Original Article

A Comparative Study Between The Therapeutic Role of Adipose Derived Mesenchymal Stem Cells and Omeprazole in Regeneration of Gastric Ulcer Induced by Aspirin in Albino Rats : Histological and Immunohistochemical Study

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ABSTRACT

Background: Aspirin induced gastric mucosal injury, which leads to gastric ulcer. Adipose Derived Mesenchymal Stem Cells (ADMSC) can be used for treatment of gastric ulcer as they can differentiate into many cell types. Omeprazole is used for treatment of gastric acid-related disorders.

The Aim of the Work: Was to compare between the therapeutic role of ADSMC and omeprazole in treatment of gastric ulcer

Material and Methods: Forty male albino rats were divided into four equal groups: 1-Control group. 2-Aspirin group in which rats were fasted for 24 hours, then given acetylsalicylic acid (200 mg/kg) once orally for induction of ulcer. 3-ADMSC-treated group in which rats were treated by (3×10^6) ADMSCs/ rat once by intravenous injection four hours after induction of ulcer. 4-Omeprazole-treated group in which rats were treated by omeprazole suspension (20 mg/kg) orally, four hours after induction of ulcer, and for five days. Gastric juice volume and acidity, length of gastric ulcer, and histopathological and immunohistochemical changes of the stomach were all evaluated.

Results: Aspirin induced erosion and loss of architecture of gastric mucosal epithelium, ADMSC treatment reduced the length of gastric ulcer, ameliorate histopathological changes and reduced the PCNA-LI also the omeprazole treatment promoted healing of the gastric ulcer induced by aspirin but the therapeutic role of ADMSC was significantly lower in ulcer index and PCNA-LI and better in PAS reaction and histopathological healing of gastric ulcer than the therapeutic role of omeprazole.

Conclusion: The therapeutic role of ADMSC was found to be superior to omeprazole in treatment of gastric ulcer.

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Key Words: ADMSC, aspirin, gastric ulcer, omeprazole.

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INTRODUCTION

The gastric ulcer caused by decline in protective factors in stomach such as mucus, prostaglandins, bicarbonates and rise in the noxious factors as hydrochloric acid, bile salts and pepsin. Aspirin, non-steroidal anti-inflammatory drug one of the most common causes of peptic ulcers[1].

Aspirin is used as antipyretic, analgesic and anti-inflammatory effects. It is taken without medical supervision as it is easily available and low cost. Some patients may exceed the maximum daily dose especially in chronic illness[2].

Aspirin stimulates secretion of hydrochloric acid (HCL) and cause failing of mucous layer which acts as barrier for gastric wall. Aspirin decreases production of prostaglandin (PG) by sup rising cyclooxygenase (COX) enzymes and reduces the production of mucus and bicarbonates. In addition, it increases reactive oxygen species (ROS) and causes mucosal injure[3].

Adipose tissue is a rich and easily available tissue for obtaining multi potent stem cells that have the same phenotypes as bone marrow mesenchymal stem cells[4].

Adipose derived mesenchymal stem cells ADMSC can differentiate into numerous cell types, as endothelial cells. Also the ADMSC secrete anti-apoptotic and angiogenic factors. They provoke renewal of injured tissue through production of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and adiponectin. They can motivate angiogenesis, get better microcirculation in the gastric mucosa, and speed up the healing rate of the gastric mucosa[5].

The Omeprazole is one of the proton pump inhibitors that reduce the amount of HCL production in the stomach. It protects the mucosa and promotes healing of mucosal injury. It is prescribed for treatment of peptic ulcer and its complications. But Omeprazole like any drug has some side effects[6].

Long term proton pump inhibitor therapy could cause moderate hyper-gastrinaemia in the majority of patients and enterochromaffin like cell hyperplasia. Gastric tumors may be induced in long-term therapy[7].

The present study was planned to compare between the role of ADMSC and omeprazole in treatment of gastric ulcer.

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MATERIAL AND METHODS

Experimental animals

Experimental study was carried out at Anatomy and Embryology Department of Benha faculty of medicine, Benha University, in this study we used forty normal adult male albino rats, aged from 3 to 4 months and body weight about 200 to 250 gm. They were obtained from Moshtohor Faculty of Veterinary Medicine. All rats were acclimatized to the laboratory environment. The rats were given normal diet. All animals had received a human care with animal care strategy of the National Institutes of Health and the Ethical Committee for scientific research had accepted the design of the experiments.

Materials

1. Acetylsalicylic: Aspocid; acetylsalicylic acid; 75 mg tablets were brought from chemical industries development (cid) Company. It was prepared by dissolving one tablet in 15ml distilled water and accordingly a volume of 1 ml of this solution contained about 5 mg, of Acetylsalicylic acid.
2. Omeprazole: Omez 20 mg capsules were obtained from Pharaonia Pharmaceuticals Company. It was ready by dissolving one capsule in 20ml distilled water, consequently a volume of 1 ml of this solution contained about 1 mg of omeprazole.
3. Adipose derived-mescenchymal stem cells: (ADMSC) these cells were obtained from Department of Histology, Kasr Al-Ainy Faculty of Medicine, Cairo University.

Preparation of AD-MSCs from rats

The pre-peritoneal adipose tissue was removed from the rats. The specimens were cleaned with phosphate buffer saline (PBS) to remove any contamination. The yellowish white tissue was minced and enzymatically digested in Dulbecco's Modified Eagle's Medium (DMEM) containing 0.075% collagenase type 2 at 37ºc for 60 min with constant shaking every10 min. The suspension was filtered by mesh then centrifuged for 10 min at 25 °C. Pellets were re-suspended in complete medium (DMEM) that, was supplemented with 10% FBS and 1% Penicillin / Streptomycin) and incubated at 37°C with 5% Co_2. Non–adhesive cells and erythrocytes were removed from the culture. The medium was distorted twice per week, and the cells were sub-cultured when 80% confluence was reached. The third passage cells were utilized in this study[8].

Induction of gastric ulcer

The rats were fasted one day before ulcer induction. The ulcer was induced by the oral administration of acetylsalicylic acid (200mg/kg body weight) as a single dose through a gastric tube. After 4 hours, two rats were anesthetized to prove occurrence of gastric ulcers by two histo-pathologists[3].

Experimental design

The animals were separated into four groups; each group consisted of ten rats:

1-Control group: Ten rats were divided into two subgroups:

Subgroup Ca: It included five rats which were fed on basal diet only; then sacrificed after five days.

Subgroup Cb: It included five rats which were intravenously injected with PBS, then sacrificed after five days.

2-Aspirin group: The rats were fasted for 24 hours, before given the acetylsalicylic acid: the latter was given in a single dose (200 mg/kg body weight acetylsalicylic acid) orally by a gastric tube. Then, rats were sacrificed after four hours[6].

3- ADMSC-treated group: The rats were fasted for 24 hours, before given the acetylsalicylic acid: the latter was given in a single dose (200 mg/kg body weight acetylsalicylic acid) orally by a gastric tube. After four hours rats had received an intravenous injection of 3×10^6 ADSCs/rat via tail vein once time, then the rats were sacrificed after five days[9].

4- Omeprazole-treated group: The rats were fasted for 24 hours, before given the acetylsalicylic acid. The latter was given in a single dose (200 mg/kg body weight acetylsalicylic acid) orally by a gastric tube. After four hours, the rats had received the omeprazole suspension in a dose of 20 mg/kg omeprazole orally by a gastric tube daily for five days then the rats were sacrificed[6].

All rats were anaesthetized by inhalation of diethyl ether. Midline abdominal incision was then performed; the stomach was removed after the esophageal end had been tied. The stomach was incised along the greater curvature and the gastric contents were put in tubes.

Calculation the gastric juice volume

The stomachs were opened then the gastric juice were collected and centrifuged at 1000 g for 10 minutes for removing any solid debris. After that we measured the volume of the supernatant[10].

Evaluation the acidity of gastric juice

A volume of 1 ml of gastric juice was diluted by ten ml of distilled water in a flask, and then added from 2 to 3 drops of Topfer's reagent as an indicator to the flask after that titrated with 0.01 NaOH until observation of canary yellow color. The volume of NaOH (ml) added was well-known corresponding to free acidity[11].

Estimation of gastric damage

The stomach was incised along the greater curvature then cleaned with saline for removal of blood clots. Each lesion was measured along the greatest length, the sum of length (mm) of these lesions for each stomach were calculated and used as ulcer index[12].

Histological study

Gastric tissue was taken from all groups, then fixed at 10% neutral buffered formalin (pH=7. 0), then dried in ethyl alcohol, and cleaned in xylol finally was put in paraffin. Four microns thickness sections were prepared and stained with Haematoxylin and Eosin to observe the morphological changes and Periodic Acid Schiff's Reaction (PAS) to study the changes in the mucous secretion on the surface epithelium and the density of PAS reaction in PAS stained sections was evaluated by the image analysis software program (Image j. 1.46version) in 10 randomly selected different microscopic fields for each specimen at a magnification power of x200 from sections of the fundic mucosa per animal in all groups[13].

PCNA Immunohistochemical stain[14]

Gastric sections were stained by anti-proliferating cell nuclear antigen) PCNA) antibody, by using mouse anti-p53 antibody and rabbit anti-MDM2 antibody correspondingly. The sections of paraffin were put in the autoclave for 15 min in TE buffer (pH 9.0) to repossess the p53 antigen then put in the autoclave for 20 min to repossess the MDM2 antigen, or the paraffin sections were put in microwave for 15 min in 10 mM citrate buffer (pH 6.0) to get back PCNA antigen. The slide was consequently incubated with the primary anti-PCNA for one hour at room temperature, and the primary anti-MDM2 for 40 min, together with their particular secondary antibodies (biotin-conjugated anti-immunoglobulin (for 10 min, and then with the streptavidin peroxidase method for 5 min.

PCNA-Labeling Index (PCNA-LI)[15]

The numbers of PCNA positive cells of the gastric fundic sections were counted by the image analysis software program (Image j. 1.46 version) in 10 randomly selected different microscopic fields for each specimen at a magnification power of x200 from sections of the fundic mucosa per animal in all groups. The numbers of PCNA positive cells were evaluated and estimated as a percentage of immuno-labeled cells to all basal cells. The mean ± SD was calculated, and then the total PCNA-LI for all groups were estimated.

CD44 Immunohistochemical stain

For detection and counting of CD44-positive cells in the gastric fundic sections, paraffin-embedded stomach sections were de-paraffinized and rehydrated. The slides were incubated in 10 % hydrogen peroxide (H_2O_2) for 10 minutes to switch off the endogenous peroxidase. the nonspecific binding sites were blocked by blocking serum. Then, slides were incubated with CD44 antibody (Thermo Fisher Scientific, USA). The binding sites of the antibody in the gastric glands were visualized by the avidin-biotin-peroxidase complex technique. The nuclei were counter stained with hematoxylin[16].

Statistical analysis

Statistical Package for Social Science (SPSS) 20 for window were used for analyzes the data. Data are expressed as the mean ± standard error (SE); by using analysis of variance (ANOVA) between different treated group followed by least significant difference (LSD), and the differences were considered significant at $P \leq 0.05$.

RESULTS

Determination of the gastric juice volume and acidity (Table1)

The volume of gastric juice and acidity obtained from rats in aspirin group were significantly increased compared with the control group. After ADMSC treatment of gastric ulcer, the volume of gastric juice and gastric acidity were significantly decreased compared with the aspirin group. After omeprazole treatment, the gastric juice volume and acidity were significantly decreased compared with the aspirin group. Also the volume of gastric juice and acidity in ADMSC-treated group were significantly decreased compared with the omeprazole-treated group.

Estimation of gastric damage in all groups (Table 2, Figure 1)

In aspirin group, the mean length of gastric ulcer was 12.3±2.5 mm and this result was significantly increased compared with the control group. After administration of (ADMSC), the mean length of gastric ulcer was 2.9±1.2 mm and this result also was significantly decreased compared with the aspirin group, and was significantly decreased compared with the mean length of gastric ulcer in omeprazole treated group. These results indicated the highly effective role of ADMSC in treatment of gastric ulcer and the improvement of gastric ulcer after administration of ADMSC was more superior to the improvement of gastric ulcer after administration of omeprazole. After administration of omeprazole, the mean length of gastric ulcer was 6.7±2.8 mm and this result was significantly decreased compared with the aspirin group.

Histopathological examination

Hematoxylin and eosin-stained sections of the gastric fundus from the control group (subgroup Ca & Cb) showed that the gastric mucosa contained gastric pit lined by surface mucous cells, gastric gland that extend from the muscularis mucosa in the lamina propria (Figure 2). The gastric gland contained parietal cell appeared pear like shape with pale stained cytoplasm and rounded nucleus, also the chief cell with dark stained cytoplasm, the apical cytoplasm occupied by the secretory granules and basal rounded nucleus (Figure 3)

Hematoxylin and eosin-stained sections of the gastric fundus from the aspirin group showed erosion of the gastric mucosa and loss of architecture of surface mucous epithelium, sloughing of the mucosa, with marked inflammatory cell infiltration, focal inflammatory cell infiltration in the lamina propria and congested blood vessel

(Figure 4). The gastric gland with a highly sloughing of its cells, degenerated cells with absent nucleus, with marked inflammatory cell infiltration and some hemorrhage were also seen (Figure 5).

Hematoxylin and eosin stained sections of the gastric fundus from the ADMSC-treated group showed normal architectures of mucosa, normal gastric gland but there was minimal inflammatory cell infiltration (Figure 6).

Hematoxylin and eosin stained sections of the gastric fundus from the omeprazol-treated group showed normal architectures of mucosa, sloughing of mucous neck cell and moderate inflammatory cell infiltration (Figure 7).

PAS stained sections of the control group showed positive PAS reaction at the surface epithelium and mucus neck cells of the gastric glands (Figure 8), PAS stained sections of the aspirin group showed areas with weak positive PAS reaction and areas with negative PAS reaction at the surface epithelium with sloughing of the surface mucosa (Figure 9), PAS stained sections of the ADMSC-treated group showed positive PAS reaction at the surface epithelium and mucus neck cells of the gastric glands (Figure 10), PAS stained sections of the omeprazole-treated group showed positive PAS reaction at the surface epithelium, positive PAS reaction at mucus neck cells of the gastric glands, but still there were areas with loss of PAS reaction were also seen (Figure 11).

Mean values of area % of PAS reaction (Table 3)

In aspirin group the mean value of PAS reaction was 3.61 ±2.4 %, this value was significantly decreased compared with the control group. In the ADMSC-treated group the mean value of PAS reaction was 7.37 ±1.2 %, this value was significantly increased compared with the aspirin group. This indicates the improvement of mucous secretion after treatment of gastric ulcer with ADMSC. In the omeprazole-treated group the value of PAS reaction was 5.91 ±1.8 %, this value was significantly increased compared with the aspirin group. This indicates the treated effect of omeprazole on gastric ulcer induced by aspirin.

Immuno-histochemical staining of the control group revealed a minimal expression of PCNA in the neck cells of the gastric glands (Figure 12), while the reactivity of the aspirin group showed a marked PCNA expression in all epithelial cells of the gastric mucosa (Figure 13). On the hand the reactivity of the ADMSC-treated group showed a mild PCNA expression in neck cells of the gastric mucosa (Figure 14) and the reactivity of the omeprazole-treated group showed a moderate PCNA expression in all epithelial cells of the gastric mucosa (Figure 15).

Immunohistochemical detection of CD44 positive cells in gastric mucosa revealed that sections in gastric fundus from the control group showed a negative reaction for CD44 antibody (Figure 16). On the other hand sections in the gastric fundus from the ADMSC-treated group showed a positive reaction for CD44 antibody, there were many branched cells with brownish cytoplasmic reaction for CD44 antibody (Figure 17).

PCNA-LI Results (Table 4)

In aspirin group, the PCNA-LI value was 53.83 ±9.5; this value was significantly increased compared with the control group. In the ADMSC-treated group the PCNA-LI value was 25.25 ±4.1; this value was significantly decreased compared with the aspirin group this indicated the treated effect of ADMSC on the gastric ulcer induced by aspirin. In the omeprazole-treated group the PCNA-LI value was 33.86 ±5.2; this value was significantly decreased compared with the aspirin group. This indicated the treated effect of omeprazole on the gastric ulcer induced by aspirin. This value was also significantly increased compared with the ADMSC-treated group. This indicated the highly effective role of ADMSC in treatment of gastric ulcer more than the omeprazole.

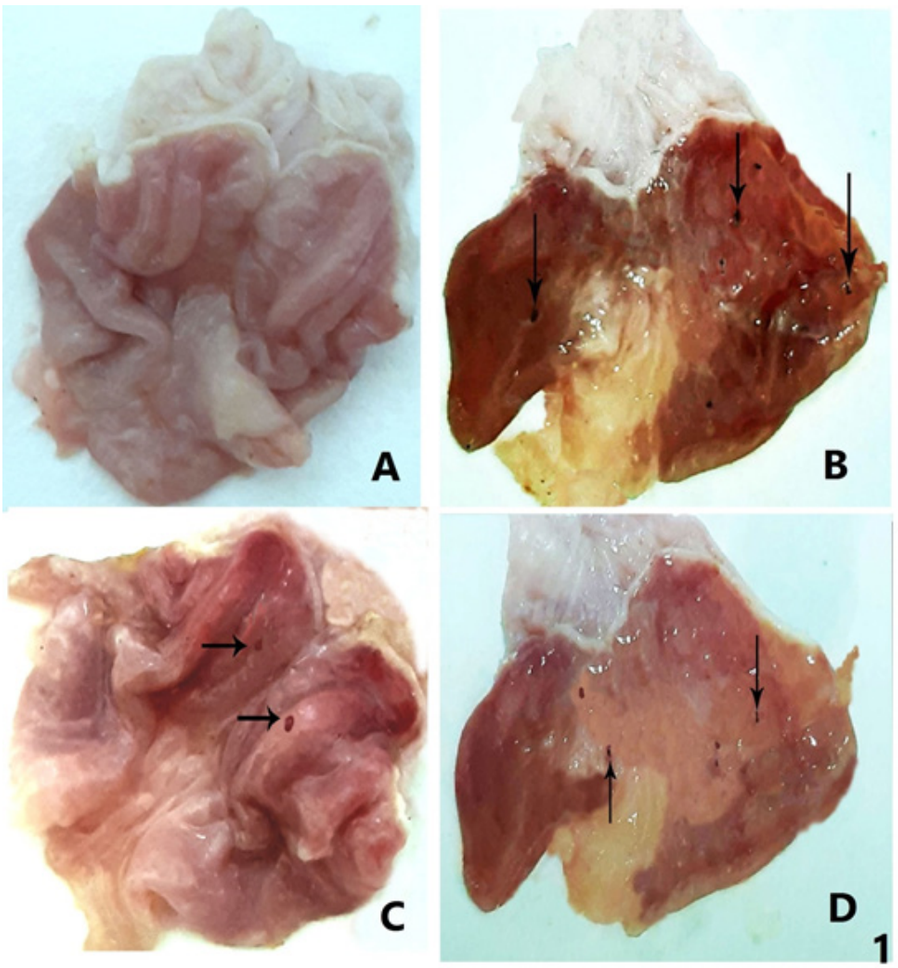

Fig 1: Photographs of opened stomach, A in control group, B in aspirin group, C in ADMSC-treated group and D in omeprazole-treated group showing gastric ulcers (arrows).

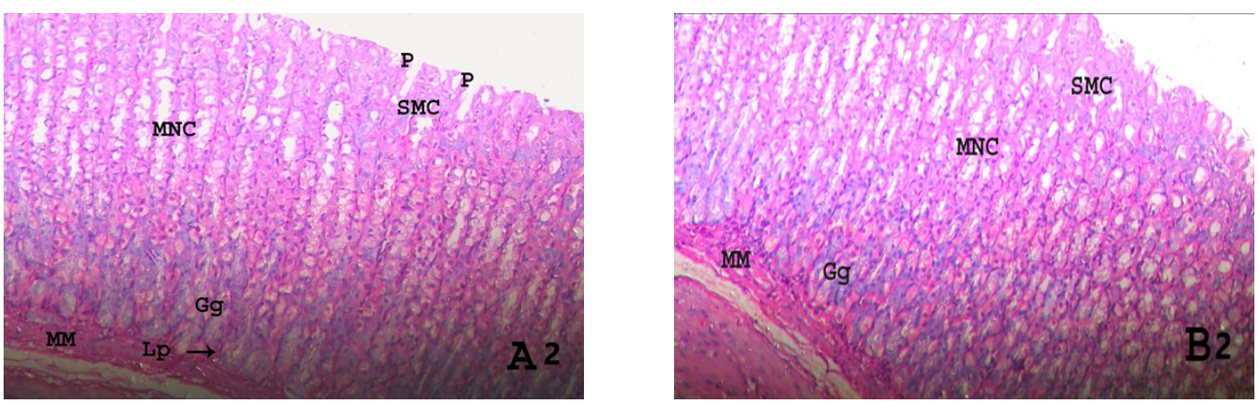

Fig. 2: A& B photomicrographs of gastric fundus from the control group A from subgroup Ca and B from subgroup Cb showing: the gastric mucosa contained the gastric pit (P) that was lined by surface mucous cell (SMC), the mucous neck cell (MNC) with lightly stained cytoplasm with mucous cup devoid of staining, gastric gland (Gg) extend from the muscularis mucosa (MM) in the lamina propria (LP). (H&E X 100).

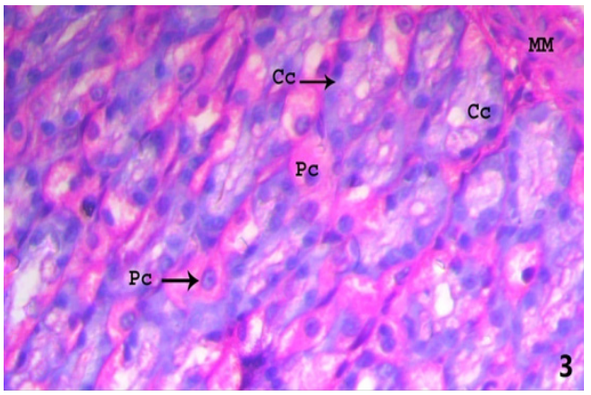

Fig. 3: A photomicrograph of a higher magnification of the previous picture from the control group showing: the gastric gland contained parietal cell (Pc) pear like shape with pale stained cytoplasm and rounded nucleus, also the chef cell (Cc) with dark stained cytoplasm, the apical cytoplasm occupied by the secretory granules and basal rounded nucleus, muscularis mucosa (MM). (H&E X 400).

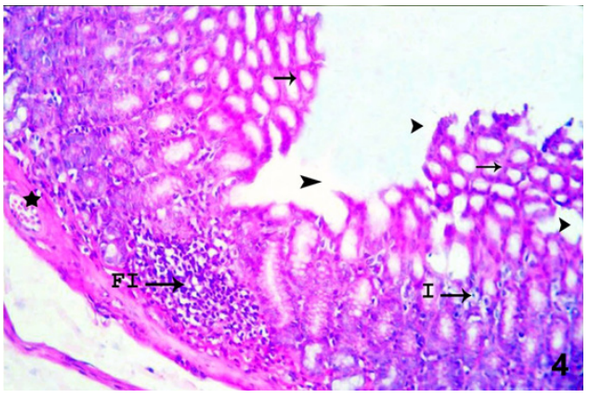

Fig. 4: A photomicrograph of gastric fundus from the aspirin group showing: loss of architecture of surface mucous epithelium (arrow heads), sloughing of the mucosa (arrows) with marked inflammatory cell infiltration (I), focal inflammatory cell infiltration (FI) and congested blood vessel (star). (H&E X 100).

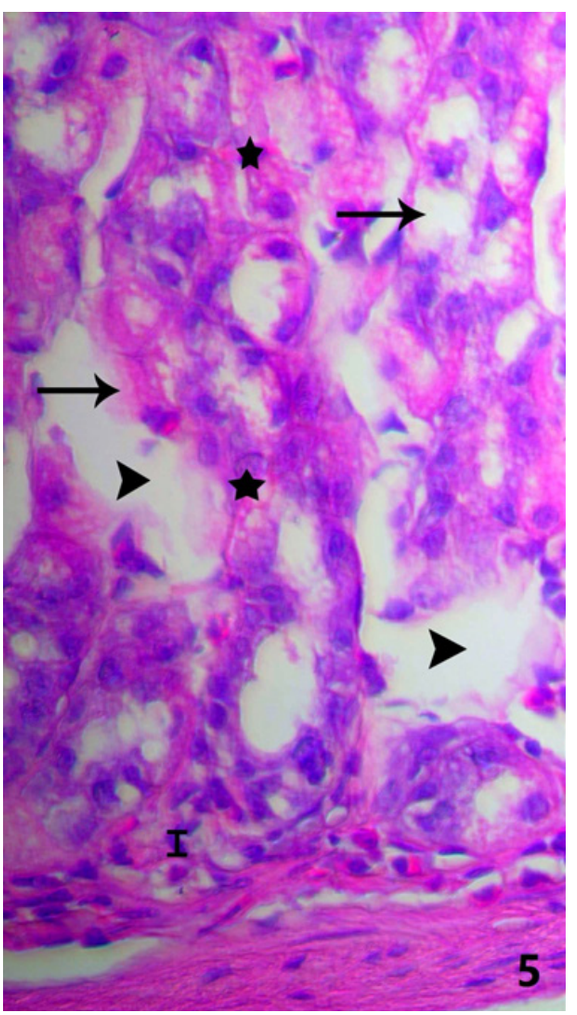

Fig. 5: A photomicrograph of a higher magnification of the previous picture from the aspirin group showing: the gastric gland with highly sloughing of its cells (arrow heads), degenerated cells with absent nucleus (arrows), with marked inflammatory cell infiltration (I) and some hemorrhage (stars). (H&E X 400).

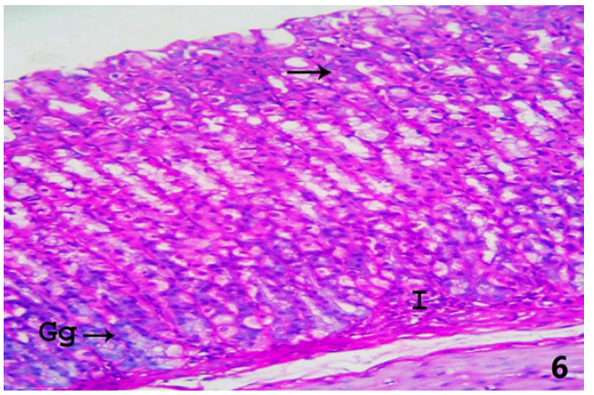

Fig. 6: A photomicrograph of gastric fundus from the ADMSC-treated group showing: normal architectures of mucosa (arrow), normal gastric gland (Gg) but there were minimal inflammatory cell infiltration (I). (H&E X 100).

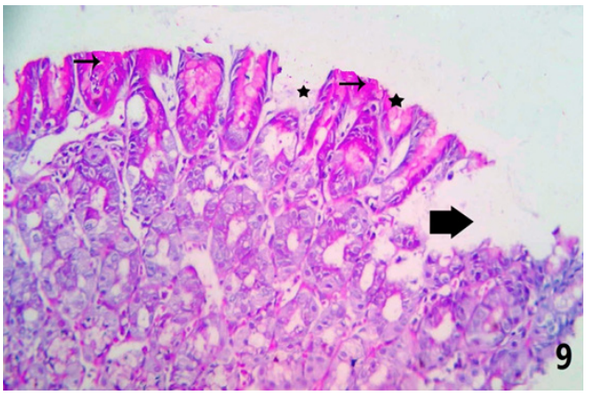

Fig. 9: A photomicrograph of gastric fundus from the aspirin group showing a weak positive PAS reaction (arrows), and a negative PAS reaction (stars) at the surface epithelium with sloughing of the mucosa (thick arrow) (PAS x 200).

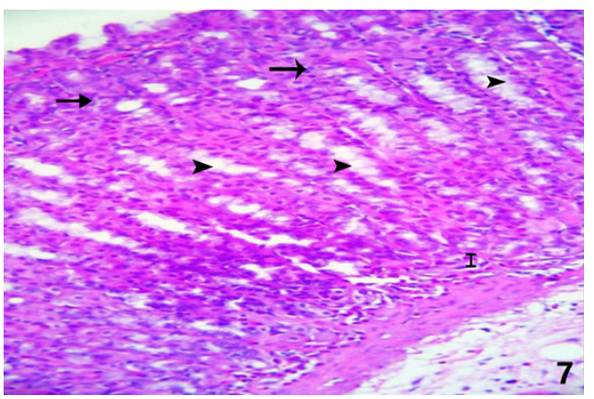

Fig. 7: A photomicrograph of gastric fundus from the omeprazoel-treated group showing: normal architectures of mucosa (arrows), sloughing of mucus neck cell (arrow heads), moderate inflammatory cell infiltration (I) (H&E X 100).

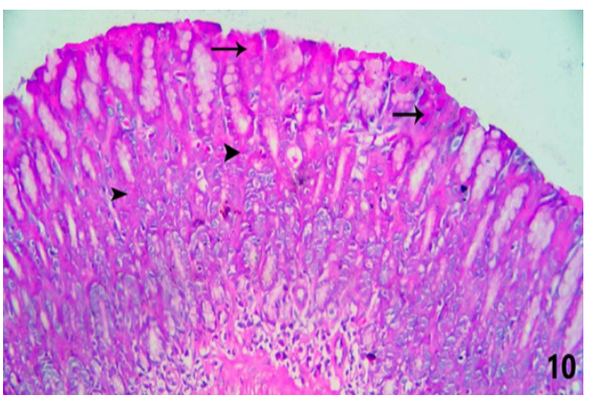

Fig. 10: A photomicrograph of gastric fundus from the ADMSC-treated group showing a positive PAS reaction at the surface epithelium (arrows) and mucus neck cells of the gastric glands (arrow heads) (PAS X 200)

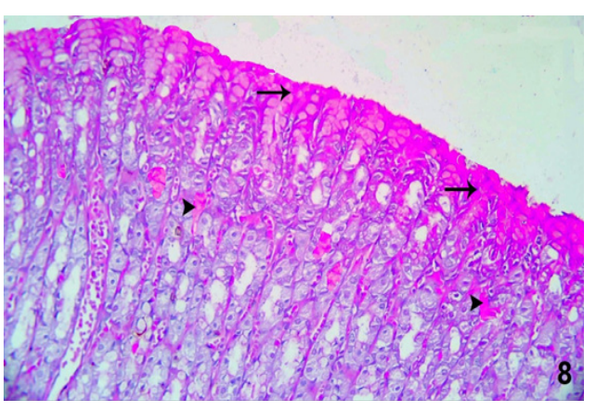

Fig. 8: A photomicrograph of gastric fundus from the control group showing a positive PAS reaction at the surface epithelium (arrows) and in mucus neck cells of the gastric glands (arrow heads) (PAS X 200)

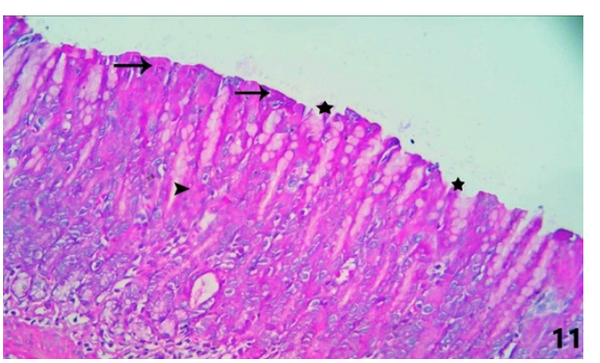

Fig. 11: A photomicrograph of gastric fundus from the omeprazole-treated group showing a positive PAS reaction at the surface epithelium (arrows), and a positive PAS reaction at mucus neck cells of the gastric glands (arrow head), but still there were areas with a negative PAS reaction (stars) at the surface epithelium (PAS x 200).

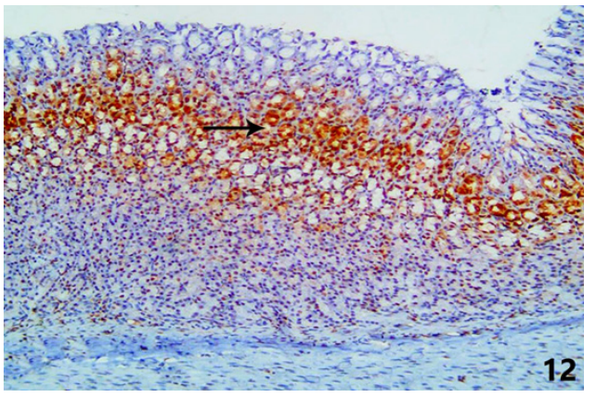

Fig. 12: A photomicrograph of gastric fundus from the control group showing: minimal PCNA expression in the neck cells of the gastric mucosa (arrow) (PCNA Immunostaining X100).

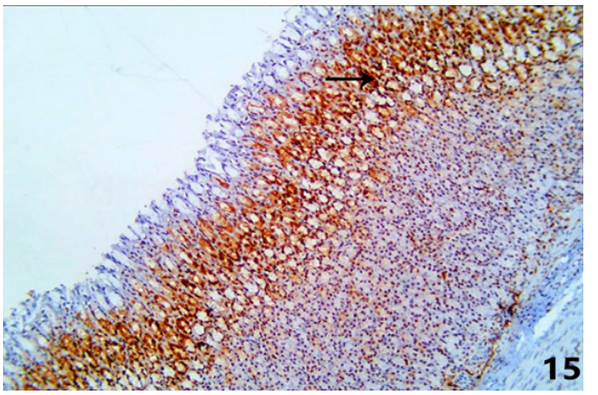

Fig. 15: A photomicrograph of gastric fundus from the omeprazole-treated group showing: moderate PCNA expression in the all epithelial cells of the gastric mucosa (arrow) (PCNA Immunostaining X100).

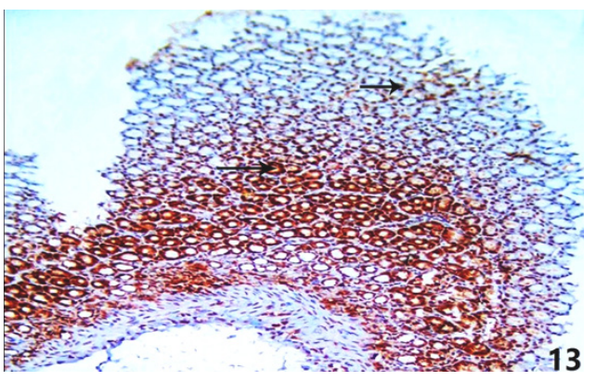

Fig. 13: A photomicrograph of gastric fundus from the aspirin group showing: marked PCNA expression in the all epithelial cells of the gastric mucosa (arrow) (PCNA Immunostaining X100).

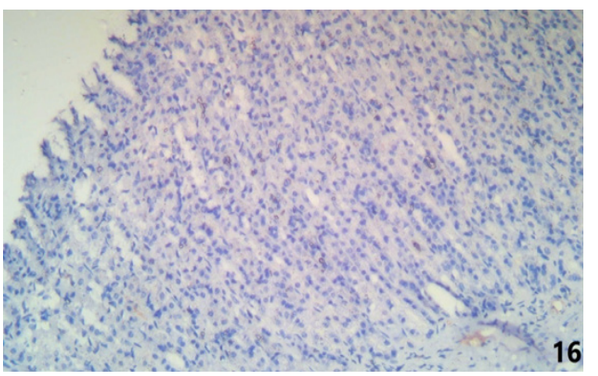

Fig. 16: A photomicrograph of gastric fundus from the control group showing: negative reaction for CD44 antibody. (Immunostaining for CD44 x 200).

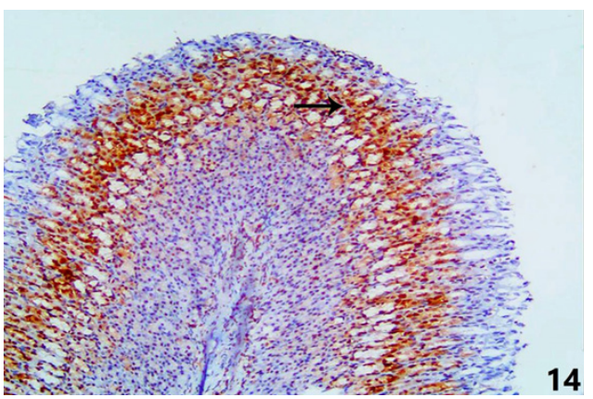

Fig. 14: A photomicrograph of gastric fundus from the ADMSC-treated group showing: mild PCNA expression in the neck cells of the gastric mucosa (arrow) (PCNA Immunostaining X 100).

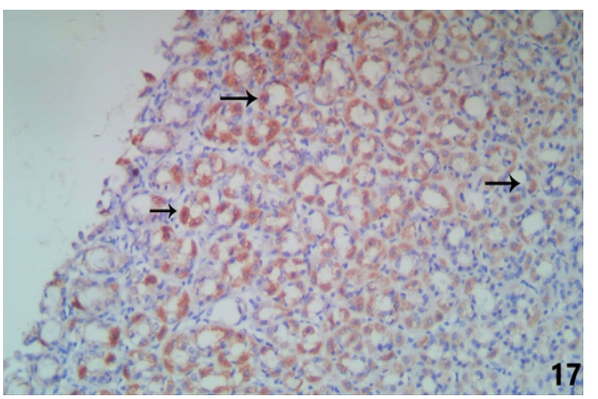

Fig. 17: A photomicrograph of gastric fundus from the ADMSC-treated group showing positive reaction for CD44 antibody as there are many branched cells with brownish cytoplasmic reaction for CD44(arrows). (Immunostaining for CD44 x 200).

Table 1: Gastric juice volume and gastric acidity in all groups

	Control group	Aspirin group	ADMSC-treated group	Omeprazole-treated group
Gastric juice volume / ml	1.49±2.7[bd]	4.98±5. 7[acd]	2.15±7. 9[cd]	3.26±8.4[abc]
Gastric acidity	9.4±1.4[bd]	50.4 ± 6.4[acd]	15.6 ±7.3[cd]	20.9 ± 4.6[abc]

Data represented by mean ± SD a: Significant difference (p<0.05) compared with control group, b: Significant difference (p<0.05) compared with aspirin group, c: Significant difference (p<0.05) compared with ADMSC group, d: Significant difference (p<0.05) compared with omeprazole group.

Table 2: Gastric ulcer index in all groups

Groups	Gastric ulcer index Mean & SD	Comparison	Sig (p<0.05)
Control group	0.0±0.0	Asprine group	0.000
		AD-MSC group	0.020
		Omebrazole group	0.000
Aspirin group	12.3 ±2.5	Control group	0.000
		AD-MSC group	0.000
		Omebrazole group	0.000
ADMSC-treated group	2.9 ±1.2	Control group	0.020
		Asprine group	0.000
		Omebrazole group	0.040
Omeprazole-treated group	6.7 ±2.8	Control group	0.000
		Asprine group	0.000
		AD-MSC group	0.040

The mean difference is significant less than 0.05 levels.

Table 3: Mean values of area % of PAS reaction in all groups

Groups	PAS reaction % Mean & SD	Comparison	Sig (p<0.05)
Control group	9.89±2.1	Asprine group	0.000
		AD-MSC group	0.022
		Omebrazole group	0.000
Aspirin group	3.61 ±2.4	Control group	0.000
		AD-MSC group	0.000
		Omebrazole group	0.000
ADMSC-treated group	7.37 ±1.2	Control group	0.022
		Asprine group	0.000
		Omebrazole group	0.046
Omeprazole-treated group	5.91 ±1.8	Control group	0.000
		Asprine group	0.000
		AD-MSC group	0.046

The mean difference is significant less than 0.05 levels.

Table 4: level of PCNA LI in all groups

Groups	PCNA-LI Mean & SD	Comparison	Sig (p<0.05)
Control group	15.38±6.1	Asprine group	0.000
		AD-MSC group	0.017
		Omebrazole group	0.000
Aspirin group	53.83 ±9.5	Control group	0.000
		AD-MSC group	0.000
		Omebrazole group	0.000
ADMSC-treated group	25.25 ±4.1	Control group	0.017
		Asprine group	0.000
		Omebrazole group	0.036
Omeprazole-treated group	33.86 ±5.2	Control group	0.000
		Asprine group	0.000
		AD-MSC group	0.036

The mean difference is significant less than 0.05 levels.

DISCUSSION

The ADMSCs enhances regeneration of damaged tissues. The immune-regulatory and anti-inflammatory properties of the ADMSCs may be the cause of its therapeutic effects[17].

Gastro-protecting drugs such as omeprazole which is a proton pump inhibitor used for management of peptic ulcer and might reduce its associated complications[2]. However, long-term adverse effects of proton pump inhibitor drugs are under researches in past years[18].

The present study designed to search a natural and safe agent for treatment of gastric-ulcer, this study compared between the therapeutic role of ADMSC and omeprazole in treatment of gastric ulcer.

In this study the volume of gastric juice and gastric acidity obtained from the rats in aspirin group were significantly increased compared with that in the control group. This was in agreement with other authors as they added that PH of gastric juice was reduced in aspirin treated group but increased after treatment with dracaena cochin-chinensis[1]. After ADMSC treatment of gastric ulcer the volume of gastric juice and gastric acidity were significantly decreased compared with that in the aspirin group. Previous findings were reported that the possibility of use of ADMSCs as a possible resource for clinical treatment of deferent gastrointestinal tract disorders[16]. Also the volume of gastric juice and acidity in ADMSC-treated group were significantly decreased compared with that in the omeprazole-treated group. After omeprazole treatment the gastric juice volume and acidity were significantly decreased compared with that in the aspirin group. These findings were explained by another study reported that omeprazole condensed the acid secretions by blocking the hydrogen potassium ATPase pump[19].

In our study estimation of the gastric damage revealed that the mean length of gastric ulcer in aspirin group was 12.3±2.5 mm and this result was significantly increased compared with that in the control group. In agreement with our results other authors found that a single oral management of aspirin (200 mg/kg.) enhanced gastric ulcer, and increased the volume of gastric juice and the total acidity with a decrease in pH value[3]. In this study after administration of ADMSC the mean length of gastric ulcer was 2.9±1.2 mm and this result was significantly decreased compared with that in the aspirin group, this indicated the highly effective role of ADMSC in treatment of gastric ulcer induced by aspirin. In concomitant with our results previous studies reported that adipose derived stem cell therapy has improved the ulcer index induced by indomethacin and their study found that the third day of the gastric ulcer was the day of highest ulcer index[9]. In omeprazole-treated group, the mean length of gastric ulcer was 6.7±2.8 mm and this result was significantly decreased compared with that in the aspirin group. Similar results were found that efficacy of omeprazole in gastro-protection of gastric ulcer enhanced by aspirin in experimental rats[6].

In the present study single dose of aspirin (200 mg/kg body wt) orally in aspirin group produced a gastric mucosal damage in the form of erosion of the gastric mucosa and loss of architecture of surface mucous epithelium, sloughing of the mucosa, with marked inflammatory cell infiltration, focal inflammatory cell infiltration in the lamina propria and congested blood vessel. The gastric gland showed highly sloughing of its cells, degenerated cells with absent nucleus, with marked inflammatory cell infiltration and some hemorrhage. Similar observations were concluded that aspirin is a potent ulcerogenic agent in rats[6,20].

In the present study, an intravenous injection of ADMSC after induction of gastric ulcer in ADMSC-treated group showed normal architectures of mucosa, normal gastric gland but there was a minimal inflammatory cell infiltration. These findings came in agreement with previous study reported that transplantation of human adipose derived stem cells intravenously facilitated the gastric ulcer regeneration that induced by indomethacin, through migration of the adipose derived stem cells into the injured tissue and the discharge of growth factors that

stimulate angiogenesis[9]. Similarly, previous reports added that 3 days after AD-MSCs transplantation, there was complete restoration of the fundic glands with a distinct reduction in inflammatory cellular infiltrate[16]. Moreover, some researchers confirmed that endoscopic submucosal injection of ADMSCs promote regeneration of non steroidal antinflammatory drug associated peptic ulcer in the pig[18]. Another study found that injection of MSC into the gastric wall surrounding the margin of the ulcer, the MSC transplantation accelerated gastric ulcer curing by their facility to secret angiogenic factors and their differentiation into gastric interstitial cells[21].

In the present study, oral daily administration of omeprazole for five days after induction of gastric ulcer in omeprazole-treated group showed a normal architecture of the mucosa with sloughing of mucous neck cell and moderate inflammatory cell infiltration. These findings came in agreement with other authors they added that treatment of the gastric ulcer induced by aspirin with omeprazole improved the congestion, haemorrhage, oedema, inflammatory and dysplastic hisopathological changes[6]. Moreover, other study found that in mixture of omeprazole with Kangfuxin liquid in treatment of patients with gastric ulcer could improve the total efficacy rate of gastroscopy and reduced the recurrence rate[22]. In the different with our results previous study reported that patients with continuance treatment with proton pump inhibitors such as omeprazole for about six months or greater were significantly more induced diffuse enterochromaffin like cell hyperplasia than patients in the control group[23].

In our study, the PAS stained sections of the aspirin group showed areas with a weak positive PAS reaction and areas with a negative PAS reaction at the surface epithelium with sloughing of the surface mucosa, and the mean value of PAS reaction was significantly decreased compared with that in the control group. This was in agreement with previous study added that aspirin significantly increased serum tumor necrotic factors-α and decreased prostaglandin E2 in the gastric mucosa[24]. PAS stained sections of the ADMSC-treated group showed a positive PAS reaction at the surface epithelium and in the mucus neck cells of the gastric glands, and the mean value of PAS reaction was a significantly increased compared with that in the aspirin group this indicated the improvement of mucous secretion after treatment of gastric ulcer with ADMSC. Similarly other authors found that there was a significantly increased in the mean value of optical density of PAS positive areas in the group treated by ADMSCs[16]. PAS stained sections of the omeprazole-treated group showed a positive PAS reaction at the surface epithelium, positive PAS reaction at the mucus neck cells of the gastric glands, but still there were areas with a negative PAS reaction and the mean value of PAS reaction was significantly increased compared with that in the aspirin group. This was in agreement with another study stated that the omeprazole has defensive effect against gastric ulcer caused by indomethacin in albino rats[25].

In this study, PCNA immunohistochemical staining was an important tissue proliferation marker. The control group revealed a minimal appearance of PCNA in the neck cells of the gastric glands, while the PCNA immunostaining reactivity of the aspirin group showed a marked PCNA expression in all epithelial cells of the gastric mucosa, and the PCNA-LI value was significantly increased compared with that in the control group. This was similarly with previous study that compared between the control group and gastric ulcer caused by acetic acid[26].

The PCNA immunostaining reactivity of the ADMSC-treated group showed a mild PCNA expression in the neck cells, and the PCNA-LI value was significantly decreased compared with that in the aspirin group. PCNA-LI decreased in ADMSC-treated group indicated that ADMSC treatment promoted healing of gastric ulcers. In the same line previous study reported that ADSC appeared to be safe and had an optimistic effect on the healing of chronic ulcers[27]. Moreover, some researchers confirmed that the vascular endothelial growth factor VEGF Immunohistochemical staining showed positive expression in the ulcer marginal mucosa of BMSC group compared with that in the control group[28].

The PCNA immunostaining reactivity of the omeprazole-treated group showed a moderate PCNA expression in all epithelial cells of the gastric mucosa, and the PCNA-LI value was significantly decreased compared with that in the aspirin group. This was in agreement with another study who reported that omeprazole is a consistent therapeutic drug for peptic ulcer, for its well recognized and broadly proved ulcer-healing and gastro-protective effects[29]. Moreover, others found that both 40 and 20-mg of esomeprazole have similar effects on healing and reduction of ulcer area induced by endoscopic submucosal dissection in human[30]. In the contrary, another study reported that long time treatment with esomeprazole in human for about 3 to 10 months may induced rhabdomyolysis in some patients[31].

In this study, the immunohistochemical detection of CD44 positive cells in gastric mucosa revealed that sections in the gastric fundus from the ADMSC-treated group showed a positive reaction for CD44 antibody, since there were many branched cells with brownish cytoplasmic reaction for CD44 immunostaining. Similar result was found by previous study who added that CD44 labels a population of undifferentiated cells in the isthmus of gastric glands. However, in rat negative CD44, the proliferation rate of stem cell was significantly decreased[16].

CONCLUSION

The present comparative study concluded that ADMSC have a greater therapeutic role in treatment of gastric ulcer more than the therapeutic role of omeprazole. This study recommended application of the ADMSCs as a new therapeutic treatment of resistant gastrointestinal ulcers.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة مقارنة بين الدور العلاجي للخلايا الجذعية الوسطية الدهنيه والأوميبرازول في تحسن قرحة المعدة التي يسببها الأسبرين في الجرذان البيضاء (دراسه هستولوجيه و هستوكميائيه مناعيه)

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المقدمه: الأسبرين يسبب قرحة المعدة عن طريق احداث جرح في الغشاء المخاطي للمعدة. تعتبر الخلايا الجذعية الوسطية المشتقه من النسيج الدهني مناسبة لعلاج قرحة المعدة بسبب قدرتها على التحور إلى أنواع عديده من الخلايا. يستخدم الاوميبرازول فى علاج الاضطرابات المعديه الناتجه عن زيادة افراز حمض المعدة.

الهدف من هذه الدراسة: المقارنة بين الدور العلاجي للخلايا الجذعية الوسطية الدهنيه والأوميبرازول في علاج قرحة المعدة.

المواد وطرق البحث: أجريت هذه الدراسه على أربعين جرز ذكر ابيض بالغ وقسمت إلى أربع مجموعات متساويه: ١- المجموعة الضابطة ٢- مجموعة الأسبرين: حيث تم صيام الجرذان لمدة ٢٤ ساعة. ثم إعطائهم الاسبرين (٢٠٠ ملغم / كغم من الاسبرين) مرة واحدة عن طريق الفم لاحداث قرحة فى المعده. ٣- المجموعة المعالجة بالخلايا الجذعيه الوسطيه الدهنيه: حيث تم اعطائها (٣×١٠^{6}) من الخلايا الجذعيه الوسطيه الدهنيه لكل جرذ مرة واحدة عن طريق الحقن في الوريد بعد أربع ساعات من احداث قرحة المعده. ٤- المجموعة المعالجه بالأوميبرازول: حيث تم اعطائها (٢٠ ملغم / كغم من الاوميبرازول) عن طريق الفم بعد أربع ساعات من احداث قرحة المعده يوميا لمدة خمسة أيام. تم تقييم حجم العصير المعدى ونسبة الحموضة و طول قرحة المعدة والتغيرات الهستولوجيه والهستوكميائيه مناعية لانسجة المعده فى كل المجموعات.

النتائج: يسبب الأسبرين تآكل وفقدان البنية الفوقيه للغشاء المخاطي للمعدة ، يقلل العلاج بالخلايا الجذعيه الوسطيه الدهنيه من طول قرحة المعدة ويقلل من التغيرات الهستوباثولوجية والهستوكميائيه المناعيه. بينما العلاج بالأوميبرازول يعزز التئام قرحة المعدة الناجم عن الأسبرين ولكن الدور العلاجي للخلايا الجذعيه الوسطيه الدهنيه أفضل في التئام أنسجة القرحة المعدية من الدور العلاجي للأوميبرازول.

الاستنتاج: الدور العلاجي للخلايا الجذعيه الوسطيه المشتقه من النسيج الدهني افضل من الأوميبرازول في علاج قرحة المعدة.