Effect of mesenchymal stem cells injection on induced stomatitis in chemotherapy treated rats

Asmaa Serry[1], Souzi F. Shinaishin[2] and Khaled El Haddad[3]

[1] lecturer assistant, oral biology department, Faculty of dentistry -BeniSeuif University, Egypt
[2]Head of oral biology department, Faculty of dentistry- Ain Shams University, Egypt
[3] Lecturer, Oral biology department, Faculty of dentistry- Ain Shams University, Egypt
dr.asmaaelgamal@yahoo.com

Abstract: Aim: This study was conducted to evaluate the effect of intravenous injection of mesenchymal bone marrow stem cells (MBMSCs) on induced stomatitis in rats receiving chemotherapy. **Methodology**: All the rats used in the study were subjected to stomatitis induction protocol by scratching the buccal mucosa and divided into 3 groups. gr.1: exposed to stomatitis induction only, gr. 2: injected with 5-FU and exposed to stomatitis induction, gr. 3: injected with 5-FU followed by stomatitis induction then injected with labelledstem cells. At day 10 all groups were sacrificed and subjected to the following investigations: oralmucositis scoring system (OMS), Routine histological examination with H&E, Immunohistochemical profile using PCNA stain and finally florescent microscope. **Results:** It was found that the treated group (gr. 3) showed better improvement than gr.2 in OMS, histological and immunohistochemical evaluation. **Conclusion**: from the present study it was found that intravenous injection of MBMSCs reduce severity of stomatitis in rats receiving chemotherapy.
[Asmaa Serry, Souzi F. Shinaishin and Khaled El Haddad. **Effect of mesenchymal stem cells injection on induced stomatitis in chemotherapy treated rats.** *J Am Sci* 2017;13(1):41-50]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 6. doi:10.7537/marsjas130117.06.

Key Words: chemotherapy induced stomatitis, oral mucositis, stomatitis induction protocol, intravenous stem cells injection, bone marrow mesenchymal stem cells.

1. Introduction:

Chemotherapeutic drugs cause damage to the cells so they are termed cytotoxic drugs. Most chemotherapeutic drugs work by impairing mitosis (cell division). They prevent mitosis by various mechanisms including damaging DNA and inhibition of the cellular organelles involved in cell division **(Makin and Hickman, 2000; Malhotra and Perry, 2003).** As chemotherapy affects cell division, tumors with high growth rates are more sensitive to chemotherapy, as a larger proportion of the targeted cells are undergoing cell division at any time. While malignancies with slower growth rates, tend to respond to chemotherapy much more modestly **(Corrie and Pippa 2008).**

Chemotherapeutic drugs that affect cells only when they are dividing are called cell-cycle specific. While those affect cells when they are at rest are called cell-cycle non-specific (**Renee and John, 2000).**

The chemotherapeutic agents are classified according to their mechanism of action into: Alkylating agents, Anti-metabolites, Anti-microtubule agents, Topoisomerase inhibitors and finally Cytotoxic antibiotics (**Rowinsky and Donehower, 1991; Lodish et al., 2000; Goodsell, 2002**; **Malhotra and Perry, 2003; Minotti, 2004; Lind, 2008; Parker, 2009**).

5-Fluorouracil is one of anti-cancer chemotherapy drug that is classified as an antimetabolite. Fluorouracil is a nucleobase analogue (to the pyrimidine uracil) that is metabolized in cells to form at least two active products; 5-fluourouridine monophosphate (FUMP) and 5-fluoro-2'-deoxyuridine 5'-phosphate (fdUMP). FUMP becomes incorporated into RNA and fdUMP inhibits the enzyme thymidylate synthase; both of which lead to cell death **(Parker, 2009)**.

Administration of chemotherapy usually associated with various side effects including toxicity to the GI tract which has a major clinicalconcernas it is a common limiting factor that prevents further dose escalation and it is often a major cause of cancer treatment- related morbidity. GIT toxicity is not simply confined to the upper gastroduodenal mucosa but also extends along the entire GI tract from the mouth to anus Stomatitis. Stomatitis (the mucositis of oral mucosa) is the best-characterized manifestation as it results in symptoms in an area accessible to routine examination **(Jaimeet al., 2003)**.

Generally, the earliest signs and symptoms of chemo/radiotherapy induced oral mucositis include erythema, edema, a burning sensation, and an increased sensitivity to hot or spicy food. Erythematous areas may develop into elevated white desquamative patches and subsequently into painful ulcers. The latter are not only often secondarily infected, but also impair nutrition and fluid intake resulting in malnutrition and dehydration **(Peterson and Dambrosio, 1992; Wolfgang et al, 2001)**. Younger patients seem to be at greater risk of

chemotherapy-induced oral mucositis because their epithelium has a higher mitotic rate and more epidermal growth factor receptors **(Karis, 2007)**.

However, there is no gold-standard protocol that is prominently better than the rest. the strategies to reduce oral mucositis are still unclear **(Kassab et al., 2009; Clarkson et al., 2010; Rodrı´guezet al., 2012)**.

There are many treatments have been studied to prevent and treat oral mucositis including: Intensive oral care protocol, Antimicrobial agents, Anti-inflammatory agents, Nutritional supplements, Natural and homoeopathic agents, Bio-stimulants, Cryotherapy, Low-energy laser therapy **(Rodrı´guez-et al., 2012)**.

One of the promising methods of controlling mucositis is the use of stem cells. Under the right conditions, stem cells have the potential to develop into mature cells that have characteristic shapes and specialized functions, such as heart cells, skin cells, or nerve cells **(Slack, 2000).**

Many authors used different type of stem cells to treatment of chronic wounds **(Evangelos and Vincent, 2003)**, accelerate wound healing **(Wu et al., 2007)**, radiation-induced intestinal injury prevention **(Kohsei et al., 2009)**, ulcer closure **(El-Menoufy et al., 2010)**.

One possible application of stem cell research is the repair of tissue injuries related to the side effects of chemotherapy and radiotherapy and ameliorating radiation-induced complications. So we will investigate whether stem cell therapy will control the mucosal damage caused by chemotherapy or not.

Aim of the Study

The aim of the present study is to evaluate the effect of intravenous injection of mesenchymal bone marrow stem cells on induced stomatitis in rats receiving chemotherapy.

2. Materials and Methods

Samples:

42 male Wister rats weighting around 250 grams were used in the present study**.** Rats were housed in wire mesh cages under controlled temperature and good ventilation. All rats were fed on standardized laboratory balanced diet.

Stem cells:

Mesenchymal bone marrow stem cells (MBSCs) were purchased from biochemistry department in faculty of medicine Cairo University. Stem cells were labeled with PKH26 die that is a red fluorescent linker die binds to the cell membrane of stem cells. The animals were injected with a dose of $1.5 \times 10^{(6)}$ cell/body **(Mahmood et al., 2003).**

Chemotherapy:

5-Fluorouracil (5-fu) had been used in this study. The administered dose was: 100mg/kg at the 1st day of the experiment followed by 65mg/kg at the 3rd day of the experiment **(Aras et al., 2013).**

Grouping of the animals:

Rats were divided into 3 groups (2 experimental and 1 control) each group consisted of 14 rats: **Group 1**(the rats were exposed to stomatitis induction without injection of 5-FU or stem cells), **Group 2**: (the rats were injected with 5-FU and exposed to stomatitis induction without stem cells injection and **Group 3**: (he rats were injected with 5-FU followed by stomatitis induction then injected by stem cells).

Each group was subdivided into 2 subgroups according to the time of scarification as follow:

Subgroup A: 1A, 2A and 3A (7 rats were sacrificed at day 8).

Subgroup B: 1B, 2B and 3B (7 rats were sacrificed at day 10).

Methodology:

1- **Administration of chemotherapy:** at the 1st day of the experiment, Intraperitoneal injection of 5-Fluorouracil (5-fu) was applied to groups 2, 3 and repeated at day 3(with the mentioned dose).

2- **Induction of stomatitis:** Stomatitis induction protocol was applied to all groups of the experiment. This protocol was performed according to **Sonis et al., (1990).** The rats were anesthetized and the left buccal mucosa was everted. Superficial scratching of mucosa was performed using a tip of 18-gauge needle by dragging it several times in a linear fashion across the everted cheek pouch at the 3rd day of the experiment and repeated again at the 4th and 5th day till the erethymetous patch appeared on the mucosa.

3- **Injection of Stem Cells:** The animals were intravenously injected with MBSCs at the tail vein at the 5th day of experiment.

4- The buccal mucosa of the rats were everted and photographed. The oral mucositis scoring system (OMS) was evaluated according to **(Parkins et al., 1983)** as shown in table 3.

Table 3: Oral mucositis scoring system.

Score	Description
0	Normal
0.5	Slight pink
1.0	Slight red
2.0	Severe reddening
3.0	Focal desquamation
4.0	Exudation covering less than one half of mucosa
5.0	Virtually complete ulceration of mucosa

5- **Weighting the animals:** The weight of the animals in all groups was monitored every 2 days.

6- **Samples collection and processing:** The animals were killed by overdose of sodium thiopental**.**

Buccal mucosa was dissected, fixed in 10% buffered formalin and processed for examination with: routine Haematoxylin and Eosin stain, Immunohistochemical stain using Proliferating Cell Nuclear Antigen (PCNA) and Fluorescent microscope.

7- **Analizing the data**: Data of OMS score, body weight loss and digital image analysis of PCNA stain were tabulated and statistically analyzed by SPSS (statistical package for social science) software using **One sample T test** was to measure the mean and standard deviation (std. deviation) of each subgroup and **Paired sample T test** to compare between the experimental groups and control group at day 8 and 10.

3. Results

Stomatitis examination:

Oral mucositis Score (OMS) was performed (fig. 1) and the data were analyzed and summarized in tables 2,3 and graph. 1:

At day 8, the highest mean value of OMS was that of subgroup 2A, then subgroup 3A and finally subgroup 1A. While at day 10, the highest mean value of OMS was that of subgroup 2B then subgroup 3B. The least mean value was of subgroup 1B. THESE results indicates better improvement in treated group specially at day 10 (as the difference between the treated group and control group was significant).

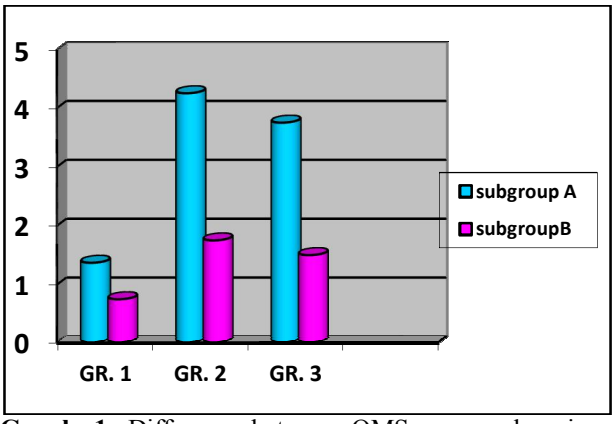
Fig.(1): differences between the 7 stages of OMS

Table (2): The mean values and standard deviation of OMS in each group

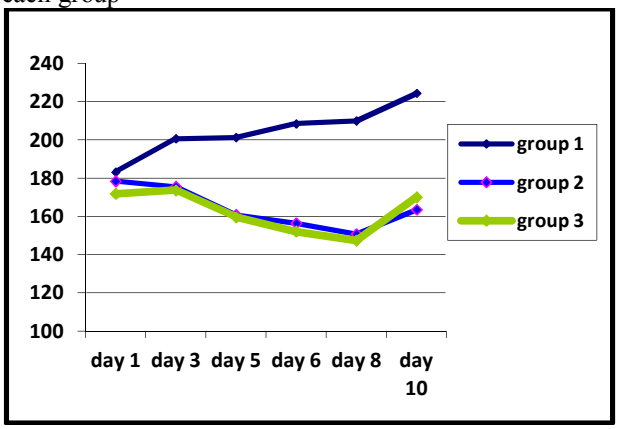
Subgroup	N	Mean	Std. Deviation	Std. Error Mean
1A	8	1.3750	.87627	.30981
1B	8	.7500	.37796	.13363
2A	8	4.2500	1.16496	.41188
2B	8	1.7500	1.00000	.35355
3A	8	3.7500	1.28174	.45316
3B	8	1.5000	1.03510	.36596

Table (3): Paired comparisons between OMS mean values of each experimental group with control group at day 8 and 10 (sub groups A and B).

Pairs of comparison	Sig.(2-tailed)		Df	T	Paired Differences				
					95% Confidence Inerval of the Difference		Std. Error Mean	Std. Deviation	Mean
					Upper	Lower			
1A – 2A	Pair 1	.001	7	-5.675	-1.67696	-4.07304	.50665	1.43303	-2.8750
1A – 3A	Pair 2	.005	7	-4.038	-.98412	-3.76588	.58820	1.66369	-2.3750
1B – 2B	Pair 3	.050	7	-2.366	-.00076	-1.99924	.42258	1.19523	-1.000
1B – 3B	Pair 4	.096	7	-1.925	.17125	-1.67125	.38960	1.10195	-0.750



Graph. 1: Difference between OMS mean values in each group



Graph. 2: monitoring of the body weight in each group

Body weight loss:

At the end of the experiment gr. 3 was the least group in weight loss compared with gr. 2 but the difference between them was non significant (graph. 2).

H&E stain:

The histological examination (fig. 2) revealed that, **Subgroup 1A** showed small ulcer filled with granulation tissue highly infiltrated with inflammatory cells and discontinuity of epithelial layer**.** While **subgroup 1B** revealed that most of the specimens showed continuous intact basal cell layer and lamina propria showed moderate infiltration of inflammatory cells, dilated blood vessels.

Subgroup 2A showed almost complete necrosis of the epithelium (except small areas) overlying degenerated C.T. the lamina propria revealed marked decrease in cellularity with low infiltration of inflammatory cells, While **Subgroup 2B** revealed necrotic epithelium and C.T. in addition, the epithelium appeared detached in some areas.

Subgroup 3A showed ulceration of mucosa as the epithelium appeared necrotic overlying C.T. with marked vasodilatation of blood vessels that was engorged with coagulated blood. Unlike **Subgroup 3B**which revealed continuous epithelial layer and the C.T. was infiltrated with inflammatory cells.

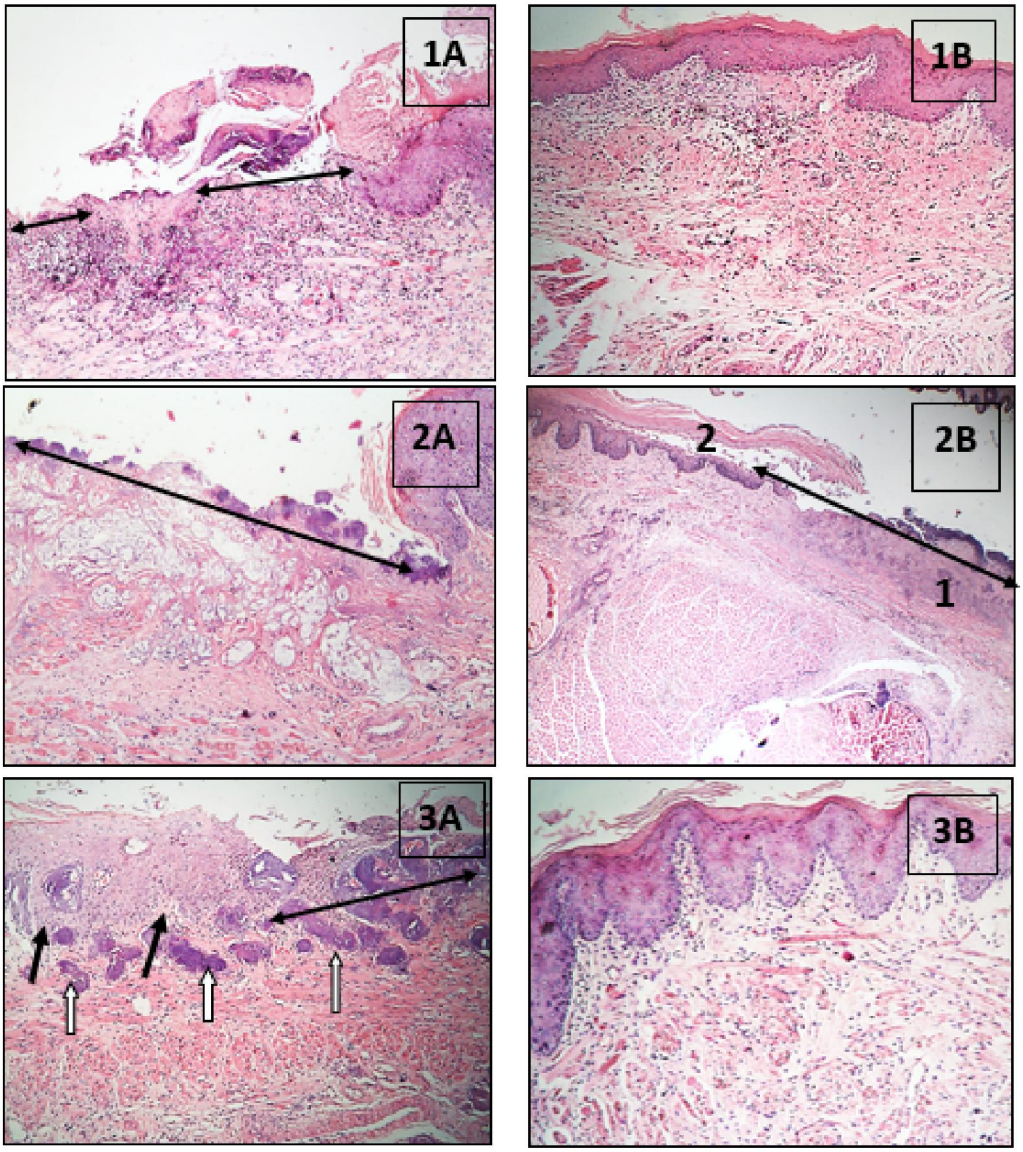


Fig. (2): showing the differences between all groups (H&E x100).
1 A: a photomicrograph of subgroup 1a showing an ulcerated mucosa
2B: intact basal cell layer, C.T. slightly infiltrated with inflammatory cell
2A: ulcerated mucosa and discontinuity of basal cell layer
2B: (1) ulcerated mucosa and (2) detached epithelium.
3A: ulceration of mucosa, disorganization of basal cells (black arrows) and enlarged bl. v. engorged with coagulated blood (white arrows)
3B: showing continuous epithelium.

Immunohistochemical results:

The data was illustrated in fig. (3), tables 4, 5 and graph. 3day 8, the mean values of PCNA stain of experimental groups showed a significant decrease than that of the control group (P-values were .001 and .000) which indicated no improvement in these subgroups.

On the other hand, at day 10 the mean values of PCNA stain of subgroup 2B showed a significant decrease than that of the control group (P-values were.000) which indicated no improvement in this group. While the experimental group 4B showed a non significant decrease in mean value than that of the control group (P-values.158) which indicated better improvement in this group.

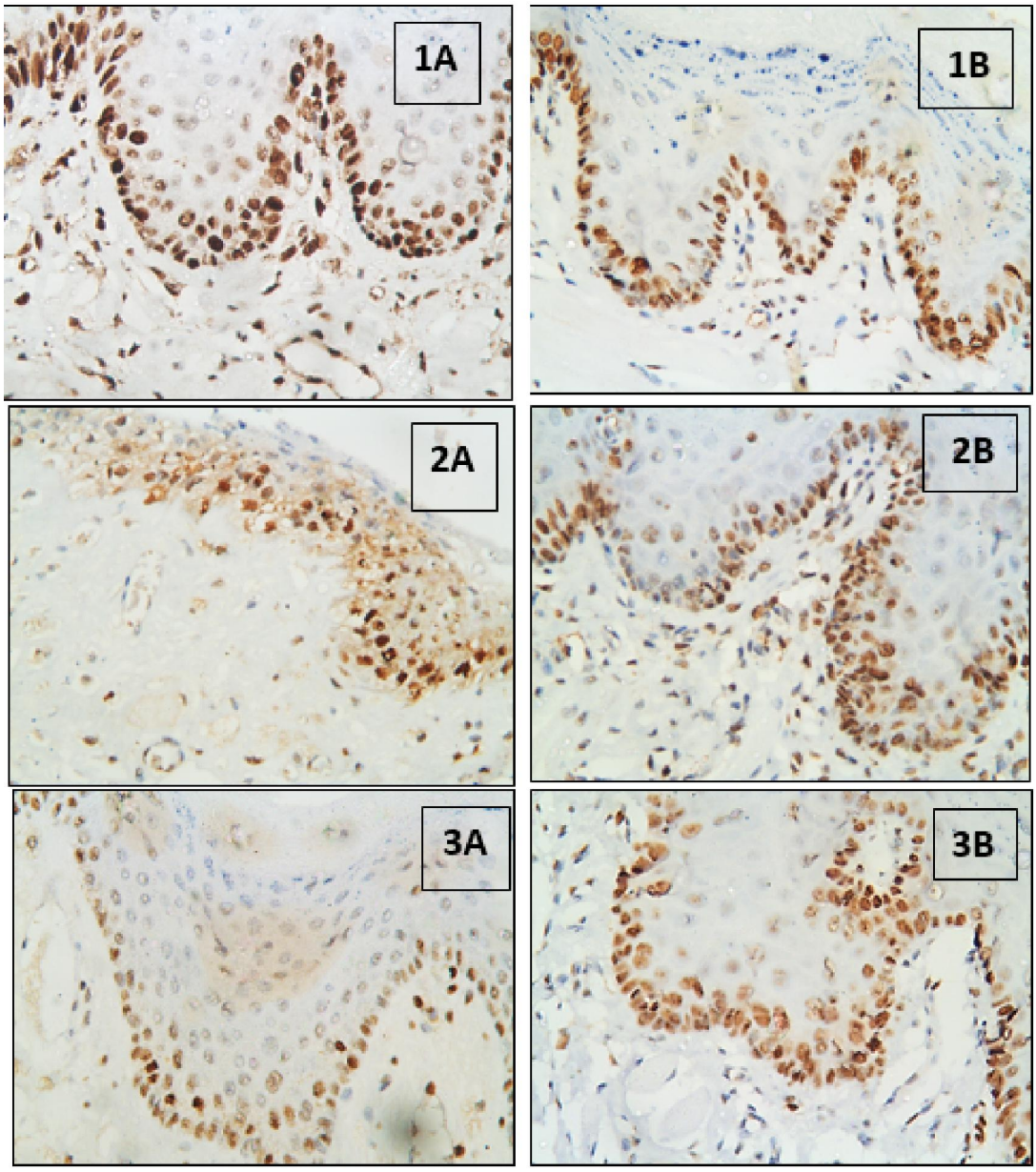


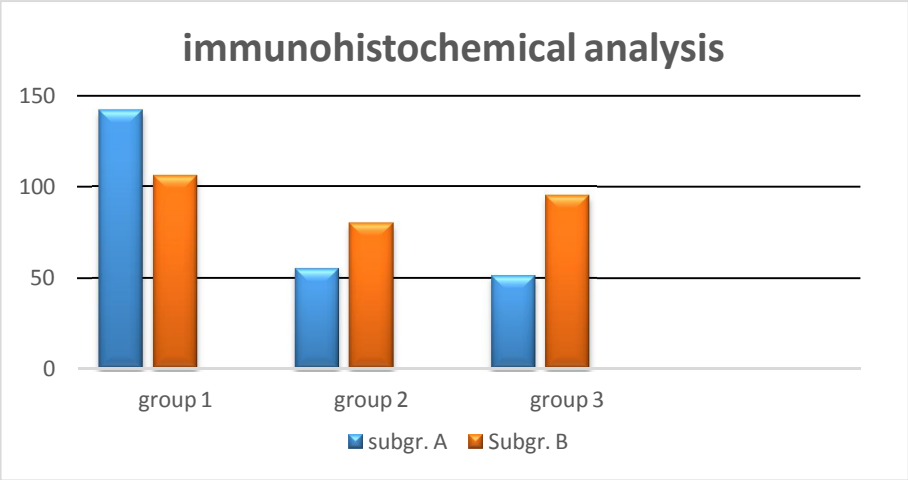
FIG.(3): demonstrate the differences of PCNA stain intensity in each group

Table (4): mean and std. deviation of PCNA intensity in each group

group	N	Mean	Std. Deviation	Std. Error Mean
GR. 1A	7	142.2857	24.49295	9.25747
GR. 2A	7	54.7143	17.21157	6.50536
GR. 3A	7	51.4286	12.73914	4.81494
GR. 1B	7	106.4286	15.41490	5.82628
GR. 2B	7	80.4286	13.74600	5.19550
GR. 3B	7	95.0000	9.64365	3.64496

Table (5): comparison of PCNA stain between the experimental groups and control group at day 8 and 10 (sub group A and B)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	1A - 2A	87.57143	36.32656	13.73015	53.97496	121.16789	6.378	6	.001
Pair 2	1A - 3A	90.85714	32.26675	12.19568	61.01538	120.69891	7.450	6	.000
Pair 3	1B - 2B	26.00000	7.02377	2.65474	19.50410	32.49590	9.794	6	.000
Pair 3	1B - 3B	11.42857	18.75151	7.08740	-5.91368-	28.77082	1.613	6	.158



Graph. 3: image analysis of PCNA stain.

Florescent microscope results:

Examination of the specimens showed increased number of stem cells aggregates in the buccal mucosa which indicated successful migration of stem cells (fig. 4).

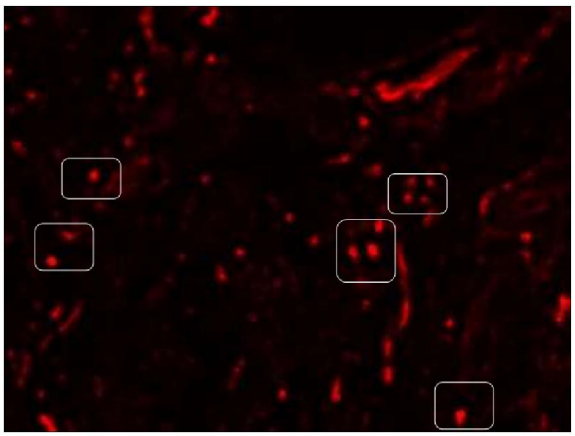


Fig.(4) Photomicrograph of group 3 showing: aggregates of MSCs labeled with PKH 26 (white rectangle)

4. Discussion:

In the present study we used 5-Florouracil (5-FU) as a chemotherapeutic drug for stomatitis induction protocol in rats as it's the first drug of choice in treatment of GIT cancer and it is known to cause severe oral GIT and mucositis **(Sonis et al., 1990)**.

Researchers have looked for years for ways to use stem cells to replace cells and tissues which are damaged or diseased. Hence, stem cells have recently received much attention**.** There are many studies were carried out to investigate the effectiveness of different types of stem cells on radiotherapy side effects as **Bensidhoum et al., (2005); Bhatt et al., (2011)** and **Aboushady et al., (2012)**.

Adult stem cells are not yet established to be tumorigenic when administered to humans. In this category, mesenchymal stromal cells (MSCs) are the right option because of the feasibility of isolation and expansion in culture in large numbers and multiple available sources **(Young et al., 1998).** Bone marrow derived-MSCs (BM-MSCs) are one of the first known MSCs and are also the most advanced in clinical trials. For these reasons, these cells generally serve as the 'gold standard' against which, other MSCs sources are

compared **(Klingemann et al., 2008)**. In agreement with these, bone marrow mesenchymal stem cells (BM-MSCs) were used in the present study.

In the present study, we preferred intravenous injection to evaluate migration of stem cell to mucositis site after chemotherapy administration due to its feasibility; it is considered the least invasive technique and finally its systemic effects that leads to treatment of several diseases induced by chemotherapeutic drug. there are many scientists also preferred intravenous injection of stem cells in treatment of different diseases as **Zhange et al., (2012)** studied its effect on chemotherapy induced stomatitis and **Bhatt et al.,(2011)** who performed a studied its effect on chemotherapy induced alimentary mucositis.

In the present study we evaluated the stem cell migration to the site of mucositis by labeling them with PKH26 fluorescent linker dye. This linker dye is ideal for in vitro cell labeling and long term in vivo cell tracking as the dye is stable and divides equally when the cells divide **(Aboushady et al., 2012)**.

BM-MSCs in the current work appeared in the buccal mucosa by florescent microscope at day 8, 10 after stem cells injection which indicates its ability to migrate to the site of injury after chemotherapy injection. This is in agreement with some studies that was performed to evaluate the migration of stem cells to the affected organs after radiotherapy treatment (**Chapel et al., 2003; Aboushady et al., 2012)** or afterinduced colitis (**Manuel et al., 2009).**

Group 1; In the present study it was noticed that, group 1 (irritated only) showed better improvement than the other groups as at day 8 and 10 which is in agreement with **Sonis et al., (1990)**.

Group 2; In contrary to group 1, group 2 (chemotherapy and irritation) showed the worst improvement (either subgroup A or B). This is in agreement with some studies that investigated the effect of chemotherapy on the mucosa as **Bultzingolowen et al., (2001)** who investigated the effect of 5-FU on the oral epithelium of rats. Also **Abou-elez et al., (2013)** studied the effect of 5-fu on the rat small intestine and he found that administration of 5-fu causes detachment and loss of epithelium surface in addition increase in intercellular spaces.

Group 3; In the present study, it was obvious that the histological evaluation of the treated group at day 8 **(subgroup 3A)** showed numerous enlarged blood vessels engorged with coagulated blood (unlike group 1, 2). This blood clotting is coincide with several studies that performed to evaluate the effect of systemic MSCs injection on the circulation as **Catalin et al., (2009)** who tracked intraarterially delivered MSCs and found that most of MSCs arrest and interrupt flow during first pass at the precapillary level, resulting in decreased flow in the feeding arteriole which by turn leads to ischemia to the supplied tissue. Also **Dario et al., (2009)** investigated the kinetics of human mesenchymal stem cells (MSCs) after intravascular administration. He found that: marked decrease in blood velocity in arteriols and venules after injection, MSCs were entrapped in capillaries and importantly, many animals died of pulmonary embolism and thrombus formation was detected in arterioles and venules of the living animals. In contrary to subgroup 3A **subgroup 3B** showed better improvement than group 2B which indicating success of MSCs in redicing stomatitis severity. there are some studies that demonstrate its therapeutic effect on stomatitis and thier results coincide with the present study as **Batt et al., (2011)** who explored the feasibility of using human gingival mesenchymal stem cells (hGMSC) to reduce the severity of induced mucositis on the ventral surface of the tongue and observed a significant reduction of mucositis at day 10. **Zhange et al., (2013)** also reported a 3D spheroid culture of gingival mesenchymal stem cells (GMSCs) to optimize stem cell properties and therapeutic effects. Then he performed a study to evaluate its effect on chemotherapy induced mucositis in comparison with normal adherent stem cell. He found a significant reduction of mucositis at day 7. Concerning the results of normal adherent stem cells, it is in agreement with our study with some differences that may led to faster improvement at day 7 (compared with the present study as the improvement started at day 10). These differences are: (1) the type of stem cells used, (2) dose of chemotherapy as he repeated it for 3 days, (3) he didn't irritate the tongue with the needle and finally (4) the mucosa used for the study was the tongue not the buccal mucosa.

Finally, the present study revealed the ability of BM-MSCs to accelerate the healing of chemotherapy induced oral mucositis in rats. This was supported by oral mucositis score (OMS), monitoring weight loss of the animals, histological, immunohistochemical and florescent examination of the specimens.

There are different studies that were performed to investigate the role of stem cells in treatment of different diseases. **El-Menoufy et al., (2010)** found that BM-MSCs accelerate healing of induced oral ulcers in dogs. He found also increased expression of collagen and VEGF (vascular endothelial growth factor) genes in MSCs-treated ulcers compared with controls. So he concluded that MSCs help in acceleration of oral ulcer healing through the induction of angiogenesis by VEGF together with increased intracellular matrix formation as detected by increased collagen gene expression. It was interested that, The high-dose group even showed better surface

epithelialization than the low-dose group. He regarded this to higher expression of VEGF and collagen gene in MSCs-treated group compared with the control group.

References

1. Aboushady I.M., Mubarak, R.T., El-mougy, S. A. F., Rashed, L.A. and El-desouky, A.A., (2012): The Effect of Transplanted Bone Marrow Stem Cells on the Tongue of Irradiated Rats (Histological and Immunohistochemical study). Journal of American Science; 8(11)553- 561.
2. Abou-Elez G.S.A., Nosseir D.A., Omar N.M. and Sarhan N.M.R. (2013): Histological and ultra structural study of 5-florouracil-induced small intestinal mucosal damage in rats. Asian journal of cell biology; 8(1):1-12.
3. Aras M.H., Sezer U., Erkilic S., Demir T. and Dagli S.N. (2013): Effect of dietary boron on 5-fluorouracil induced oral mucositis in rats. Eur J Dent; 7:310-4.
4. Aziz A.L., Menoufy H.E., Ragae A., Rashed L.A. and Sabry D., (2012): Adipose stem cells as alternatives for bone marrow mesenchymal stem cells in oral ulcer healing. Int J Stem Cells; 5:104-14.
5. Bensidhoum M., Gobin S., Chapel A., Lemaitre G., Bouet S., Waksman G., Thierry D. andMartin M.T. (2005): Therapeutic effect of human mesenchymal stem cells in skin after radiation damage. J SocBiol; 199(4):337-41.
6. Bhatt A., (2011): Mesenchymal stem cells from human gingiva ameliorate murine alimentary mucositis. a thesis presented to the faculty of the usc graduate school university of southern California. Uscthesesreloadpubvolum 62/edt-Bhatt-4495.
7. Catalin T., William R. W., Shivani B., Abigail S., and Flordeliza V. (2009): Fate of Culture-Expanded Mesenchymal Stem Cells in the Microvasculature: In Vivo Observations of Cell Kinetics. Circ Res; 104(3):398–402.
8. Chapel A., Bertho J.M., Bensidhoum M., Fouillard L., Young R.G., Frick J., Demarquay C., Cuvelier F., Mathieu E., Trompier F., Dudoignon N., Germain C., Mazurier C., Aigueperse J., Borneman J., Gorin N.C., Gourmelon P. andThierry D. (2003): Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. J Gene Med.; 5(12):1028-38.
9. Clarkson J.E., Worthington H.V., Furness S., McCabe M., Khalid T. and Meyer S. (2010): Interventions for treating oral mucositis for patientswith cancer receiving treatment. Cochrane Database Syst Rev; 4: CD001973.
10. Corrie P.G. and Pippa G., (2008): Cytotoxic chemotherapy: clinical aspects. Medicine; 36 (1): 24–28.
11. Dario F., Murat U., LeeLee O., Karen B., Erik P., Ingeborg W., Weiwei W., Can Y., (2009): Is the intravascular administration of mesenchymal stem cells safe? Mesenchymal stem cells and intravital microscopy Wenzhong L., Ralf G., Ren-ke., Brigitte V., Gustav S., Nan M. Microvascular Research 77: 370–376.
12. El-Menoufy H., Aly L.A., Aziz M.T., Atta H.M., Roshdy N.K., Rashed L.A. and Sabry D., (2010): The role of bone marrow-derived mesenchymal stem cells in treating formocresol induced oral ulcers in dogs. J Oral Pathol Med.;39:281–289.
13. Evangelos V. B. and Vincent F., (2003): Treatment of Chronic Wounds with Bone Marrow–Derived Cells. *Arch* Dermatol.; 139 (4):510-516.
14. Jaime A. Y., Xiao W. T., Kathryn A. R., Marc W. F. and Neal M. D. (2003): Chemotherapy Induced Gastrointestinal Toxicity in Rats: Involvement of Mitochondrial DNA, Gastrointestinal Permeability and Cyclooxygenase –2. J Pharm Pharmaceut Sci, 6(3):308-314.
15. Karis K.F.C., (2007): Oral mucositis, dysfunction, and distress in patients undergoing cancer therapy. J Clin Nurs; 2114–20.
16. Kassab S., Cummings M., Berkovitz S., vanHaselen R. and Fisher P., (2009): Homeopathic medicinesfor adverse effects of cancer treatments. Cochrane Database Syst Rev;15: CD004845.
17. Klingemann H., Matzilevich D. andMarchand J. (2008): Mesenchymal Stem Cells – Sources and Clinical Applications. Transfus Med Hemother; 35:272-277.
18. Kohsei K., Yong L., Kenji T., Kohetsu T., Minoru O., Dong-Liang H., Ikuo K., Hiroshi K. and Akio N.,(2009): Transplantation of mesenchymal stem cells to prevent radiation-induced intestinal injury in mice.Journal of Radiation Research; 51(1):73-9.
19. Lind M.J., M.J. (2008). Principles of cytotoxic chemotherapy. Medicine; 36 (1): 19–23.
20. Lodish H., Berk A. and Zipursky S.L., (2000): Molecular Cell Biology. 4th edition. The Role of Topoisomerases in DNA Replication. New York: W. H. Freeman.
21. Mahmood A., Lu D., Lu M. and Chopp M. (2003): Treatment of Traumatic Brain Injury in Adult Rats with Intravenous Administration of

Human Bone Marrow Stromal Cells. Neurosurgery; 53(3):697-703.
22. Makin G. and Hickman J.A. (2000): Apoptosis and cancer chemotherapy. *Cell Tissue* Res.; 301 (1): 143–52.
23. Malhotra V. and Perry M.C., (2003): Classical chemotherapy: mechanisms, toxicities and the therapeutic window. *Cancer Biol. Ther.*; 2 (4 Suppl 1): S2–4.
24. Manuel A. G., Elena G.R., Laura R., Dirk B., and Mario D., (2009): Adipose-Derived Mesenchymal Stem Cells Alleviate Experimental Colitis by Inhibiting Inflammatory and Autoimmune Responses. Gastroenterology; 136:978–989.
25. Minotti G., Menna P., Salvatorelli E., Cairo G., Gianni L. (2004): Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev.*; 56 (2): 185–229.
26. Parker W.B. (2009): Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. Chemical Reviews. 109 (7): 2880–93.
27. Parkins, C.S., Fowler, J.F., Yu, S., 1983. A murine model of lip epidermal/mucosal reactions to X-irradiation. Radiother. Oncol. 1,159–165.
28. Peterson D.E. (1992): Oral toxicity of chemotherapeutic agents. SeminOncol, 19:478-491.
29. Renee K. F. And John C.P., (2000): optimal control applied to cell-cycle-specific cancer chemotherapy. SIAM J. APPL. MATH.; 60(3): 1059–1072).
30. Rodrı´guez-Caballero A., Torres-Lagares D., Robles Garcı´a M., Pacho´n-Iba´n˜ez J., Gonza´lez-Padilla D. and Gutie´rrez-Pe´rez J. L. (2012): Cancer treatment-induced oral mucositis: a critical review. Int. J. Oral Maxillofac. Surg., 41: 225–238.
31. Rowinsky E.K., Donehower R.C. (1991): The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. Pharmacol. Ther.; 52 (1): 35–84.
32. Sonis S.T., Tracey C., Shklar G., Jenson J. and Florine D. (1990): An animal model for mucositis induced by cancer chemotherapy. Oral Surg Oral Med Oral Pathol; 69(4):437-443.
33. Von Bultzingolowen I., Jontell M., Hurst P., Nannmark U. and Kardos T. (2001): florouracil induces autophagic degeneration in rat oral keratinocytes; oral oncology; 37:537- 544.
34. Wolfgang J.K., Michael H., Catharina W., and Christoph C.Z., (2001): Oral Mucositis Complicating Chemotherapy and/or Radiotherapy: Options for Prevention and Treatment. CA Cancer J Clin; 51: 290-315).
35. Wu Y., Chen L., Scott P. G., Tredge E. E., (2007): Mesenchymal Stem Cells Enhance Wound Healing Through Differentiation and Angiogenesis. STEM CELLS, 25:2648–2659.
36. Young R.H., Butler D.L., Weber W., Caplan A.I., Gordon S.L. and Fink D.J. (1998): Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res.; 16(4):406-13.
37. Zhange Q, Andrew L. N., Shihong S., Colin H., Petra W., Tatiana B. K., and Anh D. L. (2012): Three-Dimensional Spheroid Culture of Human Gingiva-Derived Mesenchymal Stem Cells Enhances Mitigation of Chemotherapy-Induced Oral Mucositis. stem cells and development, 21(6): 937-947.

1/11/2017