A Histological Study for the Possible Therapeutic Effect of Stem Cells in Methotrexate Induced Small Intestinal Injury in a Male Rat Model

Original Article

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ABSTRACT

Background and Objectives: Methotrexate (MTX) is an antirheumatic and chemotherapeutic agent with highly adverse effect on gastrointestinal tract. Mesenchymal stem cell (MSCs) can exert a therapeutic action by regenerative capacity in intestinal damage. This study was done to demonstrate the possible therapeutic effect of MSCs in methotrexate induced intestinal injury in adult male albino rat.

Methods and Results: Male albino rats were used in this study. Group A (Control group), subgroup A-I rats received saline for 2 weeks and subgroup A-II received saline for 2 weeks then phosphate buffer saline once intraperitoneal (IP). Group B rats received MTX (14 mg/kg/week) intraperitoneal (IP) for 2 weeks. Group C, rats received MTX (14 mg/kg/week) intraperitoneal (IP) for 2 weeks. After 2 weeks, they were injected IP with MSCs (2×10^6 cells in 500 µL PBS once). Groups AI&B were anaesthetized and sacrificed after 2 weeks while groups AII&C were sacrificed after 4 weeks. Blood samples were drawn from the tail vein to measure myeloperoxidase (MPO) and vascular endothelial growth factor (VEGF). Small intestinal specimens were obtained for evaluation by light microscopy. CD4+, and CD8+ immunohistochemistry and statistical analysis were applied. Significant increase in MPO and significant decrease in VEGF were reported in Group B. MTX induced pathological alterations in small intestinal tissues, mean number of mast cells and goblet cells associated with increased CD4+ and CD8+ immunoexpression. Nearly all changes were ameliorated following MSCs therapy

Conclusion: MSCs have significant effect in repairing the deleterious action of MTX in small intestinal tissues.

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Key Words: Intestinal damage, methotrexate, stem cell.

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INTRODUCTION

Methotrexate (MTX) is equivalent to folic acid structurally and is used as antirheumatic drug, and in the treatment of malignancies as leukemia. MTX affects tissues with high cellular proliferation including both normal and tumor tissues. Gastrointestinal mucositis results from the effect of MTX on gastrointestinal tract. About 60% of cancer patients receiving chemotherapy including MTX, suffer from diarrhea, abdominal pain, and injury of the gastrointestinal tract with villous atrophy, cellular death as well as absorptive dysfunction[1].

Mesenchymal stem cells (MSCs) have marked therapeutic effect on damaged tissue because of their capacity to prevent apoptosis, reduce inflammation, enhance the growth and differentiation of local stem cells and stimulate angiogenesis. However, the mechanisms by which MSCs carry these functions remain unclear. The microenvironmental improvement after damage promote the endogenous stem and progenitor cells to regenerate the injured tissue[2].

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are currently considered one of the best applicants in the field of regenerative medicine. New predictions for cell-based therapies have been created by the genetic modification of these cells. The curative effect of stem cells should be first proved in animals due to ethical and practical limitations[3].

MATERIALS AND METHODS

Drugs

1. Methotrexate (MTX): tablets 2.5 mg (EBEWE pharma Ges.m.b.H Nfg.KG, Austria). The tablet is dissolved in 0.5 ml of saline.
2. Bone marrow derived-mesenchymal stem cells (BM-MSCs):

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Rat bone marrow derived mesenchymal stem cells were prepared in the Biochemistry Department, Kasr Al-Ainy Medical School. MSCs diluted with 1ml of normal saline were loaded in a 1-mlsterile syringe and administered intraperitoneally for each rat[4].

Animals

This study included 22 adult male albino rats with an average body weight 200-250 gm. They were provided by the animal house of Kasr Al-Ainy, Faculty of Medicine, Cairo University. They were housed in hygienic cages according to the guidelines for animal research issued by the National Institute of Health and approved by Animal Ethics Committee, Cairo University. The rats received chow, water ad libitum.

A. 6 rats were used in the biochemistry department

For the preparation of bone marrow derived mesenchymal stem cells.

Preparation of bone marrow derived –mesenchymal stem cell (BM-MSCs)

Six-week old albino rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The tibia and femur were flushed with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL) to collect the bone marrow. A density gradient [Ficoll / Paque (Pharmacia)] was used to isolate the nucleated cells and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Then cells were incubated in 5% humidified CO2 at 37 °C for 12–14 days as primary culture or till formation of large colonies. The cultures were washed twice with phosphate buffer saline (PBS) when large colonies were established (80–90% confluence) then the cells were trypsinized with 0.25% trypsin in 1ml EDTA (GIB- CO/BRL) at 37 °C for 5 min. Cells were resuspended with serum-supplemented medium after centrifugation and incubated in 50 cm2 culture flask (Falcon). The resulting cultures were considered as the first-passage cultures. Identification of mesenchymal stem cell (MSC) in culture done by their fusiform shape, adhesiveness and by detection of one of the surface markers of rat mesenchymal stem cell (cluster of differentiation- CD29) by flow cytometry[5].

B. 16 rats were divided into the following groups

Group A (Control group): 6 rats, rats were further subdivided into 2 subgroups:

Subgroup A-I: 3 rats were injected intraperitoneally with 0.5 ml of normal saline for 2 weeks.

Subgroup A-II: 3 rats were injected intraperitoneally with 0.5 ml of normal saline for 2 weeks. After 2 weeks, rats were injected once intraperitoneally with 0.5 ml of phosphate buffer saline (PBS).

Group B (MTX-injected group): 5 rats received MTX (14 mg/kg/week) intraperitoneal for 2 weeks[6].

Group C (MTX & MSC-treated group): 5 rats received MTX (14 mg/kg/week) intraperitoneal for 2 weeks. After 2 weeks, they were injected intraperitoneally with MSCs (2×10^6 cells in 500 µL PBS once[6].

After 2 weeks, rats from subgroup AI and group B were anaesthetized and sacrificed. While rats of subgroup AII and group C were sacrificed after 4 weeks. Blood samples were collected from tail veins to measure Myeloperoxidase (MPO) and Vascular endothelial growth factor (VEGF) in the Biochemistry Department, Faculty of Medicine, Cairo University. MPO is a lysosomal protein in azurophilic granules of neutrophils and released during degranulation. VEGF is a signal protein induces blood vessels formation.

Small intestinal specimens were obtained from the same part of the duodenum in all groups and subjected to the following:

1. Part (I): Specimens were fixed in 10% formol saline solution to be processed into paraffin blocks. Serial sections at 7 um thicknesses were cut using a microtome and mounted on glass slides. Other sections were mounted on positive charged slides for immunohistochemistry.
2. Part (II): Specimens obtained from the same part of the duodenum were fixed in glutaraldehyde to be prepared for semithin sections.

Histological study

(A) Light microscopic study

Paraffin sections were subjected to the following stains:

1-Haematoxylin and Eosin[7].

2-Immunohistochemical staining[8]:

Small intestinal sections were mounted on positive charged slides, deparafinized and rehydrated. Boiling tissue sections in 10 mM citrate buffer was done for antigen retrieval. Then, incubating the sections in hydrogen peroxide to block endogenous peroxidase activity. Sections were incubated overnight with:

A. Rabbit monoclonal anti-CD4+ antibody [EPR6855] (ab133616), Abcam plc, England, was done to detect T-helper cells.

B. Rabbit polyclonal anti-CD8+ antibody (ab4055), Abcam plc, England, was done to detect cytotoxic T-cells.

The labeled avidin-biotin-peroxidase complex (Histostain SP kit, Zymed Laboratories Inc, San Francisco, USA) was used to detect the bound primary antibody. Diaminobenzidine (DAB) was used as a chromogen and Meyer's haematoxylin as a counterstain. In order to create immunohistochemical-staining specificity, negative control serial sections were processed in the

same manner aside from replacing the primary antibody by phosphate buffer saline. Positive tissue control for CD4+ immunostaining was human colon with a brownish cytoplasmic immunoreaction while that of CD8+; the positive control was human spleen tissue with a brownish cytoplasmic immunoreaction.

(B) Semithin sections

Small pieces of small intestinal specimen with average size of 1 mm3 were immediately cut and rapidly fixed in 3 % phosphate buffered glutaraldehyde (PH 7.2). Post fixed in 1% osmium tetroxide and embedded in resin. Semithin sections (2 µm) were cut and stained with toluidine blue for 45 seconds to be examined with the light microscope[9].

(C) Morphometric study

Data were obtained using "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, UK). To calculate the mean length of small intestinal mucosa in H&E-stained sections using interactive measurement menu, mean area % of CD 4 +ve cells and CD8 +ve cells in immunostained sections, these measurements were done using binary mode in 10 non- overlapping fields at a magnification of x400.

(D) Statistical Analysis

SPSS software version 9 (SPSS, Chicago, IL) was used to analyze the obtained measurements. Analysis of variance (ANOVA) followed by post hoc Tukey test were used to compare between different groups. The results were revealed as means ± standard deviation (SD). The differences were regarded as statistically significant when "*p value*" was < 0.05.

RESULTS

Biochemical results (Histogram 1)

The mean value of serum myeloperoxidase (MPO) in control group was (42.55±1.49 U/I). In MTX-injected group was (63.86±4.75 U/I), which was significantly increased (P<0.05) in comparison with control group. Mean value of serum MPO in MTX & MSC-treated group was (45.35±1.37 U/I), which was significantly decreased (P<0.05) in comparison with MTX-injected group and non-significant difference when compared to control group.

While the mean value of serum vascular endothelial growth factor (VEGF) in control group was (83.3±1.42 pg/ml). In MTX-injected group was (52.5±4.4 pg/ml), which was significantly decreased (P<0.05) in comparison with control group. Mean value of serum VEGF in MTX & MSC-treated group was (94.7±3.27 pg/ml), which was significantly increased (P<0.05) in comparison with control and MTX-injected groups.

Hematoxylin and Eosin histological results

Examination of H&E-stained sections from control group showed normal small intestinal architecture where villi in the form of finger like projections lined with tall columnar cells having acidophilic cytoplasm, basal oval nuclei, and apical brush border (enterocytes) and few goblet cells were seen. Connective tissue forms the core of the villus having central lacteals and few inflammatory cells. The crypts were invaginating into the connective tissue (Figure 1A,B).

Small intestinal sections from Methotrexate (MTX) treated group revealed marked pathological alterations in the form of villous atrophy, sloughing of the epithelium. Some crypts were enlarged, and their cells were atrophied. The underlying lamina propria was studded with inflammatory cells (Figure 2A,B).

Sections in the small intestinal tissue of MTX & MSC-treated group showed villi lined with tall columnar cells having acidophilic cytoplasm, basal oval nuclei, and apical brush border (enterocytes). Large number of cells with rounded nuclei and goblet cells were seen. Connective tissue forms the core of the villus having central lacteals and few inflammatory cells. The crypts were invaginating into the connective tissue (Figure 3A,B).

Morphometric results

As regarding the mean length of small intestinal mucosa in the control group was (478.77±16.31 µm). In MTX-injected group was (223.15±33.67 µm), which was significantly decreased (P<0.05) in comparison with control group. While in MTX & MSC-treated group was (475.19±15.17 µm), which was significantly elevated (P<0.05) compared to MTX-injected group and non-significant different when compared with control group (Histogram 2).

Semithin Sections stained with toluidine blue results

Sections in the control group showed small intestinal villi lined with columnar cells (enterocytes) having basal pale vesicular nucleus with clear apical brush border. Goblet cells were present in between the enterocytes with large number of basolateral lymphocytes (Figure 4 A).

MTX-injected group showed absent intestinal villi with desquamated cells, only crypts are present. The small intestinal crypts are lined with columnar cells having basal pale vesicular nucleus with large number of goblet cells in between. Lymphocytes and mast cells are present (Figure 4B).

Sections in MTX & MSC-treated group showed small intestinal villi lined with columnar cells (enterocytes) having basal pale vesicular nucleus with clear apical brush border. Goblet cells are present in between the enterocytes with few basolateral lymphocytes (Figure 4 C).

Examination of small intestinal crypts in control group exhibited columnar cells having basal pale nucleus and Paneth cells present at the bottom of the crypt having apical cytoplasmic granules. Goblet cells are present in between the enterocytes. Note the presence of mast cell (Figure 5 A).

Sections in MTX-injected group showed crypts lined with columnar cells having pale vesicular nucleus and no Paneth cells could be detected. Large number of goblet cells and mitotic figures are existing with obvious presence of mast cells (Figure 5 B).

Small intestinal crypts in MTX & MSC-treated group showed apparently normal histological architecture (Figure 5 C).

Morphometric results

As regarding the mean number of mast cells in the crypts of the control group was (0.4±0.52). In MTX-injected group was (4.2±0.63), which was significantly increased (P<0.05) in comparison with control group. While in MTX & MSC-treated group was (0.7±0.48), which was significantly decreased (P<0.05) compared to MTX-injected group, with non-significant difference when compared with control group (Histogram 3A).

As regarding the mean number of goblet cells in the crypts of the control group was (4±1.54). In MTX-injected group was (11.3±1.15), which was significantly increased (P<0.05) in comparison with control group. While in MTX & MSC-treated group was (5±0.81), which was significantly decreased (P<0.05) compared to MTX-injected group but there was a non-significant difference versus the control group (Histogram 3B).

Immunohistochemical results

Sections in the control group showed some positive CD4+ cells in connective tissue corium of intestinal villi (Figure 6A). While sections in MTX-injected group showed numerous positive CD4+ cells in connective tissue corium of small intestinal villi and inside dilated blood vessels (Figure 6B). Sections in MTX & MSC-treated group exhibited some positive CD4+ cells in lamina propria (Figure 6C).

Examination of small intestinal sections in control group showed some immunostained cells with CD8+ in connective tissue corium of intestinal villi (Figure 7A). Sections in MTX-injected group revealed multiple immunostained cells with CD8+ in connective tissue corium of small intestinal villi and shedded in intestinal lumen (Figure 7B). Sections in MTX & MSC-treated group showed few immunostained cells with CD8+ in connective tissue corium of small intestinal villi (Figure 7C)

The mean area % of CD4+ immunoreactivity in control group was (8.39±1.23). In MTX-injected group was (17.57±3.47), which was significantly elevated (P<0.05) in comparison with control group. Mean area % of CD4+ immunoreactivity in MTX & MSC-treated group was (8.24±1.23), which was significantly decreased (P<0.05) in comparison with MTX -injected group and non-significant difference versus control group (Histogram 4A).

In the CD8+ immune stained sections the mean area % of CD8+ immunoreactivity in control group was (14.05±2.27), in MTX-injected group was (16.73±1.15), which was significantly elevated (P<0.05) in comparison with control group. Mean area % of CD8+ immunoreactivity in MTX & MSC-treated group was (13.9±2.17), which was significantly decreased (P<0.05) comparable to MTX-injected group and non-significant difference when compared to control group (Histogram 4B).

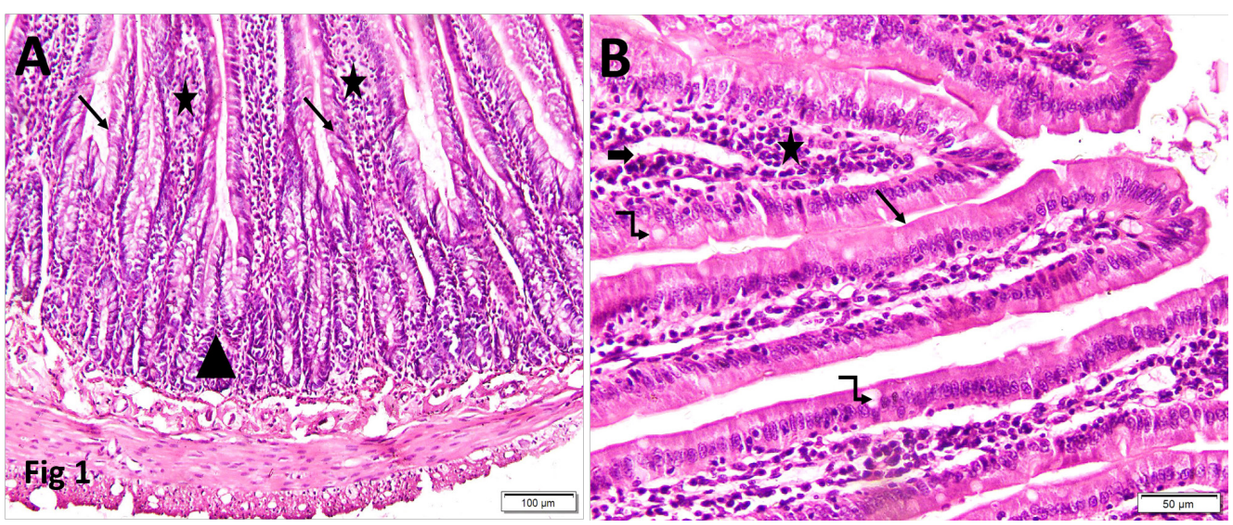


Fig. 1: (H&E): sections in the small intestinal tissue of rats in control group. A: showing normal small intestinal architecture, villi (astrex) lined with tall columnar cells having acidophilic cytoplasm, basal oval nuclei, and apical brush border (enterocytes) (arrows), the crypts (triangle). B: goblet cells (kinked arrows), central lacteals (thick arrow) and few inflammatory cells (astrex), intestinal villi (thin arrow). (A: x100) (B: x200)

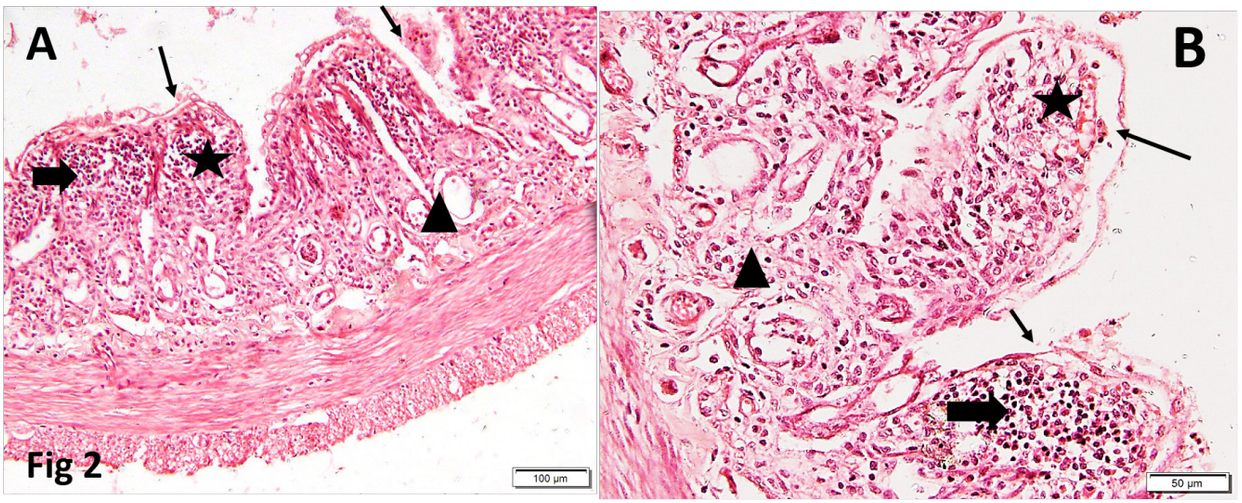


Fig. 2: (H&E): sections in the small intestinal tissue of rats in MTX-injected group. (A): showing villous atrophy (astrex), sloughing of the epithelium (thin arrows), some crypts are enlarged, and their cells are atrophied (triangles). The underlying lamina propria is studded with inflammatory cells (thick arrows). (A: x100) (B: x200)

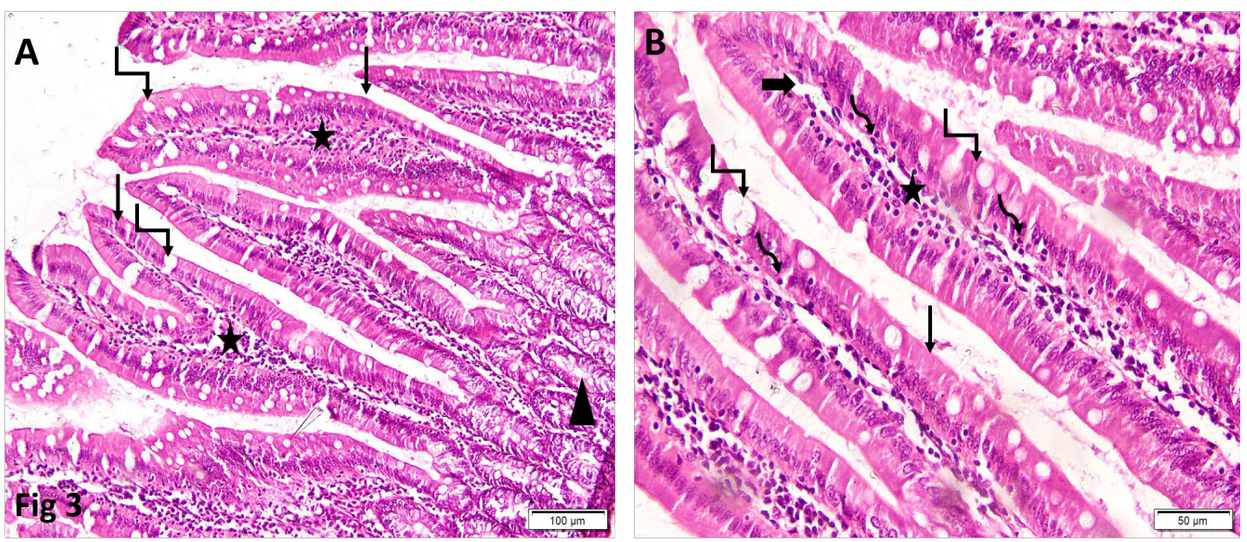


Fig. 3: (H&E): sections in the small intestinal tissue of MTX & MSC-treated group rats showing, (A): Villi (astrex) lined with tall columnar cells having acidophilic cytoplasm, basal oval nuclei, and apical brush border (enterocytes) (thin arrows) goblet cells (kinked arrows); The crypts (triangle). (B): large numbers of cells with rounded nuclei (curved arrows), goblet cells (kinked arrows), central lacteals (thick arrow), few inflammatory cells (astrex). (A: x100) (B: x200)

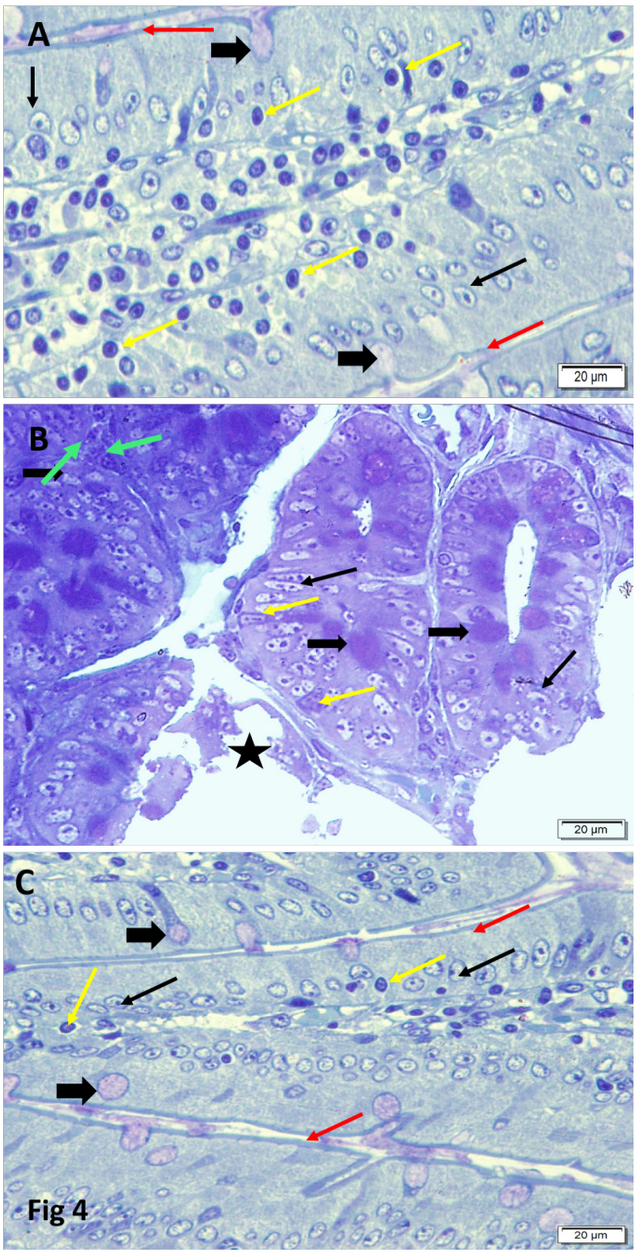


Fig. 4: Photomicrographs of semithin sections in rat intestinal villi stained with toluidine blue (x:1000). A: (control group), intestinal villi lined with columnar cells (enterocytes) having basal pale vesicular nucleus (thin arrows) with clear apical brush border (red arrows). Goblet cells (thick arrows) are present in between the enterocytes with large number of basolateral lymphocytes (yellow arrows). B (Methotrexate-injected group): exhibiting absent intestinal villi with desquamated cells (astrex) only crypts are present. The intestinal crypts are lined with columnar cells having basal pale vesicular nucleus (thin arrows) with large number of goblet cells (thick arrows) in between. Lymphocytes (yellow arrows) and mast cells (light green arrow) are present. C: (MTX & MSC-treated group): intestinal villi lined with columnar cells (enterocytes) having basal pale vesicular nucleus (thin arrows) with clear apical brush border (red arrows). Goblet cells (thick arrows) are present in between the enterocytes with few numbers of basolateral lymphocytes (yellow arrows).

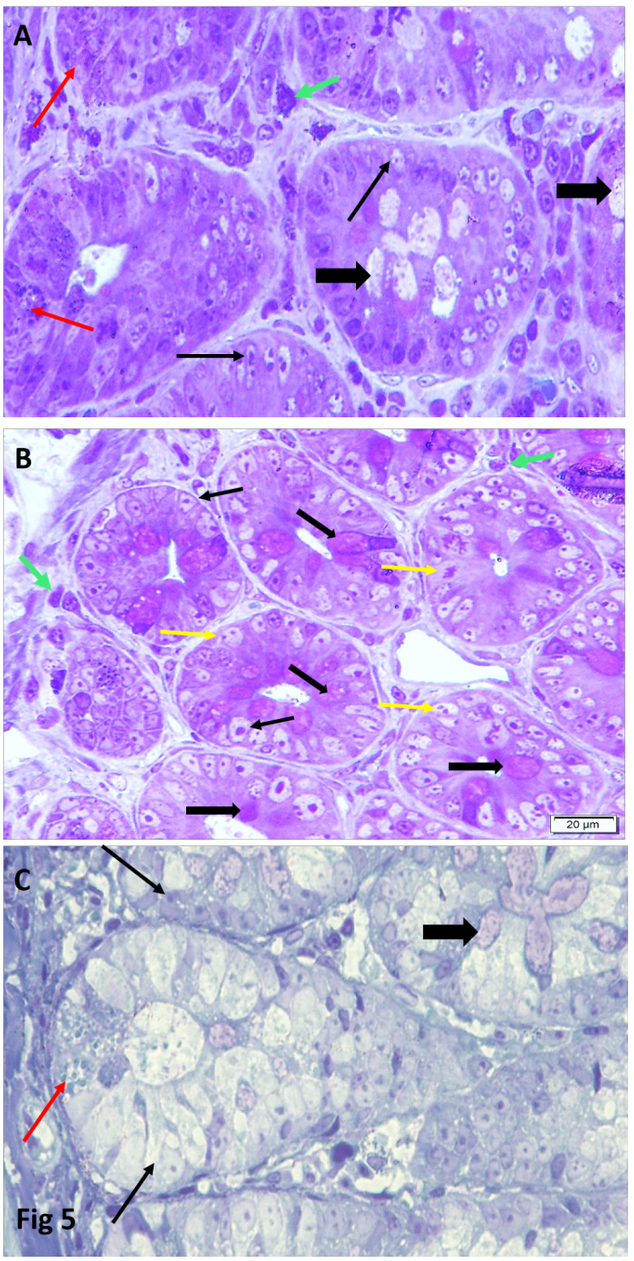


Fig. 5: Photomicrographs of semithin sections in rat small intestinal crypt stained with toluidine blue (x:1000). A (control group): showing crypts lined with columnar cells having basal pale vesicular nucleus (thin arrows) and Paneth cells (red arrows) are present at the bottom of the crypt having apical cytoplasmic granules. Goblet cells (thick arrows) are present in between the enterocytes. Note the presence of mast cell (light green arrow). B:(Methotrexate-injected group): exhibiting crypts lined with columnar cells having pale vesicular nucleus (thin arrows) and No Paneth cells could be detected. Large number of goblet cells (thick arrows) and mitotic figures (yellow arrows) are present. Note the presence of mast cells (light green arrows). C: (MTX & MSC-treated group): showing crypts lined with columnar cells having basal pale nucleus and pale vacuolated cytoplasm (thin arrows) with Paneth cells (red arrows), Goblet cells (thick arrow).

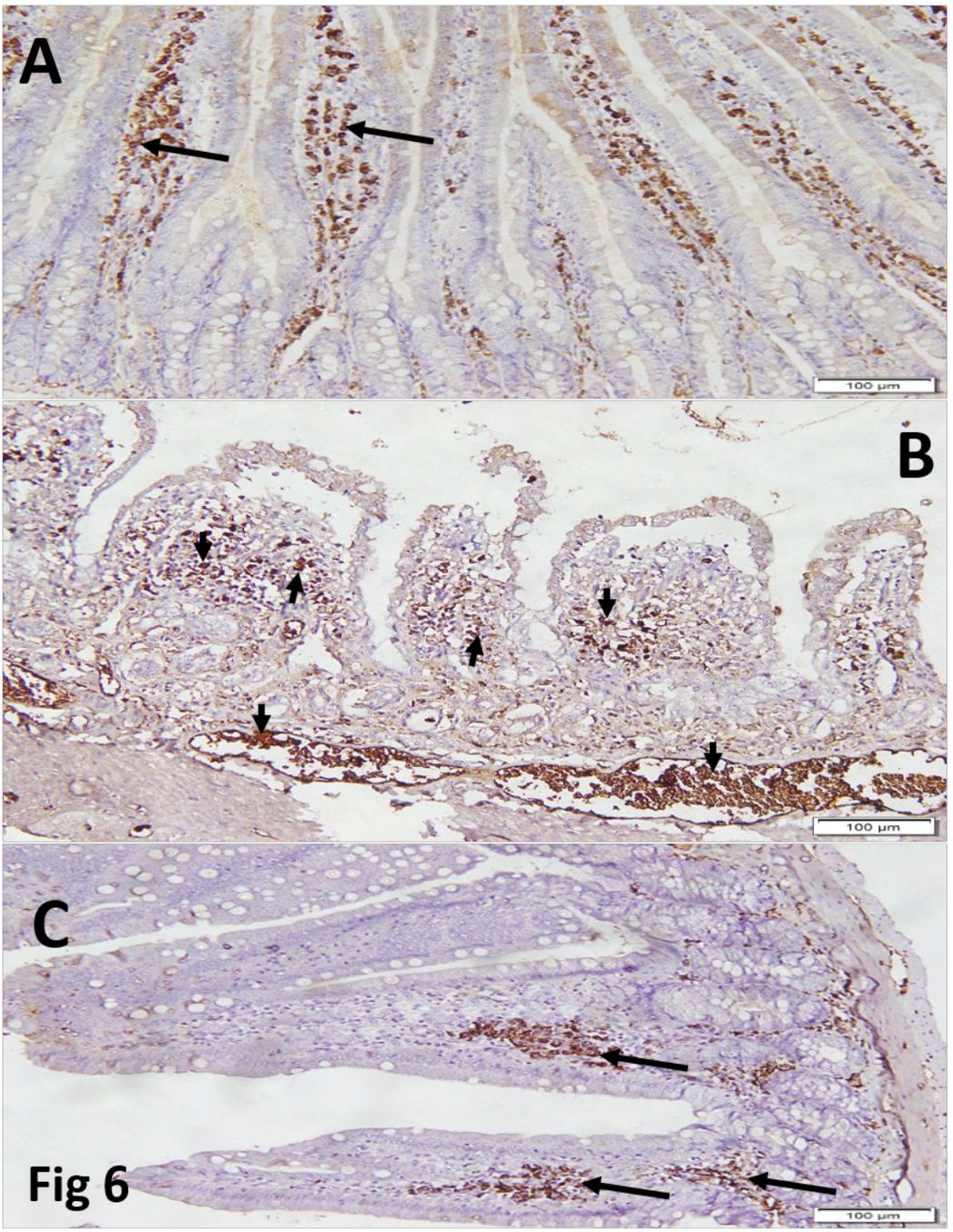


Fig. 6: Sections in the rat small intestinal tissues immunostained with CD4+ (X: 100). A: (control group): revealing moderate immunostaining of CD4+ (arrows) in connective tissue corium of intestinal villi. B: (MTX-injected group): showing marked immunostaining of CD4+ (arrows) in connective tissue corium of intestinal villi and inside dilated blood vessels. C: (MTX & MSC-treated group): showing minimal immunostaining of CD4+ (arrows) in connective tissue corium of intestinal villi.

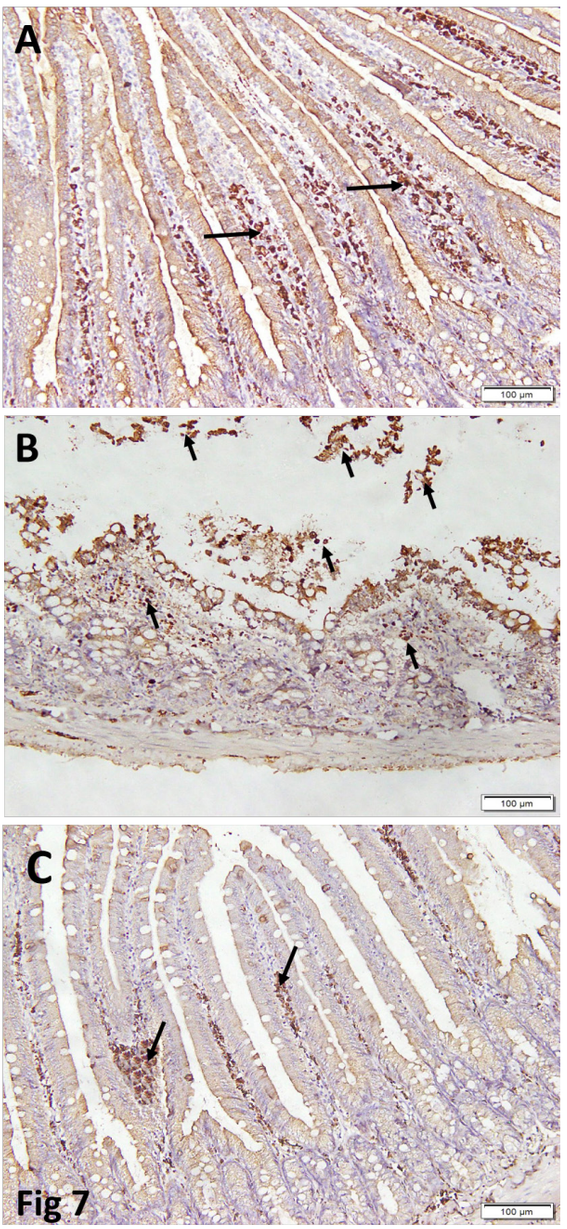
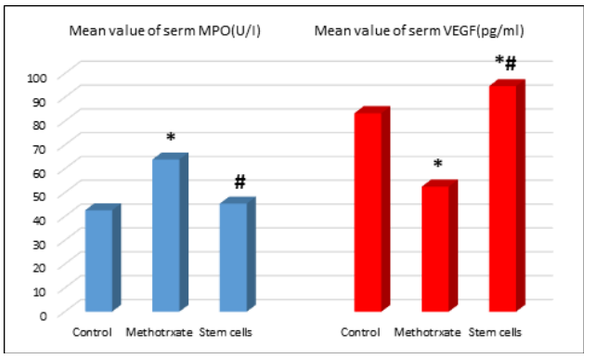
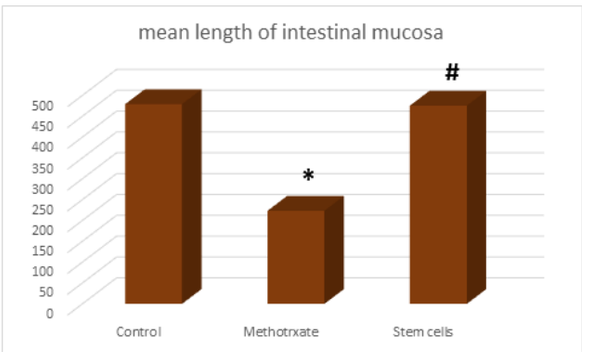


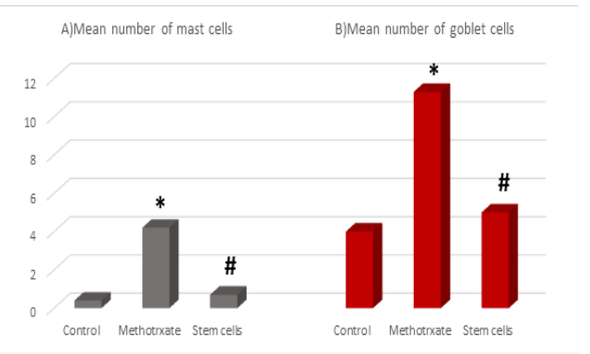
Fig. 7: Sections in the rat small intestinal tissues immunostained with CD8+ (X: 100). A: (control group): revealing moderate immunostaining of CD8+ (arrows) in connective tissue corium of intestinal villi. B: (MTX-injected group): showing marked immunostaining of CD8+ (arrows) in connective tissue corium of intestinal villi and shedded cells in intestinal lumen. C: (MTX & MSC-treated group): showing minimal immunostaining of CD8+ in connective tissue corium of intestinal villi (arrow).



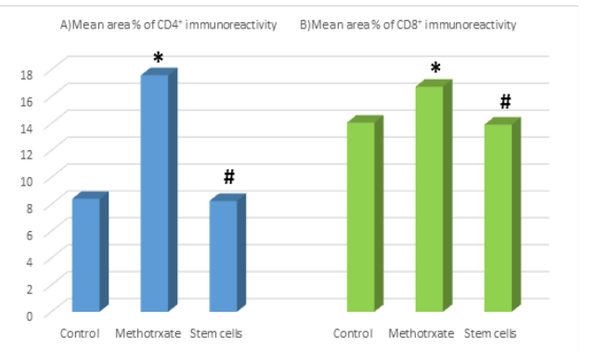
Histogram 1: Mean serum level of MPO and VEGF in the studied groups
*Significant as compared to control group (p<0.05).
#Significant as compared to MTX-injected group (p<0.05).



Histogram 2: Mean length of intestinal mucosa in the investigated groups
*Significant as compared to control group (p<0.05).
#Significant as compared to MTX-injected group (**p**<0.05).



Histogram 3: A) Mean number of mast cells, B) Mean number of goblet cells in the investigated groups
*Significant as compared to control group (p<0.05).
#Significant as compared to MTX-injected group (p<0.05).



Histogram 4: A) Mean area % of CD4+ immunoreactivity, B) Mean area % of CD8+ immunoreactivity in the investigated groups
*Significant as compared to control group (p<0.05).
#Significant as compared to MTX-injected group (p<0.05).

DISCUSSION

The present study was done to investigate the possible therapeutic effect of mesenchymal stem cells (MSCs) on Methotrexate (MTX) induced small intestinal injury in male albino rats. This was performed via laboratory, histopathological, immunohistochemical and morphometrical studies.

The rat was chosen to be an appropriate sample for studying the pathogenesis of chemotherapeutic toxicity on gastrointestinal tract. After MTX administration, the rats showed a histopathological verification of drug induced small intestinal enteropathy and villous atrophy as seen in humans[10].

In MTX treated group, serum Myeloperoxidase (MPO) levels showed significant increase in comparison with the control group. This was in accordance with Kolli *et al*.,[11] who stated that MTX administration activate neutrophils in the small intestinal tissues, the activated neutrophils secrete enzymes including myeloperoxidase (MPO). MPO plays an important role in superoxide production. Nitric oxide directly reacts with the superoxide anion to form the peroxynitrite. The increase in peroxynitrite production, results in loss of barrier function, induction of enterocyte apoptosis and villous atrophy by several methods including suppression of mitochondrial function, depletion of ATP and stimulation of caspases by cytochrome C.

Angiogenesis is important to provide new blood vessels, characterized by multiple steps including endothelial cells, cytokines, growth factors and extracellular matrix. Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis inducing endothelial cells migration and proliferation, stimulating smooth muscle growth, nerve regeneration and stabilizing neovascularization[12].

In our study, the mean value of serum VEGF in MTX-injected group was significantly decreased in comparison with control group. This finding agrees with Saadoon *et al*.[13] who stated that MTX administration showed obvious decrease in serum level of VEGF as MTX has an anti-angiogenic effect. The exact mechanism by which MTX inhibit angiogenesis is still unclarified. It might be attributed to suppression of the structural fitting of α, β, and γ VEGF receptors subunits, or due to modification of adhesion cells[14].

In the current study, MTX administration resulted in a marked pathological alteration documented by loss of normal small intestinal architecture with villous atrophy,

sloughing of the epithelium. Some crypts were enlarged, and their cells were atrophied. The underlying lamina propria was studded with inflammatory cells. In agreement, it was stated that the significant morphological changes caused by MTX, was small intestinal mucosal damage by suppressing crypt mitotic activity, blocking dihydrofolate reductase and consequently harming folic acid absorption, leading to a decrease of intracellular folate and impairing DNA synthesis[15].

Morphometrical results declared that the mean length of small intestinal mucosa significantly decreased in MTX treated group in comparison with control group. It was reported previously by other authors that MTX-induced gastrointestinal toxicity to induce alimentary mucositis (AM) by passing through five phases: initiation, up-regulation and generation of messenger signals, signal amplification, ulceration, and healing". Studies from both human and animal support this theory. In addition, these phases can cause gastrointestinal injury, including cell death, villous atrophy and crypt ablation in the small intestine[16].

Examination of semithin sections stained with toluidine blue showed that mast cell more pronounced in MTX injected group than other experimental groups. This is consistent with significant increase of mean number of mast cells in small intestinal crypts in MTX-injected group than control group. It has been reported that mast cells are tissue-resident immune cells which are distributed between barrier tissues as the skin, the mucosa, and the intestinal intraepithelial tissue playing an important physiological role initiating and regulating the immune response[17].

During inflammation, Treg cell function is weakened by mast cell activation, stopping tissue damage and chronic inflammation development. Clinical studies have concerned the contribution of mast cell in transmitting the immune signal, maintenance and controlling epithelial permeability in addition to consequent tissue remodeling[18]. Mast cells protects the intestinal tract by mediating IL-13 and IL-22 production to coordinate the immune response to reduce inflammation and stimulate epithelial repair[19].

In MTX injected groups, the semithin section examination appeared with large number of goblet cell, presented with significant increase in mean number of goblet cells. In consistent with Pelaseyed *et al*.,[20] who reported that, goblet cells were spared at villus tips and persisted functional Mucus2 and trefoil factor 3 expression after MTX exposure, contribute to epithelial defensive mechanism. Mucus secreted by goblet cells, is formed of glycan-covered proteins, or mucins, to create a protective gel-like structure above the epithelium. Alterations in mucin level or in mucin glycan are related to colitis and inflammation[21]. Previous studies also showed that the trefoil not only protects the intestinal mucosa from injury but can also facilitate repair after injury[22].

In the current study, CD4+ and CD8+ immunostained sections revealed some +ve CD4+ and CD8+ T-lymphocytes in lamina propria in control group. It is well known that the adaptive and innate immune systems cooperate at mucosal borders such as the skin, lung, and intestine to preserve barrier integrity and homeostasis[23].

Reported that T-lymphocytes identify foreign antigens by their surface T cell receptor (TCR) Konjar *et al*.,[24]. A unique TCR is expressed with each T cell. So, all foreign antigens can be identified by T cells. The antigens processed by antigen presenting cells, are presented in major histocompatibility complexes class II (MHCII) can be identified by CD4+ T helper (TH) cells. TH cells have a significant arranging role, differentiating into [type 1 (TH1), type 2 (TH2), and type 3 (TH17)] with definite functions in immune response.

Stated that cytotoxic T cells expressing CD8+ are derived as native cells from the thymus Sukhotnik *et al* and Konjar *et al*[1,24]. They chiefly distinguish antigens presented by MHC-I. These antigens resulting from viral infections and intracellular bacterial infections, are results of target cells' transcriptional machinery and cytosolic proteins degradation by the proteasome. CD8+ T cells differentiate into effector cells, which is a part of innate immunity.

In MTX treated group, CD4+ and CD8+ immunostanied sections showed multiple +ve CD4+ and CD8+ lymphocytes confirmed by a significant increase in the mean area % of +ve CD4+ and CD8+ immunoexpression. One of a highly proliferative tissues is small intestinal epithelium; chemotherapy breaks DNA strands resulting in direct small intestinal cellular injury. Additionally, chemotherapy may generate reactive oxygen species causing cell-damage, via enzymatic pathways or transcription factor (NF-κB) activation. The transcription factor (NF-κB) causes upregulation of genes responsible for the secretion of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. It has been reported that inflammation induced by MTX mediated by the pervious pro-inflammatory cytokines enhance T cell proliferation[1].

Sated that MTX induces severe small intestinal damage activating the mononuclear phagocytic system including dendritic cells and macrophages Zhou *et al*.,[23]. These cells will initiate the adaptive and innate immune systems by inducing the proliferation of CD4 and CD8 T-cells, secreting inflammatory cytokines (as TNF-α, IFN-γ and IL-1β).

In MTX & MSC-treated group, mean serum level of MPO was significantly decreased as compared with MTX-injected group, which was concomitant with Abbasi-Kenarsaria *et al*.,[25] who stated that mesenchymal stem cells have immunomodulatory property through affecting the development of various inflammatory cells including neutrophils. MSCs decreases the number of neutrophils thus decreasing the production of MPO.

In addition, MSCs markedly increased the mean serum level of VEGF is in comparison with MTX- injected group.

It has been suggested that MSCs may actively secrete a broad range of bioactive molecules with angiogenic (TGF-β1 and VEGF), immunomodulatory (PGE2, IL-1, IL-10 and IL-6), anti-apoptotic (STC-1 and SFRP2) and/or mitogenic (EGF, IGF-1and TGFα/β) properties that function to regulate the regenerative media at the site of injury. Upon re-establishment of the microenvironment, the damaged tissue can be completely regenerated by the surviving endogenous progenitor and stem cells[26].

Small intestinal sections showed regression of marked morphological changes detected by light microscopy that were induced by MTX administration. In addition, MTX & MSC-treated group exhibited significant decrease in mean number of mast cells and goblet cells associated with significant decrease in mean area % of CD4+ and CD8+ immunoreactivity.

Demonstrated that great curative effect of MSCs; as it proficiently responds to inflammatory cytokines and secrets immunomodulatory molecules that interact with the innate and adaptive immune components to regulate inflammation development via modulating NK cell, T cell, B cell, macrophage, and producing extracellular vesicles as exosomes Ocansey *et al*.,[27]. These vesicles are efficient intercellular transporters, transporting lipids, nucleic acids, and proteins. Therefore, they convey numerous physiological functions between parent cells and recipient cells including the development and repair of injured tissues[27,28].

Intestinal stem cells (ISCs) are settled in the base of the crypts and are responsible for preserving the intestinal epithelial homeostasis and regeneration following injury. Two populations of stem cells are identified in the small intestine of mice called Bmi1+ and Lgr5+ ISCs. Lgr5+ ISCs, also known as crypt base columnar cells (CBCs), are rapidly dividing stem cells and are scattered between the Paneth cells. A single Lgr5+ ISC can grow to create 'enteroids' that develop in the intestinal crypt into all the differentiated cell types[29,30].

Showed that the growth of endogenous Lgr5+ ISCs is supported by MSCs, thus inducing small intestinal repair Gong *et al*[31]. This is mediated by deactivation of the Wnt/β-catenin signaling pathway.

CONCLUSION

Based on the pervious results, MTX could induce detrimental effect in small intestinal tissues by disrupting normal epithelial function in mucosal barrier. MSCs could have positive effect in repairing the damaging effect of MTX in small intestinal tissues.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة هستولوجيه للتأثير العلاجي المحتمل للخلايا الجذعيه ضد الاصابة المعوية التي يسببها الميثوتريكسات في نموذج الفئران

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الخلفيه والهدف من العمل: ميثوتريكسات يستخدم كمضاد للروماتيزم وعلاج كيميائي, له تأثير ضار للغاية على الجهاز الهضمي. يمكن للخلايا الجذعية الوسيطة أن تمارس تأثيرًا علاجيًا من خلال القدرة على تجدد الأمعاء الناتج من تلفها بالميثوتريكسات. أجريت لدراسة التأثير العلاجي المحتمل للخلايا الجذعية الوسيطة ضد الإصابة المعوية التي قد يسببها الميثوتريكسات في ذكور الجرذان البيضاء البالغة.

الأساليب و النتائج: تم استخدام الجرذان الذكور في التجربه. المجموعة أ (المجموعة الضابطة) ، المجموعة الفرعية (أ-١) تلقت محلولًا ملحيًا لمدة أسبوعين بينما تلقت المجموعة الفرعية (أ-٢) محلولًا ملحيًا لمدة أسبوعين ثم تلقت محلول ملحي عازل الفوسفات مرة واحدة داخل الصفاق. تلقت جرذان المجموعة (ب), ميثوتريكسات (١٤ مجم / كجم / أسبوع) داخل الصفاق لمدة أسبوعين. المجموعة(ج), تلقت الفئران ميثوتريكسات (١٤ مغ / كغ / أسبوع) داخل الصفاق لمدة أسبوعين. بعد أسبوعين ، تم حقنهم بـالخلايا الجذعية الوسيطة (٢ × ١٠ خلايا في ٥٠٠ ميكرولتر PBS مرة واحدة). تم تخدير المجموعات أ-١ و ب بعد أسبوعين بينما تم التضحية بمجموعات أ-٢ و ج بعد ٤ أسابيع. تم سحب عينات الدم من الوريد لقياس عامل نمو بطانة الأوعية الدموية (MPO) وعامل نمو البطانة الوعائية (VEGF). تم الحصول على عينات صغيرة من الأمعاء للتقييم عن طريق الفحص المجهري الضوئي. تم تطبيق CD٤ + و CD٨ + الكيمياء المناعية والتحليل الإحصائي. تم الإبلاغ عن زيادة ملحوظة في عامل نمو بطانة الأوعية الدموية وانخفاض كبير في عامل نمو البطانة الوعائية في المجموعة B. التغيرات المرضية التي يسببها ميثوتريكسات في أنسجة الأمعاء الدقيقة ، ومتوسط عدد الخلايا البدينة والخلايا الكأسية المرتبطة بزيادة التعبير المناعي CD٤ + و CD٨ +. تم تحسين جميع التغييرات تقريبًا بعد العلاج بالخلايا الجذعية الوسيطة.

الاستنتاج: الخلايا الجذعية الوسيطة لها تأثير إيجابي في إصلاح التأثير الضار للميثوتريكسات في أنسجة الأمعاء الدقيقة.