in resin Epon 812 and prepared for examination by transmission electron microscope[12].

Blood samples for osmotic fragility test

Blood samples were taken from each group at the end of the experimental period, to monitor the percentage of hemolysis as a systemic effect of cisplatin, ADSCs and PRP to support and confirm our histological results. Two ml of blood from each rat in the experimental groups were taken from the retro-orbital plexus. Hematocrit and erythrocytes mean osmotic fragility were determined on heparinized blood using Sysmex hemocytometer[13].

Histomorphometric analysis was done using image J (Image J, v1.41a, NIH, USA) and a digital video camera (C5060, Olympus, Japan) in order to assess:

1. Area fraction of acini: Five H&E sections of the SMG from each group were chosen to run the morphometric study and five fields from each section were examined for area fraction of acini under L.M, at x200 magnification.
2. Area fraction of apoptotic cells: Five caspase-3 sections of the SMG from each group were chosen to run the morphometric study and five fields from each section were examined for area fraction of apoptotic cells under L.M, at x200 magnification.

N.B: Platelet concentration before and after separation were measured to ensure proper preparation of the PRP[8].

Image analysis

For both H&E and anti-active caspase-3 antibody photomicrograph's:

1. Images were first corrected for brightness and contrast.

2. Images were then transferred to the Image J program (Image J, v1.41a, NIH, USA).
3. Sampling was performed to select the area of interest, while the remaining area (which were excluded) were covered by white pixels.
4. The images were then converted into 8-bit monochrome type.
5. Color threshold was then performed automatically, and area fraction was calculated.

Figure plates containing original images were compiled to compare the histological variations.

Statistical analysis of the mean area of acini of all groups was performed using SPSS (IBM SPSS Statistics 21.0, IBM Corporation, and Somers, NY, USA). Comparison between groups were tested using one-way analysis of variance (ANOVA) followed by a Post hoc test. Numerical data of groups was also analyzed (a *p-value* <0.05 was selected as significant). Values were determined as means ± standard deviation.

RESULTS

1- Hematoxylin & Eosin results

Submandibular salivary glands of control group revealed normal appearance of both serous acini and glandular lobules which were infiltrated by normal vasculature. On the other hand, SMG of cisplatin group revealed apparent shrinkage of both serous acini and glandular lobules with increased interstitial edema in between. Serous acini and granular convoluted tubules (GCTs) showed irregular outline, multiple vacuoles, pyknotic and hyperchromatic nuclei and many degenerated cells. Excretory ducts (EDs) revealed loss of pseudo-stratification, degeneration in some areas, vacuoles, wide lumen, stagnation of secretion and many desquamated cells. Extravasated RBCs were seen between the acini (Figures 1A, 2A, 2B).

In our study, SMG of ADSCs group showed almost normal appearance of the majority of serous acini and ductal system except for stagnation of secretions and some areas of shrinkage. Apparently large lobules were separated by relatively thin connective tissue septa. Some serous acini with cytoplasmic vacuoles and hyperchromatic nuclei were detected. EDs were lined by flat to cuboidal epithelium and revealed loss of pseudo-stratification in some areas. Areas of degeneration and cytoplasmic vacuoles, surrounded by apparently normal fibrous connective tissue septa were detected (Figures 1B, 2C). Regarding SMG of PRP group, it showed normal appearance of the majority of serous acini and glandular lobules with apparent decrease in both interstitial edema and extravasated RBCs as well. Serous acini, GCTs with normal outline and striated ducts (SDs) with basal striation were also detected. EDs were lined by pseudostratified columnar epithelium with goblet cells with few areas of cytoplasmic vacuoles and relatively large sized blood vessel which were surrounded by fibrous connective tissue septa (Figures 1C, 1D, 2D).

2- Immuno-histochemical results (Anti-active Caspase 3 antibody)

In the current study, SMG of the control group revealed negative reactions in most serous acini, however few samples showed faint positive reactions. Both intercalated ducts (IDs) and SDs showed almost negative reactions. In addition, few positive reactions in GCTs were seen (Figure 3A). On the other hand, cisplatin group revealed intense positive reactions in serous acini, IDs, SDs and GCTs (Figure 3B).

Regarding ADSCs group, SMG revealed positive reactions in some serous acini, IDs and faint positive cytoplasmic reactions in the SDs and GCTs. Some SDs revealed negative reactions (Figure 3C). SMG of PRP group showed negative reactions in most serous acini, IDs, SDs and GCTs (Figure 3D).

3-Electron-microscopic results

In the current study, ultra-structure examination of SMG of control group revealed acini with basally situated rounded nucleus (N) and normal appearance of secretory granules (SG). SDs showed deep basal enfolding, between which numerous mitochondria are radially packed, rough endoplasmic reticulum (rER) and nucleus with peripheral chromatin condensation. GCTs revealed basally situated rounded nucleus, secretory granules, rER, mitochondria with internal lamellar cristae and many Golgi saccules (Figures 4A, 5A, 6A).

On the other hand, ultrastructural examination of cisplatin group revealed atypical and atrophied acini with pyknotic and shrunken nuclei, multiple cytoplasmic vacuoles, interstitial edema and loss of junctions between acini. SDs appeared with multiple cytoplasmic vacuoles, stagnation of secretion, and loss of basal striations as well as most of organelles. Thickening of the basement membrane of the SDs also was detected. GCTs with ruptured nuclear membrane, swollen mitochondria, dilated rER and multiple cytoplasmic vacuoles were seen (Figures 4B, 5B, 6B).

ADSCs group showed spaced acini with pyknotic nuclei and indented nuclear membrane, some cytoplasmic vacuoles and interstitial edema. SDs revealed partial loss of basal enfolding, with some mitochondria, open faced nucleus with peripheral chromatin condensation and narrow lumen. Some nuclei were displaced. Some GCTs revealed open faced nucleus, many mitochondria, well organized rER and prominent Golgi apparatus (Figures 4C, 5C, 6C).

PRP group revealed acini with open faced nucleus, prominent nucleolus parallel arrays of rER radiating in the basal and lateral portion of the cell, well observed mitochondrial cristae and secretory granules. SDs appeared with deep basal enfolding, between which numerous mitochondria were radially packed and nucleus with peripheral chromatin condensation. (Figures 4D, 5D, 6D).

4- Statistical results

- Platelet count was done before and after separation of PRP to ensure proper concentration and preparation for the PRP (Table 1).

- Both ADSCs and PRP successfully enhanced the regeneration of submandibular salivary gland tissue after cisplatin induced cytotoxicity; this was manifested by significant increase in the area fraction of acini, hemolysis percentage and significant decrease in area fraction of apoptotic cells in both groups compared to cisplatin group (Table 2).
- Regarding comparison between ADSCs and PRP, PRP revealed better result represented by non-significant difference in the area fraction of apoptotic cells between this group and the control group, however there was a significant increase in the area fraction of apoptotic cells in ADSCs group compared to the control group. In addition, there were non-significant difference between ADSCs group and PRP group regarding area fraction of acini, area fraction of apoptotic cells and percentage of hemolysis (Table 2).

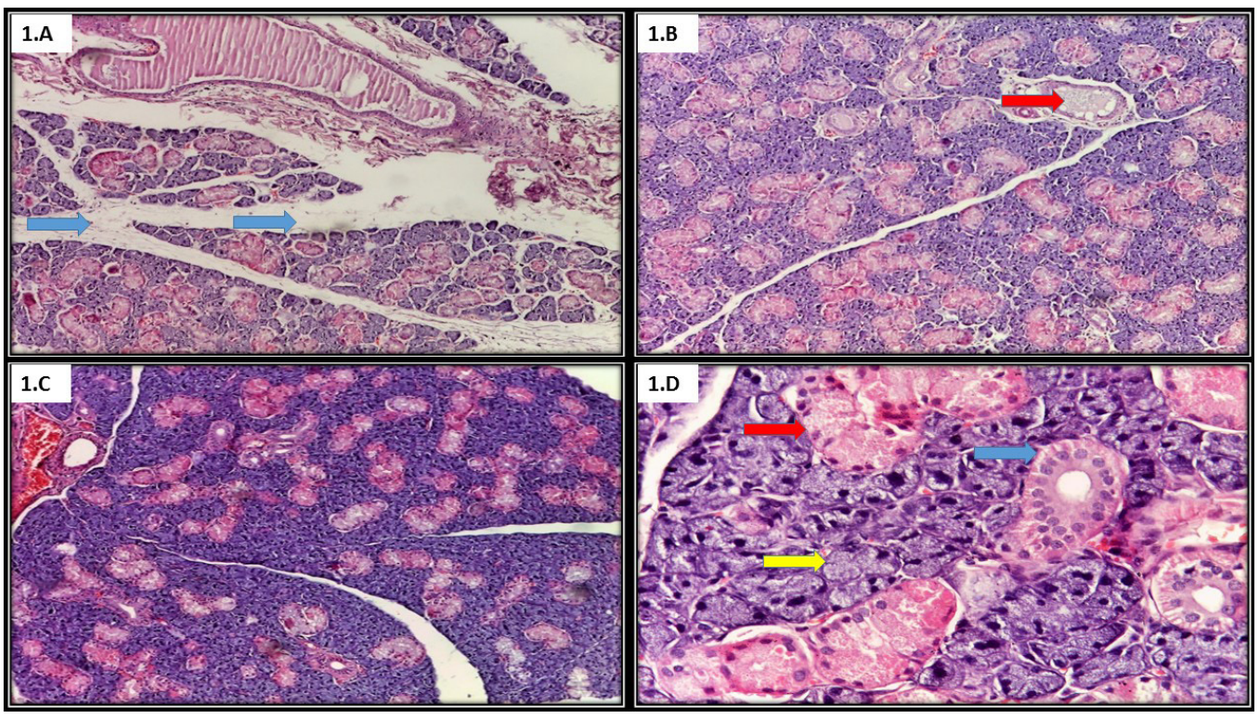

Fig. 1: (A): A photomicrograph of SMG of cisplatin group showing apparent shrinkage of both serous acini and ductal element separated by interstitial edema (blue arrows). **(B)**: ADSCs group showing almost normal serous acini and ductal system except for stagnation of secretion (red arrow). Note the apparently large lobules separated by relatively thin connective tissue septa. **(C)**: PRP group showing serous acini and glandular lobules with apparent decrease in the interstitial edema (H&E, x100). **(D)**: Higher magnification of the previous section showing serous acini (yellow arrow), GCT (red arrow) with normal outline, SD (blue arrow) with basal striation (H&E, x400).

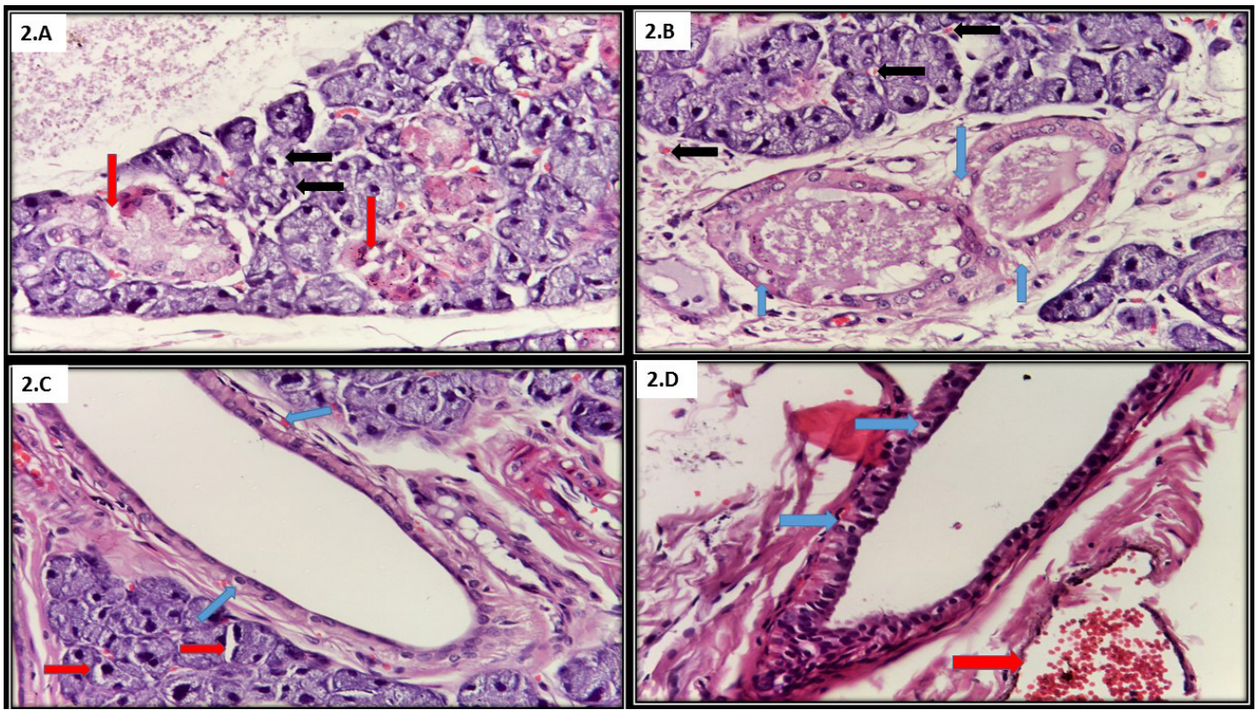

Fig. 2: (A): A photomicrograph of SMG of cisplatin group showing irregular outline and multiple vacuoles in serous acini (black arrows), GCT (red arrows). (B): Cisplatin group showing ED with loss of pseudo-stratification, stagnation of secretion, many desquamated cells (blue arrows) and extravasated RBCs (black arrows) (C): ADSCs group showing ED lined by flat to cuboidal epithelium with loss of pseudo-stratification in some areas, areas of degeneration and cytoplasmic vacuoles (blue arrows). Note, serous acini with cytoplasmic vacuoles and hyperchromatic nuclei (red arrows) (D): PRP group showing ED with few areas of cytoplasmic vacuoles (blue arrows) and relatively large sized blood vessel (red arrow) (H&E, x400).

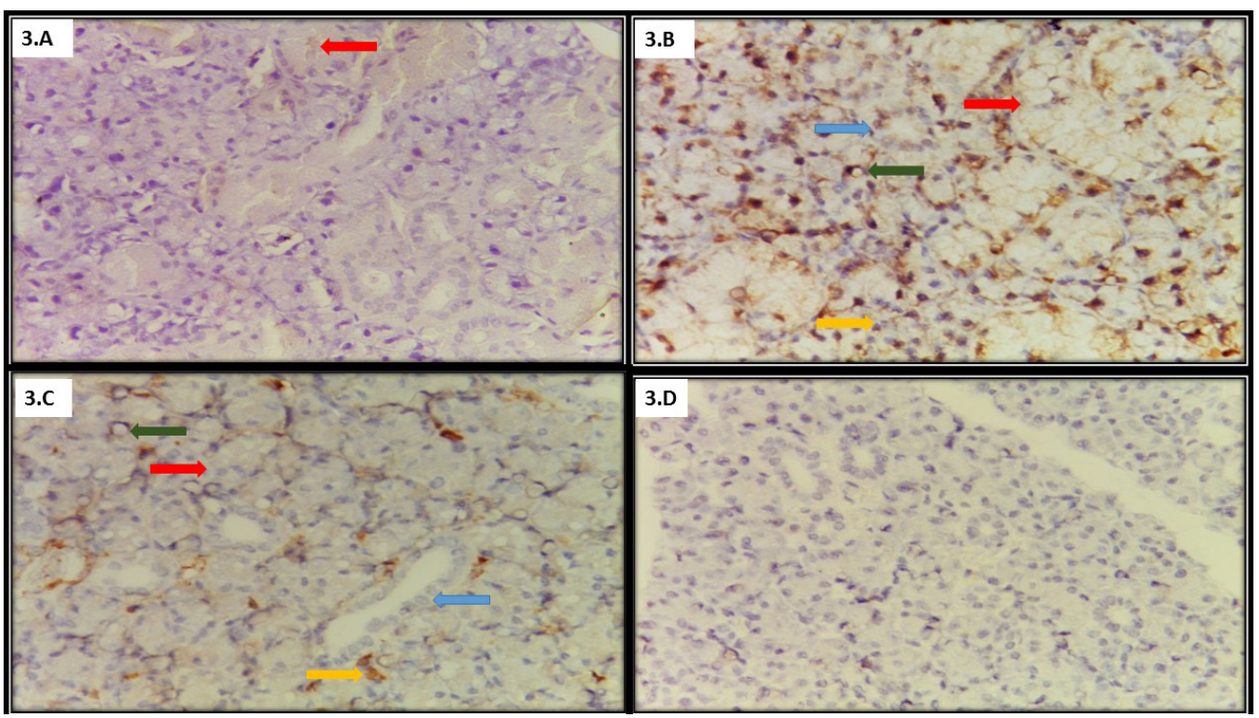

Fig. 3: (A): A photomicrograph of control group showing negative reactions in most serous acini, IDs, and SDs. Note the few positive reactions in GCTs (red arrow). (B): Cisplatin group showing intense positive reactions in serous acini (yellow arrow), ID (green arrow), SD (blue arrow) and GCT (red arrow). (C): ADSCs group showing positive reactions in some serous acini (yellow arrow), ID (green arrow), faint positive cytoplasmic reactions in GCT (red arrow) and almost negative reactions in the SD (blue arrow). (D): PRP group showing negative reactions in serous acini, IDs, SDs and GCTs (Anti-active caspase-3 antibody x400).

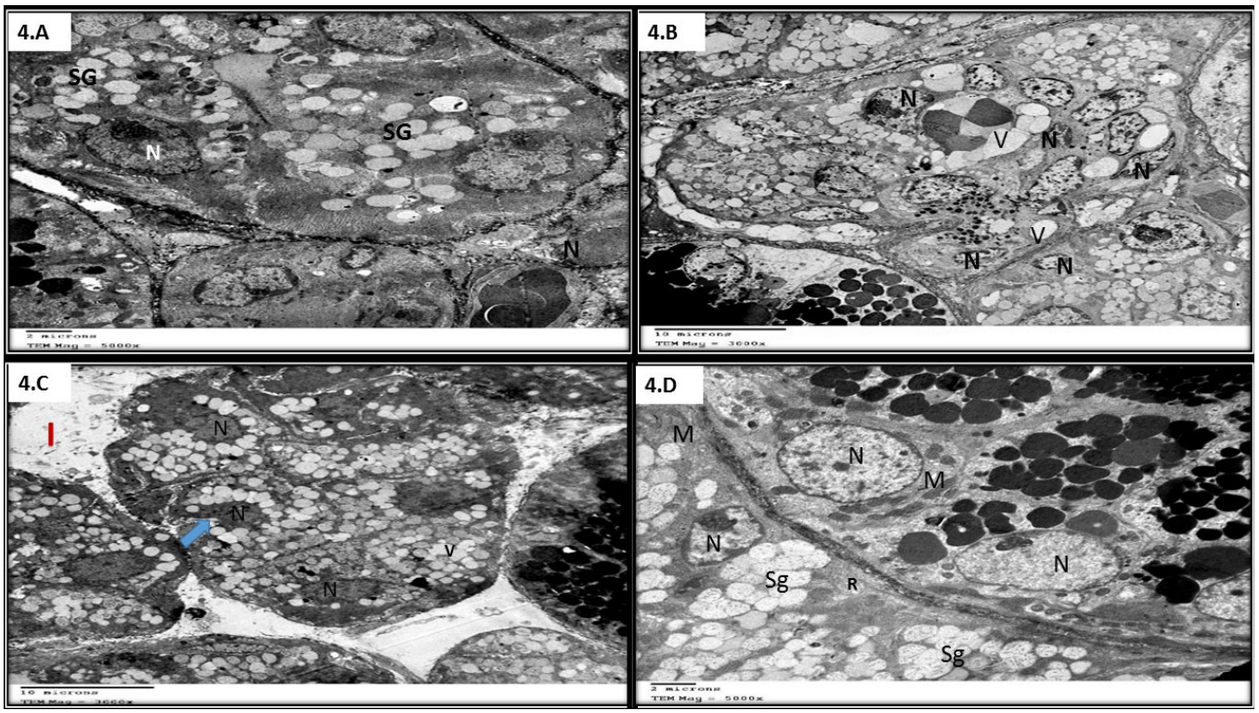

Fig. 4: (A): Electron micrograph of control group acinus showing open faced nucleus (N) and many secretory granules (SG) (x5000). (B): Cisplatin group showing atypical and atrophied acini with pyknotic and shrunken nuclei (N) and multiple cytoplasmic vacuoles (V) (x3000). (C): ADSCs group showing acini with pyknotic nuclei (N) indented nuclear membrane (blue arrows), some cytoplasmic vacuoles (V) and interstitial edema (I) (x3000). (D): PRP group showing acinar cell facing the basal part of GCT. Some acini show open faced nucleus (N) The GCT shows open faced nucleus (N), Secretory granules (Sg) and mitochondria laterally and basally (M) (x5000).

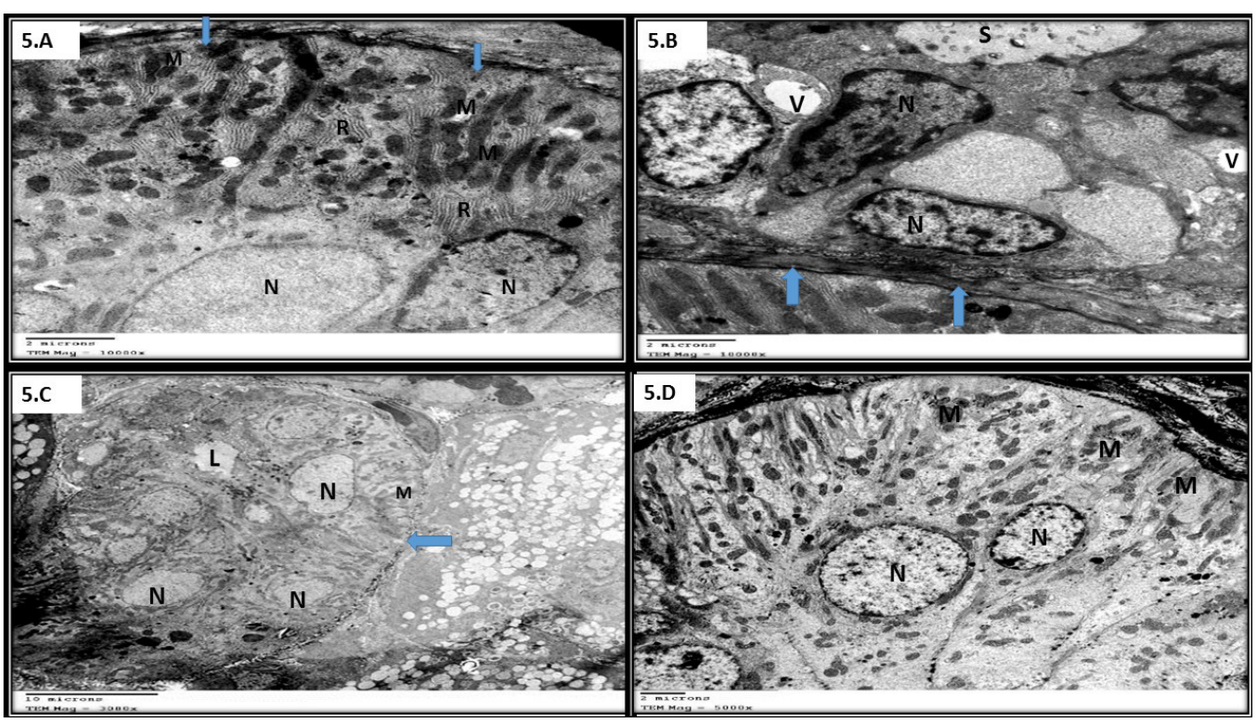

Fig. 5: (A): Electron micrograph of control group showing SD having basal enfolding (blue arrows), numerous mitochondria (M), RER (R) and open-faced nucleus (N) (x10000). (B): Cisplatin group showing SD with multiple cytoplasmic vacuoles (V), stagnation of secretion (S), pyknotic nuclei (N) and loss of basal striations , thickening of the basement membrane of the SD (blue arrows) (x10000). (C): ADSCs group showing SD having partial loss of basal enfolding (blue arrow), with some mitochondria (M), open faced nucleus (N) and narrow lumen (L) (x3000). (D): PRP group showing SD with radial mitochondria (M) and deep basal enfolding (x5000).

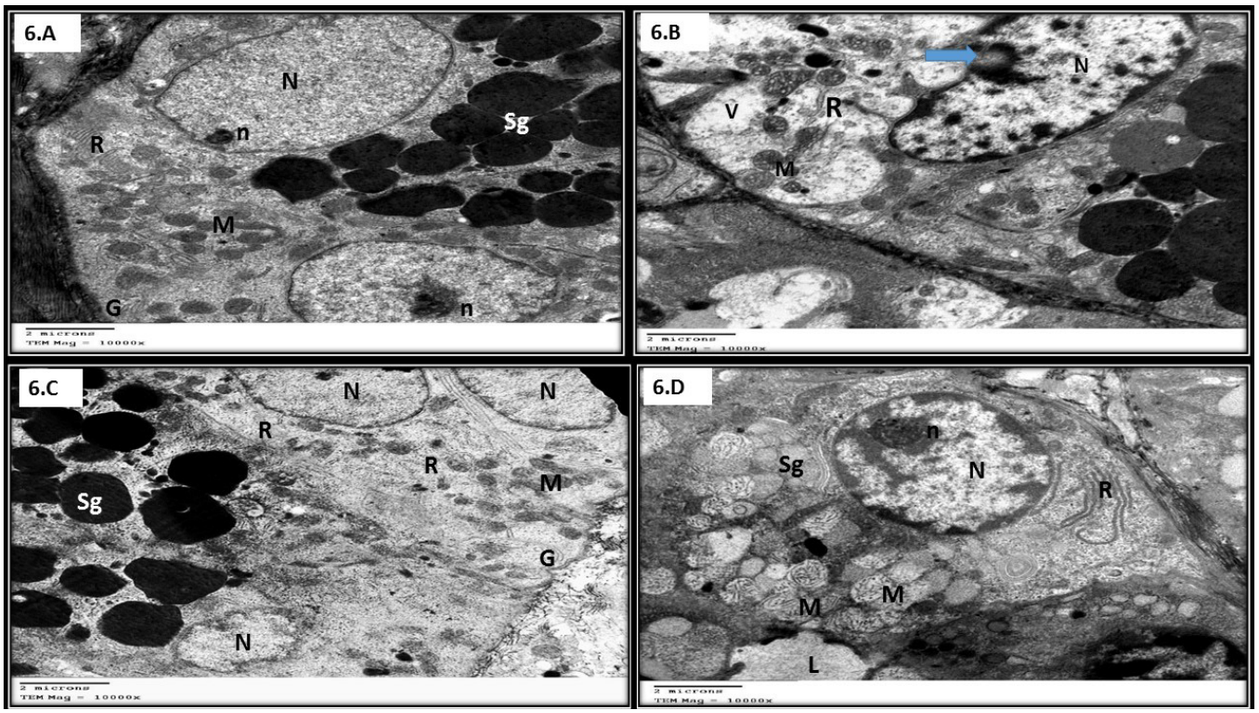

Fig. 6: (A): Electron micrograph of control group GCT (basal part) showing basally situated rounded nucleus (N), prominent nucleoli (n), secretory granules (Sg), rER (R), mitochondria (M) and many Golgi saccules (G) (x10000). (B): Cisplatin group showing GCT with ruptured nuclear membrane (blue arrow), swollen mitochondria (M), dilated rER (R) and multiple cytoplasmic vacuoles (V) (x10000). (C): ADSCs group showing GCT with some open-faced nucleus (N), secretory granules (Sg), many mitochondria (M), well organized rER (R) and prominent Golgi apparatus (G) (x10000). (D): PRP group acinus showing basally situated rounded open-faced nucleus (N) with prominent nucleolus (n), secretory granules (Sg), rER (R), mitochondria (M) and the lumen (L) with regular outline (x10000).

Table 1: Platelet concentration before and after separation (PRP/ ul)

	Mean± Standard Deviation	*P value*
platelet count before separation	618777.78±93456.91	<0.001
platelet count after separation	1196888.89±84229.51	

Values are presented as mean ±SD.
P-values less than 0.05 were considered as statistically significant

Table 2: Area fraction of acini, apoptosis, and percentage of hemolysis in all groups

	Control group	Cisplatin group	ADSCs group	PRP Group	*P value*
Area fraction of acini % (H&E)	93.64±2.11	56.77±4.96*	71.42±7*#	75.63±8.42*#	< 0.001
Area fraction of Apoptosis% (Immuno- Caspase 3)	19.5±13.16	47.97±22.44*	31.93±11.9*#	25.33±14.44#	0.004
The Percentage of Hemolysis	54.14±11.10	10.14±1.77*	25.00±3.27*#	21.14±3.13*#	<0.001

Values are presented as mean ±SD
*: statistically significant compared to corresponding value in control group (*P*<0.05)
#: statistically significant compared to corresponding value in cisplatin group (*P*<0.05)
$: statistically significant compared to corresponding value in ADSCs group (*P*<0.05)

DISCUSSION

In the present study, cisplatin group revealed apparent shrinkage of both serous acini and glandular lobules with presence of interstitial edema. Serous acini, intercalated ducts, striated ducts, granular convoluted tubules, and excretory ducts showed multiple vacuoles, pyknotic and hyperchromatic nuclei and many degenerated cells. These results were in consistent with Terzi *et al*.,[14] which could be attributed to accelerated acinus cell apoptosis and degeneration induced by cisplatin administration[15,5]. Presence of intra cytoplasmic vacuoles might be caused by the released free radicals which cause damage to the cellular components[16,17]. Also, other cellular alternations in the form of pyknotic nuclei, irregular membrane outline, hyperchromatism and polymorphism could be attributed to nuclear damage which in turn causes DNA platination and results eventually in apoptosis[5].

The SDs in cisplatin group exhibited loss of basal striations and swollen mitochondria in H&E and electron-microscopic results respectively. This finding agrees with

Labah and Wahba,[18] who found the same results on day 4 after intraperitoneal injection with a single dose (6 mg/kg body weight in 0.9% saline) of cisplatin in rats. This could be explained by the damage of mitochondria which may be attributed to its sensitivity to cisplatin as cisplatin is hydrolyzed to generate a positively charged metabolite which preferentially accumulates within the negatively charged mitochondria[19].

In the current study, H&E results of the EDs in cisplatin group revealed loss of pseudo-striation, wide lumen, degenerated epithelial lining and stagnation of secretion. The wide lumen and stagnation of secretion could be due to the pathological effect of chemotherapy on myoepithelial cells that embracing the EDs leading to failure of expelling the secretion into the oral cavity and subsequently result in xerostomia[20].

On the other hand, our results regarding cisplatin group revealed a significant increase in the area fraction of apoptosis compared to all other groups, manifested by many intense positive nuclear and cytoplasmic reaction for anti-active caspase 3 antibody in almost all acinar and ductal elements. This result come in agreement with Mubarak and Ali[21]; in their examination of BAX (indicating apoptotic activity) in rat parotid salivary glands, they detected intense BAX positive immune reactivity in acinar and ductal cells in the group that received cisplatin (7mg/kg). The increased area fraction of apoptosis could be explained by Conklin[17], who stated that free radicals produced by cisplatin cause damage to the cellular components.

Using osmotic fragility test (reflects the oxidative stress), cisplatin group showed statistically significant decrease in the percentage of hemolyzed RBCs compared to the corresponding groups, which is indicative for hemolytic anemia. This result is in concomitant with Cvetkovic *et al*.,[22] who reported that cisplatin administration resulted in hematotoxicity at both high (4-16 mg/kg) and low (10 -8 -10 -12 g/ml per rat) concentrations, with the development of anemic conditions. These results also come in accordance with Harris *et al*.,[23] who reported that increased oxidative stress might promote this pathological status progression in cisplatin-based chemotherapy. This can be attributed to the cisplatin-induced anemia (microcytic hypochromic anemia) which results in decreased hematological parameters as a result of suppression of erythropoiesis[24]. Hence the significant decrease in blood analysis in cisplatin group in the current study could be explained by decreased erythrocyte number caused by the cisplatin induced anemia[25].

Cisplatin is proven to cause deterioration on salivary glands after 72 hours of systemic administration[15]; we chose to start the possible therapy either ADSCs or PRP on day 4 to make sure that cisplatin-induced tissue damage has already been established. Moreover, no self-regeneration was observed in cisplatin group when a dose of (5mg/kg/week) was used[6].

In general, ADSCs group revealed superior histological result over cisplatin group represented by apparent decrease in the shrinkage of both serous acini and glandular lobules. Serous acini, intercalated ducts, striated ducts, granular convoluted tubules and excretory ducts showed less vacuoles, some open-faced nuclei, many mitochondria, well organized rER and prominent Golgi apparatus. ADSCs group also revealed significant increase in the acini area fraction and percentage of hemolysis as well as significant decrease in the area fraction of apoptosis compared to cisplatin group.

These findings confirm the regenerative effect of ADCSs on the cisplatin-induced salivary gland damage and prove that ADSCs enhance the healing potential and the proliferative capacity of the SMG gland cells after cisplatin administration. These results come in accordance with Hany *et al*.[5]; in their study, systemic intravenous bone marrow stem cells (BMSCs) administration resulted in significant increase in the average number of positive immunolabeled proliferating cell nuclear antigen (PCNA) cells compared to the cisplatin group indicating higher proliferative capacity and lower apoptotic profile. Similarly, in the study by Sumita *et al*.,[26] intravenous BMSCs administration led to increased PCNA proliferation marker in irradiated submandibular glands.

Such regenerative effect of MSCs observed in the chemotherapeutic rat model, may be mediated through many mechanisms, such as trans-differentiation to tissue-specific cells, fusion with the existing original cells to enhance the organ performance by directing their own genetic and cellular material[27] or through their paracrine mediators secreted by MSCs, which could participate in the repair process[28].

In several studies, it was established that, MSCs secrete a wide array of cytokines in vitro including IL-8, G- colony stimulating factor (G-CSF), IL-11, IL-15, IL-10, and basic fibroblast growth factor (bFGF)[29], vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF-I). IGF-I seems to play a major role in MSCs restorative effect[28].

IGF-I prevents cell apoptosis and promotes functional recovery as suggested by Xu *et al*.,[30] who denoted that, IGF-I and other paracrine mediators such as VEGF, bFGF directly up-regulated Bcl-2 in cardiomyocytes. The homodimers of Bcl-2 may stabilize the mitochondrial membrane and prevent the activation of downstream apoptotic signaling. These reports may explain our statistical results regarding the significant decrease in the apoptotic area fraction, apparent decrease in the acinar and ductal intra cytoplasmic vacuoles and normal mitochondrial membrane in ADSCs group compared to the corresponding cisplatin group.

The significant increase in the hemolysis percentage in ADSCs group compared to cisplatin group reflect the increase in number of RBCs and subsequently enhancement of hematopoiesis as through the effect of ADCSs on the cisplatin treated rats. These hematological results could be due to the fact that ADSCs are rich in CXCL12, a critical regulator of hematopoiesis. ADSCs express CXCL12 at a

statistically higher level than that in BMSCs. In addition, ADSCs exhibit a perivascular phenotype in vitro and in vivo. Moreover, ADSCs are similar to CXCL12- reticular cells in their phenotype and CXCL12 expression, which may explain why ADSCs can efficiently support hematopoiesis[31].

In contrast to our results, Wang *et al*.,[32] reported a non-significant difference in the ADSCs group compared to the irradiated group regarding apoptosis and cellular proliferation. In their study, (2×105 ADSCs/rat) were injected intra-glandular weekly for three consecutive weeks after 12 weeks post radiation. This controversy in results may be due to difference in the route of administration chosen in each study.

Despite using ADSCs as possible treatment for induced tissue damage, we can't neglect studies that are concerned with the drawbacks. It was reported that MSCs secrete several fatty acids that confer resistance to platinum chemotherapy in breast cancer cells[33].

In addition, ADSCs may secrete IL-6 to promote the tumor-initiating diseases of colon and breast cancers, also IL-10 which is secreted from ADSCs during hypoxia may promote tumor growth in Burkett lymphoma[34,30]. More recently it was found that conditioned medium of ADSCs could support epithelial ovarian cancer cell survival in the presence of cisplatin. On the light of the previously mentioned studies, MSCs seem to provide non-specific cell protection against cisplatin induced apoptosis in both cancerous and non-cancerous cells[35].

In the present study, PRP group revealed apparent decrease in the shrinkage of both serous acini and glandular lobules. Serous acini, intercalated ducts, striated ducts, granular convoluted tubules and excretory ducts showed less vacuoles, many open-faced nuclei, many mitochondria, well organized rER and prominent Golgi apparatus. These results regarding the curative effect of PRP are in consistence with another study which reported that PRP appeared to be histologically effective in the regeneration of submandibular salivary glands after severe irreversible atrophic changes caused by the gland delegation[36]. Also, another study reported a reno-protective effect of PRP on cisplatin-induced nephrotoxicity in adult male Wistar rats[9].

These histological improvements were also observed by El-Sharouny *et al*.[37], who recorded that treatment of cisplatin induced hepatotoxicity in rats with PRP revealed improvement of the hepatic parenchymal architecture and minimal marked improvement in the parameter of hepatic enzymes, comparable with the control group.

This capacity of PRP to ameliorate cisplatin induced salivary gland damage, is represented by the improved histological and hematological results in PRP group. It may be mediated through natural cocktail of growth factors and bioactive substances within α-granules in the PRP which orchestrating tissue regeneration. These growth factors include platelet derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF), VEGF, (b-FGF) and connective tissue growth factor (CTGF[38].

On the other hand, the significant decrease in the area fraction of apoptosis in PRP group compared to cisplatin group could be due to the fact that PRP has anti-apoptotic activities via downregulating the expression of apoptotic genes as DAPK1 and BIM mRNA and inhibiting p53, BAX, and caspase-3 levels[39]. In addition, PRP may suppress caspase-3 by enhancing the PI3K/Akt pathway which curbs reactive oxygen species generation, thereby downregulating nuclear factor Kappa B (NF-κB) activation and increasing resistance to oxidation[9]. This finding also could explain the significant increase in the hemolysis percentage (possibly less oxidative stress) which was detected by the osmotic fragility test and the significant decrease in the area fraction of apoptosis when compared to the cisplatin group.

In the present work, we considered the significant increase in the acini area fraction in PRP group compared to cisplatin group as a sign of tissue repair, in addition decreased inflammatory signs was presented by apparent decrease in the interstitial edema and the acini shrinkage. This could be due to the fact that PRP increase the intracellular expression of the anti-inflammatory mediators (IL-4, IL-10, and IL-13) which is known to play a major role in inhibiting inflammation and decreasing IL-1β-mediated catabolic effect. Moreover, IGF-1 is one of the growth factors in PRP which stimulates the release of growth hormones, which help in tissue repair[40].

In this work, we detected apparent a decrease in extravasated RBCs between the acini in PRP group. This result could be related to the fact that PRP extract promotes angiogenesis through the angiopoietin1-Tie2 pathway that stimulates growth, migration, and differentiation of endothelial cells[41].

In contrast to our results, a study reported a non-significant difference in the platelet rich fibrin (PRF) group compared to the irradiated group regarding both the cell proliferation using (PCNA) and the cellular apoptosis using "TUNEL" (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling) to detect DNA cleavage and chromatin condensation[32]. This controversy in results may be due to first, different route of administration (they used local administration while we used systemic one). Second, different types of plasma concentrate since they used PRF while we used PRP (fibrin free).

In the present study, we found a statistically non-significant difference between ADSCs group & PRP group regarding the area fraction of acini and area fraction of apoptosis. This comes in accordance with Wang *et al*.,[32], who reported a non-significant difference in the ADSCs group compared to the PRF regarding both the cell proliferation using (PCNA) and the cellular apoptosis using "TUNEL" to detect DNA cleavage and chromatin condensation.

In view of our results and in conjunction with the previously mentioned studies[42]; PRP stand as a strong and profound compeer to the ADSCs. In addition, PRP is safe,

autologous and has no reported anti-immune or toxic effect on the tissues[9]. In addition, our current study proved that the protocols of PRP administration (multiple injection), is more profound than the single injection protocol of ADSCs regarding the repeated treatment with cisplatin.

CONCLUSION

Both ADSCs and PRP could be considered as effective treatments for the cisplatin induced submandibular salivary gland damage. Both showed nearly the same results at the end of our experiment with no significant difference between both groups regarding all comparative parameters used in this study. In addition, there was a non-significant difference in the area fraction of apoptotic cells between PRP group and the control group, but we detected a significant difference between ADSCs group and the control group regarding the same parameter. Accordingly, we recommend the usage of PRP over ADSCs as a potent and effective treatment of the cisplatin-induced salivary gland damage due to its safety, easy accessibility and proved treatment effect.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Mittal BB, Pauloski BR, Rademaker AW, Discekici-Harris M, Helenowski IB, Mellot A, Agulnik M and Logemann JA: Effect of induction chemotherapy on swallow physiology and saliva production in patients with head and neck cancer: a pilot study. Head Neck. (2015) 37(4): 567-572.
2. Yoo C, Vines JB, Alexander G, Murdock K, Hwang P and Jun HW: Adult stem cells and tissue engineering strategies for salivary gland regeneration: a review. Biomater Res. (2014) 18(9): 2-12.
3. Lombaert I, Movahednia MM, Adine C and Ferreira JN: Concise Review: Salivary Gland Regeneration: Therapeutic Approaches from Stem Cells to Tissue Organoids. Stem Cells. (2017) 35(1): 97–105.
4. Pavlovic V, Ciric M, Jovanovic V and Stojanovic P: Platelet rich plasma: A short overview of certain bioactive components. Open Med. (2016) 11(1): 242–247.
5. Hany E, Sobh M, ElKhier M, ElSabaa H and Zaher A: The Effect of Different Routes of Injection of Bone Marrow Mesenchymal Stem Cells on Parotid Glands of Rats Receiving Cisplatin: A Comparative Study. Int J Stem Cells. (2017) 10(2): 169–178.
6. Lin MT, Ko JL, Liu TC, Chao PT and Ou CC: Protective Effect of D-Methionine on Body Weight Loss, Anorexia, and Nephrotoxicity in Cisplatin-Induced Chronic Toxicity in Rats. Integr Cancer Ther. (2018) 17(3): 813–824.
7. Pazzini JM, Nardi AB, Huppes RR, Gering AP, Ferreira MG, Silveira CP, Luzzi MC and Santos R: Method to obtain platelet-rich plasma from rabbits (Oryctolagus cuniculus). Pesqui Vet Bras. (2016) 36(1): 39–44.
8. Kazemnejad S, Allameh A, Gharehbaghian A, Soleimani M, Amirizadeh N and Azayeri M: Efficient replacing of fetal bovine serum with human platelet release during propagation and differentiation of human bone marrow-derived mesenchymal stem cells to functional hepatocytes-like cells. Vox Sang. (2008) 95(2): 149– 158.
9. Salem N, Helmi N and Assaf N: Renoprotective Effect of Platelet-Rich Plasma on Cisplatin-Induced Nephrotoxicity in Rats. Oxid Med Cell Longev. (2018) 2018: 9658230.
10. PR B, Krishnapillai R, Thomas P and Palani J: Facts in artifacts. J Oral Maxillofac Pathol. JOMFP. (2013) 17(3): 397-401.
11. Vyas D, Roertson C, Stromberg P, Martin J, Dunne M, Houchen C, Barrett T, Ayala A, Perl M, Buchman T and Coopersmith C: Epithelial apoptosis in mechanistically distinct methods of injury in the murine small intestine. Histol Histopathol. (2007) 22: 623-630.
12. King R: Principles and Techniques of Electron Microscopy: Biological Application. J Anat. (1992) 181(Pt 2): 385–386.
13. Karale S and Kamath JV: Effect of daidzein on cisplatin-induced hematotoxicity and hepatotoxicity in experimental rats. Indian J Pharmacol. (2017) 49(1): 49–54.
14. Terzi S, Özgür A, Mercantepe T, Çeliker M, Tümkaya L and Dursun E: The effect of astaxanthin on salivary gland damage caused by cisplatin in the rat. Int J Res Med Sci. (2017) 5(4): 1410-1414.
15. Kitashima S: Morphological alterations of submandibular glands caused by cisplatin in the rat. Kurume Med J. (2005) 52(1-2): 29–38.
16. Elemam H and Amin L: Role of Ghrelin on cisplatin induced morphological changes on submandibular salivary glands. Egypt Dent J. (2019) 63: 3225-3233.
17. Conklin KA: Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness. Integr Cancer Ther. (2004) 3(4): 294-300.
18. Labah DA and Wahba OM: Flaxseed oil: an emerging nutraceutical decimates cisplatin-induced submandibular salivary glands damage in rats. Tanta Dent j. (2017) 14(3): 105-111.
19. Qian W, Nishikawa M, Haque AM, Hirose M, Mashimo M, Sato E and Inoue M: Mitochondrial density determines the cellular sensitivity to cisplatin-induced cell death. Am J Physiol, Cell Physiol. (2005) 289: 1466- 1475.
20. Al-Refai AS, Khaleel AK and Ali S: The effect of green tea extract on submandibular salivary gland of methotrexate treated Albino rats. Immuno-histochemical study. J Cytol Histol. (2014) 5:212.

21. Mubarak R and Ali ZH: Protective effects of L-Carnitine on Cisplatin induced toxicity in rat parotid salivary glands. J Am Sci. (2012) 8(2): 137-144.

22. Cvetkovic D, Milutinović M, Ognjanović, BI, Štajn, AS and Obradović A: Alteration of oxidative stress parameters in red blood cells of rats after chronic in Vivo treatment with cisplatin and selenium. Archives of Biological Sciences. (2011) 63: 991-1000.

23. Harris AG, Sinitsina I and Messmer K: Validation of OPS imaging for microvascular measurements during isovolumic hemodilution and low hematocrits. Am J Physiol Heart Circ Physiol. (2002) 282: 1502–1509.

24. Gao LP, Li Z, Guo ZY and Zhao YM: The effects of vitamin C on DDP-induced anemia in rats. Toxicol Mech Methods. (2013) 23: 383–388.

25. Geyikoğlu F, Çolak S, Türkez H, Bakır M, Koç K, Hosseinigouzdagani MK, Çerig S and Sönmez M: Oleuropein Ameliorates Cisplatin-induced Hematological Damages Via Restraining Oxidative Stress and DNA Injury. Indian J Hematol Blood Transfus. (2017) 33(3): 348-354

26. Sumita Y, Liu Y, Khalili S, Maria OM, Xia D, Key S, Cotrim AP, Mezey E and Tran SD: Bone marrow-derived cells rescue salivary gland function in mice with head and neck irradiation. Int J Biochem Cell Biol. (2011) 43(1): 80–87.

27. Wang F, Wang L, Yao X, Lai D and Guo L: Human amniotic epithelial cells can differentiate into granulosa cells and restore folliculogenesis in a mouse model of chemotherapy-induced premature ovarian failure. Stem Cell Res Ther. (2013) 4(5): 124.

28. Amin N and Reyad O: Role of mesenchymal stem cell therapy in restoring ovarian function in a rat model of chemotherapy-induced ovarian failure: A histological and immunohistochemical study. Egypt J Histol. (2013) 36(1): 114–126.

29. Choi M, Kim H, Park J , Lee T, Baik C, Chai Y, Jung K, Park K, Roh W, Kim K and Kim S: election of optimal passage of bone marrow-derived mesenchymal stem cells for stem cell therapy in patients with amyotrophic lateral sclerosis. Neurosci letters. (2010) 472: 94-8.

30. Xu L, Wang X, Wang J, Liu D, Wang Y, Huang Z, and Tan H: Hypoxia-induced secretion of IL-10 from adipose-derived mesenchymal stem cell promotes growth and cancer stem cell properties of Burkitt lymphoma. Tumour Biol. (2016) 37: 7835–7842.

31. Nakao N, Nakayama T, Yahata T, Muguruma Y, Saito S, Miyata Y, Yamamoto K. and Naoe T: Adipose tissue-derived mesenchymal stem cells facilitate hematopoiesis in vitro and in vivo: advantages over bone marrow-derived mesenchymal stem cells. Am J Pathol. (2010) 177(2): 547-54.

32. Wang Z, Xing H, Hu H, Dai T, Wang Y, Li Z, An R, Xu H, Liu Y and Liu B: Intraglandular transplantation of adipose-derived stem cells combined with platelet-rich fibrin extract for the treatment of irradiation-induced salivary gland damage. Exp Ther Med. (2018) 15(1): 795–805.

33. Roodhart JM, Daenen LG, Stigter EC, Prins HJ, Gerrits J, Houthuijzen JM, Gerritsen MG, Schipper HS, Backer MJ and Van Amersfoort M, *et al*. Mesenchymal stem cells induce resistance to chemotherapy through the release of platinum-induced fatty acids. Cancer Cell. (2011) 20(3): 370–383.

34. Wei HJ, Zeng R, Lu JH, Lai WF, Chen WH, Liu HY, Chang YT and Deng WP: Adipose-derived stem cells promote tumor initiation and accelerate tumor growth by interleukin-6 production. Oncotarget. (2015) 6(10): 7713–26.

35. Wen Y, Guo Y, Huang Z, Cai J, and Wang Z: Adipose derived mesenchymal stem cells attenuate cisplatin induced apoptosis in epithelial ovarian cancer cells. Mol Med Rep. (2017) 16(6): 9587-9592.

36. Anwar S, Yahia W and Gaballah O: Effect of Platelet Rich Plasma on Regeneration of Submandibular Salivary Gland of Albino Rats. N Y Sci J. (2018) 11(12): 27-40.

37. El-Sharouny SH, Rizk A, Rashed L, Sayed W and Abd Elmoneam M: Analysis of the therapeutic role of platelet-rich plasma against cisplatin-induced hepatotoxicity in rats: controversy between oxidative and apoptotic markers. Eur J Anat. 2019 23(3): 201-213.

38. Halpern B, Chaudhury S and Rodeo S: The role of platelet-rich plasma in inducing musculoskeletal tissue healing. HSS J. (2012) 8(2): 137–145.

39. Jia C, Lu Y, Bi B, Chen L, Yang Q, Yang P, Guo Y, Zhu J, Zhu N and Liu T: Platelet-rich plasma ameliorates senescence-like phenotypes in a cellular photoaging model. RSC Adv. (2017) 7(6): 3152–3160.

40. Moghadam A, Talaei-Khozani T, Mafi A, Namavar MR and Dehghani F: Effects of platelet-rich plasma on kidney regeneration in gentamicin-induced nephrotoxicity. J Korean Méd.Sci. (2017) 32(1): 13–21.

41. Mammoto T, Jiang A, Jiang E and Mammoto A: Platelet rich plasma extract promotes angiogenesis through the angiopoietin1-Tie2 pathway. Microvasc Res. (2013) 89: 15–24.

42. Suzuki E, Fujita D, Takahashi M, Oba S and Nishimatsu H: Adipose tissue-derived stem cells as a therapeutic tool for cardiovascular disease. World J Cardiol. (2015) 7(8): 454–465.

الملخص العربى

دراسة لمقارنة تأثير البلازما الغنية بالصفائح الدموية مقابل الخلايا الجذعية المستخلصة من النسيج الدهني علي الغدد اللعابية تحت الفكية المعالجة المتضررة من السيسبلاتين

شيماء ممدوح دسوقي، أحمد محمود حلاوة، إيمان أحمد فتحي، دينا محمد قاشقوش

قسم بيولوجيا الفم-كلية طب الأسنان-جامعة عين شمس

المقدمة: يعتبر السيسبلاتين ذو فاعلية كبيرة ضد العديد من الأمراض السرطانية ولكن يصاحبه الكثير من الأعراض الجانبية. كما أثبتت الكثير من الدراسات فاعلية كل من الخلايا الجذعية المستخلصة من النسيج الدهني وكذلك البلازما الغنية بالصفائح الدموية في علاج الكثير من الأمراض وأنسجة الجسم المختلفة.

الهدف من الدراسة: دراسة تأثير تقنية العلاج بالبلازما الغنية بالصفائح الدموية وتأثير الخلايا الجذعية المستخلصة من النسيج الدهني علي الغدد اللعابية تحت الفكية التي تعرضت لتأثير عقار السيسبلاتين في الفئران البيضاء عن طريق الميكرسكوب الضوئي والميكرسكوب الإلكتروني النافذ.

مواد وطرق البحث: تم استخدام ٣٨ فأرا ذكرا بالغا، عشرة منهم لاستخلاص البلازما الغنية بالصفائح الدموية والباقي انقسم إلي أربعة مجموعات أساسية: المجموعة الأولي ضابطة سالبة واشتملت علي ٧ فئران. والمجموعة الثانية ضابطة ايجابية واشتملت علي ٧ فئران تم حقنهم بعقار السيسبلاتين داخل الصفاق بجرعة ٥ ميلليجرام/كيلوجرام في اليوم الأول والثامن. المجموعة الثالثة (٧ فئران) تم حقنهم بعقار السيسبلاتين داخل الصفاق بجرعة ٥ ميلليجرام/ كيلوجرام في اليوم الأول والثامن وفي اليوم الثالث من بدء التجربة تم حقنهم بالخلايا الجذعية المستخلصة من النسيج الدهني مرة واحدة عبر وريد الذيل بجرعة ٢ × ١٠٦خلية لكل فأر. المجموعة الرابعة (٧ فئران) تم حقنهم بعقار السيسبلاتين داخل الصفاق بجرعة ٥ ميلليجرام/كيلوجرام في اليوم الأول والثامن وفي اليوم الثالث من بدء التجربة تم حقنهم بالبلازما الغنية بالصفائح الدموية بجرعة ٠,٥ ميلجرام/كيلو جرام ثلاث مرات أسبوعيا. بعد انقضاء المدة الزمنية للتجربة (١٨ يوم) تم أخذ عينات الدم وعينات الغدد اللعابية تحت الفكية من جميع المجموعات وجهزت للفحص بالمجهر الضوئي والإلكتروني.

النتائج: أظهرت نتائج المجموعة الثالثة والرابعة ارتفاعا ذو دلالة إحصائية في المساحة النسبية في الحويصلات وانخفاض ذو دلالة إحصائية في المساحة النسبية للخلايا المفقودة بالموت المبرمج. كما أظهرت النتائج ارتفاع ذو دلالة إحصائية في اختبار الهشاشة الأسموزي لكلا المجموعتين بالمقارنة بالمجموعة الثانية. كما أظهرت النتائج تحسن في التغيرات النسيجية في المجموعتين الثالثة والرابعة علي مستوي المجهر الضوئي والإلكتروني في حين بدت المجموعة الرابعة هي الأقرب للمجموعة الضابطة السالبة.

الخلاصة: تبين من الدراسة أن الخلايا الجذعية المستخلصة من النسيج الدهني وكذلك البلازما الغنية بالصفائح اثبتت فاعليتها في تحسين التأثير الضار لعقار السيسبلاتين علي الغدد اللعابية تحت الفكية، لكن البلازما الغنية بالصفائح الدموية تتميز بأنها أكثر أمانا ولم يثبت لها تأثير ضار علي الخلايا السرطانية او مناعة الجسم.