Study of the Effect of Mesenchymal Stem Cells on Colitis: Possible Role of Galectins.

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ABSTRACT: Background: The anti-inflammatory and reparative properties of mesenchymal stem cells (MSCs) make them a promising tool for treating inflammatory and immune-mediated disorders. T cell dysfunction is undoubtedly a key feature in the pathogenesis of inflammatory bowel disease (IBD). MSCs suppress proliferation and alloreactivity of T cells, where several signaling molecules contribute to this effect. Galectins, a family of β-galactoside binding proteins, now emerge as a main regulator of MSCs immunomodulatory function. However, whether MSCs can be used for treatment of IBD still remains unclear. **Aim:** In this study, a dextran sulfate sodium (DSS) - induced colitis model was used to test the effect of infused bone marrow-derived MSCs on immunomodulatory molecules and if they could exert anti-inflammatory effects against experimental colitis.**Methods:** The study was carried on female albino rats, which were divided into three groups; Group 1 [Control group], Group 2 [Dextran sulfate sodium (DSS)-induced colitis group] and Group 3 [MSCs treated group]. Serum values of pro-inflammatory cytokines [tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL6)] as well as anti-inflammatory cytokines [interleukin 10 (IL10) and prostaglandin E_2 (PGE_2)] in the three groups were evaluated quantitatively by enzyme-linked immunosorbent assay (ELISA). Quantitative analysis of galectins 1, 2, 3 and 4 as well as basic fibroblast growth factor (bFGF) gene expression was done by Real Time PCR. Colon sections were stained with hematoxylin and eosin and examined for histopathological changes. **Results:** DSS-induced colitis group showed similar findings to that of ulcerative colitis in human, including body weight loss, bloody diarrhea, mucosal inflammation and ulceration. PKH26 labeled bone marrow-derived MSCs accumulated in inflamed regions of the colon, mainly in the submucosa and significantly ameliorated the clinical and histopathologic severity of DSS-induced colitis. Pro-inflammatory cytokines (TNF-α and IL6) were significantly lower in MSCs-treated rats compared to DSS-induced colitis rats. On the contrary, anti-inflammatory cytokines IL10, PGE2 and bFGF were significantly higher in MSCs-treated rats compared to DSS-induced colitis rats. Galectin 1 (Gal1), Galectin 2 (Gal2), Galectin 3 (Gal3) and Galectin 4 (Gal4) were significantly higher in MSCs-treated rats compared to DSS-induced colitis rats. **Conclusions:** Systemic infusion of bone marrow-derived MSCs may exert therapeutic efficacy on acute DSS-induced colitis in rats through their immonumodulatory and anti-inflammatory effects, which demonstrates the feasibility of using bone marrow-derived MSCs to treat IBD. Also the results presented in this study illustrate the involvement of the measured members of the endogenous galectin family (galectins 1, 2, 3 and 4) in the experimental model of colitis. The changes in their levels during inflammation evidenced that they play important role in MSCs immunomodulatory and anti-inflammatory actions.

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1. Introduction

Mesenchymal stem cells (MSCs) are multi-potent progenitor cells that are isolated from the bone marrow as well as several adult and fetal tissues [1]. In addition to their multilineage differentiation capacity, these cells also possess remarkable immunomodulatory properties. They can inhibit the proliferation and function of the major immune cell populations, including T cells, B cells, natural killer (NK) cells and dendritic cells [2-5]. They induce regulatory T cells which enhance their immunomodulatory effects [6-7]. Owing to their immunomodulatory effects, MSCs also alleviate adverse inflammatory reactions such as those in autoimmune disorders and after allogenic hematopoetic stem cell transplantation [8-10].

The immunomodulatory effect of MSCs is mediated by a non-specific anti- proliferative action of these cells, which is dependent on cell-cell contact or secreted soluble factors such as indoleamine 2,3-dioxygenase (IDO), nitric oxide (NO), histocompatibility leucocyte antigen-G (HLA-G), transforming growth factor-beta (TGF)-β, interleukin 10 (IL10) and prostaglandin E2 (PGE2) [11]. However whether these factors represent the only triggers involved in immunosuppression is not clear. Indeed, adding IDO inhibitor or neutralizing antibodies against TGF-β and IL-10 to mixed lymphocyte reaction cultures failed to prevent the T-

cell suppression induced by MSCs, suggesting that there is either redundancy in the mechanisms of immunosuppression or the involvement of other factors yet to be described [12].

Galectins, previously termed S-type lectins, are a family of β-galactoside binding proteins which now emerge as a main regulator of MSCs immunosuppressive function. Members of galectin family are found in mammals, birds, fishes as well as lower organisms. The mammalian galectins are a family of 15 proteins characterized by binding affinity for β-galactosides, such as lactose and N-acetyllactosamine, in free form or contained in glycoproteins or glycolipids. Some galectins contain one carbohydrate recognition domain (CRD) such as galectin-1, -2, - 5, -7, -10, -11, -13, -14 and -15, whereas other galectins such as galectin -4, -6, -8, -9 and -12, contain two CRDs connected by a short linker region. Galectin-3 uniquely occurs as a chimeric protein with one CRD and an additional non lectin domain, which is involved in the oligomerization of this protein [13-15]. Galectins are located intracellularly or extracellularly. They are involved in a variety of biological and pathological processes. They seem to be master regulators of immune cell homeostasis and inflammations [16-17]. Accumulating evidence has shown that galectins play a role in the initiation and resolution phases of inflammatory responses by promoting pro- or anti-inflammatory effects. In this regard, it has been hypothesized that the same galectin may exert pro- or anti-inflammatory effects depending on multiple factors, such as the concentration reached in inflammatory foci, the extracellular microenvironment and the particular target cells impacted [18]. Thus, recombinant proteins or specific galectin inhibitors may be used as therapeutic agents in inflammatory diseases [19]. Galectin-1 and galectin-3 are constitutively expressed and secreted by human bone marrow MSCs. Inhibition of galectin-1 and galectin-3 gene expression with small interfering RNAs abrogated the suppressive effect of MSCs on allogeneic T cells [20]. Moreover, suppression of T-cell proliferation by MSCs could be abrogated by exogenous addition of β lactose, a competitive inhibitor for galectin binding to cell surface glycoproteins, indicates that the carbohydrate-recognition domain of galectins is responsible for the immunosuppression of T cells [21]. Inflammatory bowel disease (IBD) is an autoimmune disease characterized by two forms of intestinal inflammations, Chron's disease and ulcerative colitis, and is associated with increased abnormal T cell activity. An increase in understanding of MSC suppressor mechanisms will offer an insight into the use of MSCs in human therapy such as the treatment of IBD [22].

In the present study bone marrow derived MSCs were isolated from male rats and were used to detect their possible anti-inflammatory effects against DSS-induced colitis. In this study we focused on four members of galectins (galectins 1, 2, 3 and 4), which have been studied regarding intestinal inflammation. Quantitative analysis of gene expression of these four galectin members together with the basic fibroblast factor (bFGF) was done by Real Time PCR in all control, DSS-induced colitis and MSCs treated animal groups, in order to detect changes in the level of mRNA of these four galectins members in colonic tissue during inflammation, and to study the effect of administration of MSCs on their levels as they reported to be immunomodulators for MSCs. Also serum values of pro-inflammatory cytokines [tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL6)] as well as anti-inflammatory cytokines [interleukin 10 (IL10) and prostaglandin E2 (PGE2)] in the three groups were evaluated quantitatively by enzyme-linked immunosorbent assay (ELISA).

2.Material & Methods

-Preparation of the animal model:

Experimental Animals: The study was carried on 30 female white albino rats, of an average weight 150-200 gm. Rats were bred and maintained in an air-conditioned animal house with specific pathogen-free conditions, and were subjected to a 12:12-h daylight/darkness and allowed unlimited access to chow and water. All the ethical protocols for animal treatment were followed and supervised by the animal unit, Faculty of Medicine, Cairo University. They were divided into 3 groups as follow:

- **Group 1 [Control group]:** consisted of 10 rats, used as healthy control group.
- **Group 2 [Dextran sulfate sodium (DSS)-induced colitis group]:** consisted of 10 rats which received 4% DSS in drinking water for 7 days followed by administration phosphate buffer saline (Wirtz *et al.,* 2007 [23] and Tanaka *et al.,* 2008 [24]).
- **Group 3 [Mesenchymal stem cells (MSCs) treated group]:** consisted of 10 rats with DSS-induced colitis rats that received (MSCs), in a single dose of 10^6 cells per rat, given by intravenous infusion at the rat tail vein.

After 21 days animals were scarified and intestinal tissues were carefully dissected and examined for:

- Presence of MSCs homing in intestinal tissue after labeling with PKH26 dye, by fluorescent microscope to detect red fluorescence.
- Presence of histopathological changes where specimen of intestine were fixed, sectioned and

stained with hematoxylin and eosin, and examined by light microscope.

- Quantitative analysis of galactins 1, 2, 3 and 4 and bFGF gene expression by Real Time PCR.
- Blood samples were withdrawn before animal scarification and serum values of pro-inflammatory cytokines [tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL6)], as well as, anti-inflammatory cytokines [interleukin 10 (IL10) and prostaglandin E2 (PGE2)] in the three groups were evaluated quantitatively by enzyme-linked immunosorbent assay (ELISA).

2-Preparation of bone marrow-derived mesenchymal stem cells from rats:

Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO_2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5 min at 37°C. After centrifugation, cells were resuspended in serum-supplemented medium and incubated in 50 cm2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures. MSCs in culture were characterized by their adhesiveness and fusiform shape Also CD29 gene expression was detected by RT-PCR as a marker of MSCs (ALDAH).

Labeling of MSCs with PKH26:

MSCs were harvested during the 4th passage and were labeled with PKH26, which is a red fluorochrome. It has excitation (551 nm) and emission (567 nm) characteristics compatible with rhodamine or phycoerythrin detection systems. The linkers are physiologically stable and show little to no toxic side-effects on cell systems. Labeled cells retain both biological and proliferating activity, and are ideal for in vitro cell labeling, in vitro proliferation studies and in vivo cell tracking. In the current work, MSCs were labeled with PKH26 from Sigma Company (Saint Louis, Missouri USA). Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution. Cells were injected intravenously into rat tail vein. Intestinal tissue was examined with a fluorescence microscope to detect and trace the cells.

3-Real-time quantitative analyses for galectins and bFGF genes expression:

Total RNA was extracted from intestinal tissue homogenate using RNeasy purification reagent (Qiagen, Valencia, CA). cDNA was generated from 5 μg of total RNA extracted with 1 μl (20 pmol) antisense primer and 0.8 μl superscript AMV reverse transcriptase for 60 min at 37 °C.

The relative abundance of mRNA species was assessed using the SYBR® Green method on an ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA). PCR primers were designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from GenBank (Table 1) according to Klima *et al.* (2009) [25]. All primer sets had a calculated annealing temperature of 60°. Quantitative RT-PCR was performed in duplicate in a 25-μl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer and 2–3 μl of cDNA. Amplification conditions were 2 min at 50°, 10 min at 95° and 40 cycles of denaturation for 15 s and annealing/extension at 60° for 10 min. Data from real-time assays were calculated using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied mRNA was calculated using the comparative Ct method as previously described. All values were normalized to the beta actin genes and reported as fold change over background levels detected in colitis.

4-Detection of TNF-α, IL-6, IL-10 and PGE2:

TNF-α, IL-6, IL-10 were measured in serum samples using ELISA kit supplied by Quantikine R & D system according to manufacturer's instruction. PGE2 was estimated by using Parameter™ PGE2 Assay supplied by (R & D system Inc.).

5-Statistical methods:

Data were coded using the statistical package SPSS version 15. Data were summarized using mean, standard deviation and range for the quantitative variable. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables. While non parametrical Kruscal-Wallis test and Mann-Whitney test were used for non normally distributed quantitative variables. Correlations were done to test for linear relations between quantitative variables. *P* values less than or equal to 0.05 were considered as statistically significant.

3.Results

MSCs culture, identification and homing:

Isolated and cultured undifferentiated MSCs reach 70-80% confluence at 14 days. MSCs were identified by shape and surface markers CD29 (+) by PCR. MSCs labeled with PKH26 fluorescent dye was

detected in the intestinal tissues confirming that these cells homed into the intestinal tissue (Figs. 1-2).

Body weight:

The mean body weight of rats was significantly lower in DSS-induced colitis group compared to control group; however, it was significantly higher in MSCs treated group compared to DSS-induced colitis group. (Table 2, Graph 1).

Histopathological changes:

-Control group:

Examination of histological sections of the colon of this group showed apparently normal mucosa, showing crypts lined by columnar cells with intervening large number of Goblet cells, with underlying submucosa formed of loose vascular connective tissue and musculosa (Figs. 3-4).

-DSS-induced colitis group:

Examination of histological sections of the colon of this group showed degenerated crypts lined with degenerated cells with pyknotic nuclei and decreased number of Goblets cells. The mucosa revealed heavy inflammatory cellular infiltrate (Figs. 5-8).

-MSCs treated group:

Examination of histological sections of the colon of this group showed marked improvement compared to the previous group. Proliferating crypts, lined with regular columnar cells together with increased number of Goblets cells and a marked reduction in the inflammatory cellular infiltrate were observed in this group (Figs. 9-10).

TNF-α and IL6:

TNF-α and IL6 were significantly higher in DSS-induced colitis group compared to control group. Also they were significantly lower in MSCs treated group compared to DSS-induced colitis group (Table 2, Graphs 2-3).

IL10, PGE2 and bFGF:

IL10, PGE2 and bFGF were significantly lower in DSS-induced colitis group compared to control group. Also they were significantly higher in MSCs treated group compared to DSS-induced colitis group (Table 2, Graphs 4-6).

Gal 1, Gal 2, Gal 3 and Gal 4:

Gal 1, Gal 2, Gal 3 and Gal 4 were significantly lower in DSS-induced colitis group compared to control group. Also they were significantly higher in MSCs treated group compared to DSS-induced colitis group (Table 2, Graphs 7-10).

Table 1: Sequence of the primers used for real-time PCR:

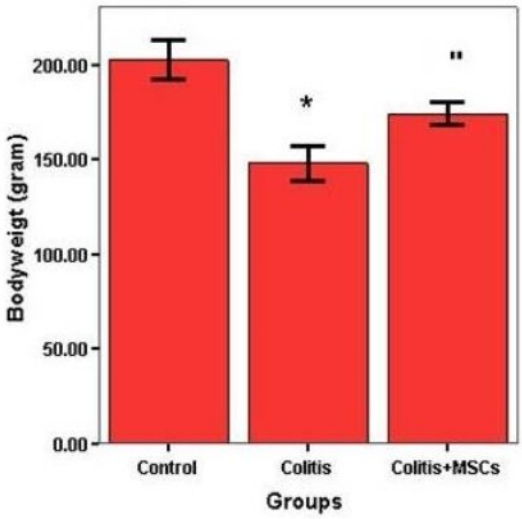
	Primer sequence
Galectin 1	Forward primer :5′-GGCAAAGACAGCAACAACCT-3’ Reverse primer: 5′-GGCCACACACTTGATCTTGAA-3’
Galectin 2	Forward primer : 5’-ATGACGGGGGAACTTGAGGTT-3’ Reverse primer: 5’-TTACGCTCAGGTAGCTCAGGT-3’
Galectin 3	Forward primer :5′-TGCCTCGCATGCTGATAACA-3’ Reverse primer: 5′-GGTTCAACCAGGACTTGTAT-3’
Galectin 4	Forward primer: 5’-TGGTAAATGGAAATCCCTTCTATG-3’ Reverse primer: 5‘-GAGCTGTGAGCCCTCCTT-3’
bFGF	Forward primer :5’-CCAGGCTGGATTGCAGTT- 3’ Reverse primer: 5’-GATCACGAGGTCAGGAGATG-3’
β-actin	Forward primer :5’-CCAGGCTGGATTGCAGTT- 3’ Reverse primer:5’-GATCACGAGGTCAGGAGATG-3’

Table (2): Comparison of mean ± SD values of the parameters in the studied groups:

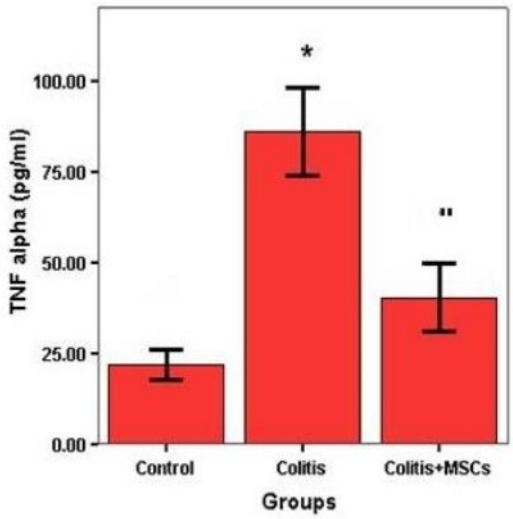
	Control group (n=10)	**DSS-induced colitis group** (n=10)	**MSCs treated group** (n=10)
Body weight (gram)	202.24±10.34	147.73±9.05*	173.80±6.08"
TNF alpha (pg/ml)	21.94±4.17	86.02±11.97*	40.29±9.37"
IL6 (pg/ml)	21.10±2.92	87.22±15.19*	53.74±13.10"
IL10 (pg/ml)	105.14±10.42	61.84±15.37*	84.74±14.21"
PGE2 (pg/ml)	221.54±12.39	120.79±17.39*	177±23.31"
bFGF	0.71±0.19	0.21±0.5*	0.42±0.12"
Galectin 1 (Gal1)	1.22±0.28	0.16±0.13*	0.48±0.21"
Galectin 2 (Gal2)	0.71±0.25	0.09±0.02*	0.31±0.14"
Galectin 3 (Gal3)	2.14±0.67	1.27±0.21*	1.82±0.47"
Galectin4 (Gal4)	0.88±0.11	0.22±0.13*	0.64±0.13"

* significant difference in the comparison to control group ($p < 0.05$)

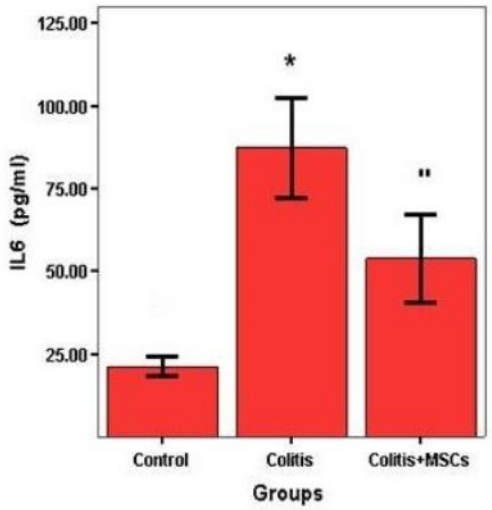
" significant difference in the comparison to colitis group ($p < 0.05$)



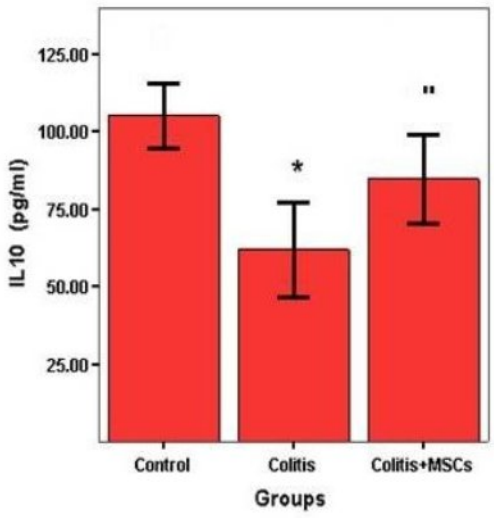
Graph 1: Mean body weight in different groups



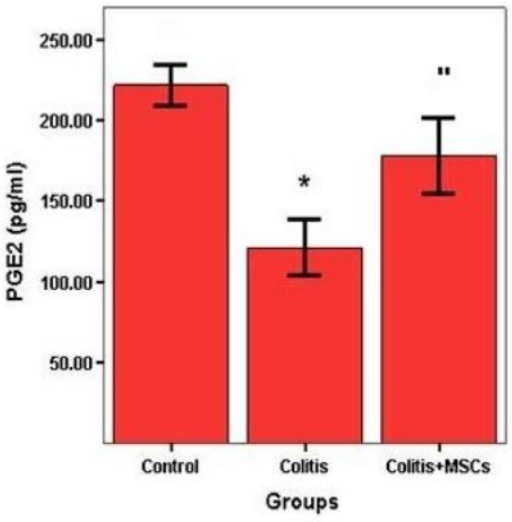
Graph 2: TNF-α in different groups



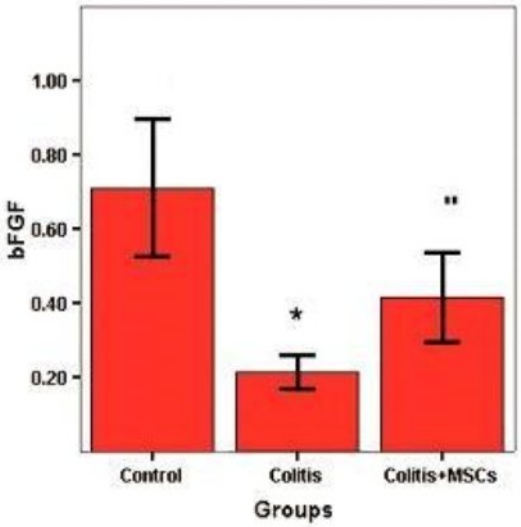
Graph 3: IL6 in different groups



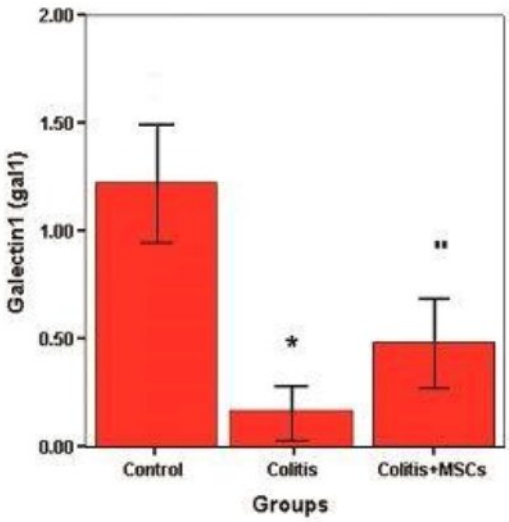
Graph 4: IL10 in different groups



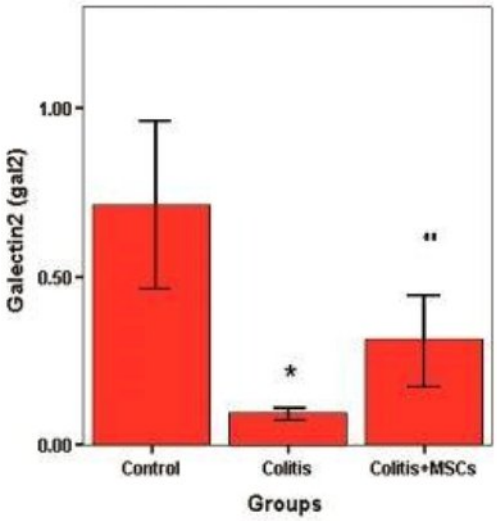
Graph 5: PGE2 in different groups



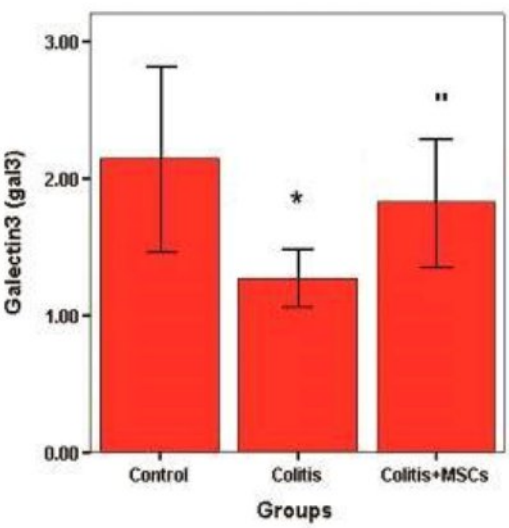
Graph 6: bFGF in different groups



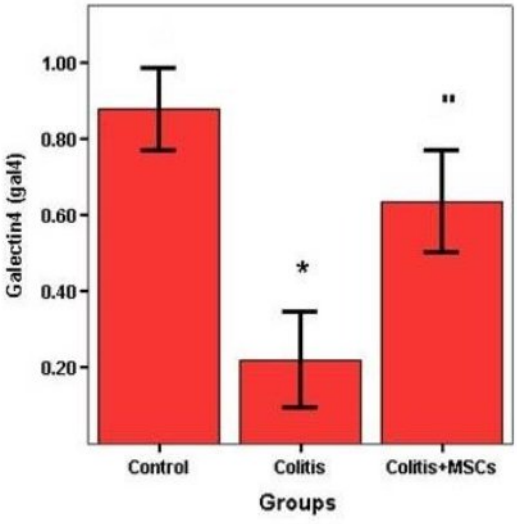
Graph 7: Gal-1 in different groups



Graph 8: Gal-2 in different groups



Graph 9: Gal-3 in different groups



Graph 10: Gal-4 in different groups

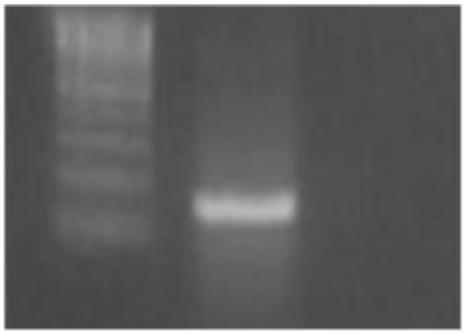


Fig. 1: Agarose gel electrophoresis showing PCR products of CD29 gene expression in MSC culture. Lane M: DNA marker with 100 bp., Lane 1: MSCs culture.

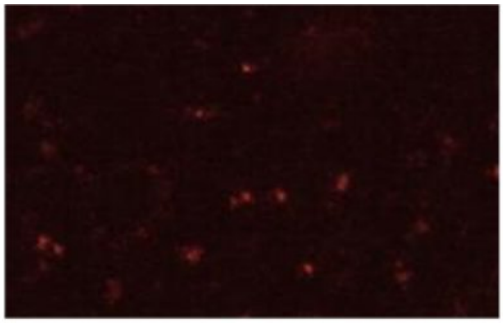


Fig. 2: Labeling of MSC with PKH26 dye.

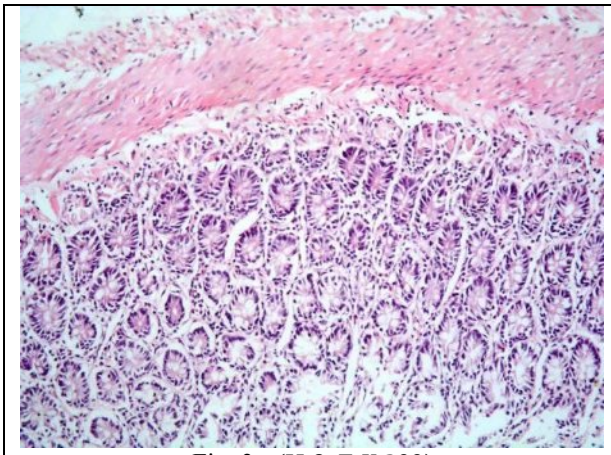


Fig. 3 *(H & E X 200)*

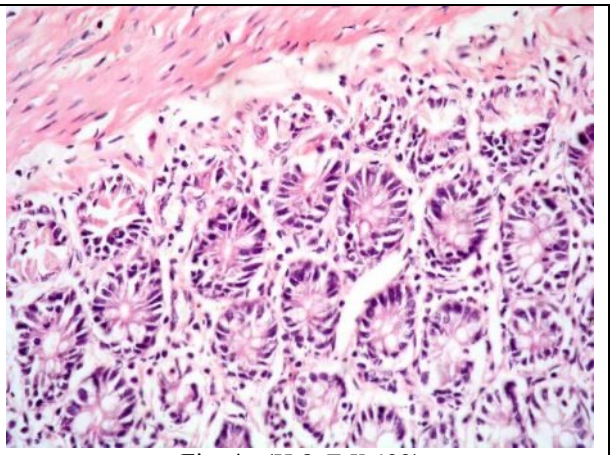


Fig. 4 *(H & E X 400)*

Group 1 ***(Control group):*** showing normal mucosa with crypts lined by columnar cells with intervening Goblet cells, with underlying submucosa.

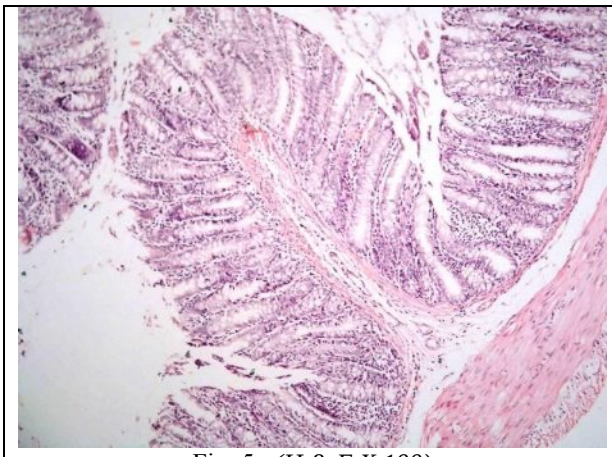


Fig. 5 *(H & E X 100)*

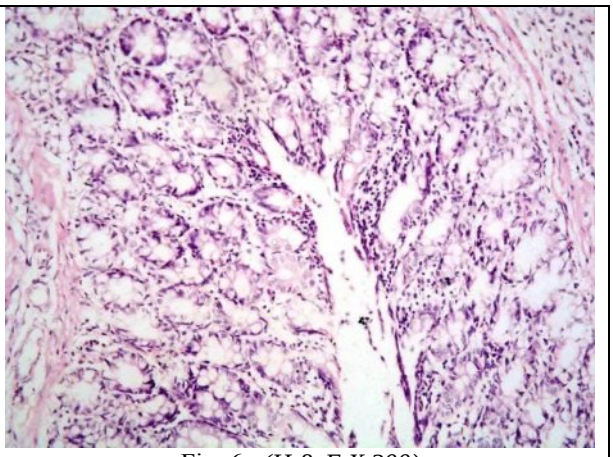


Fig. 6 *(H & E X 200)*

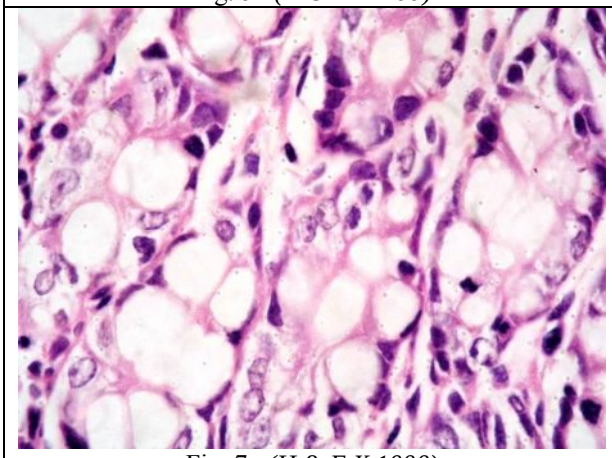


Fig. 7 *(H & E X 1000)*

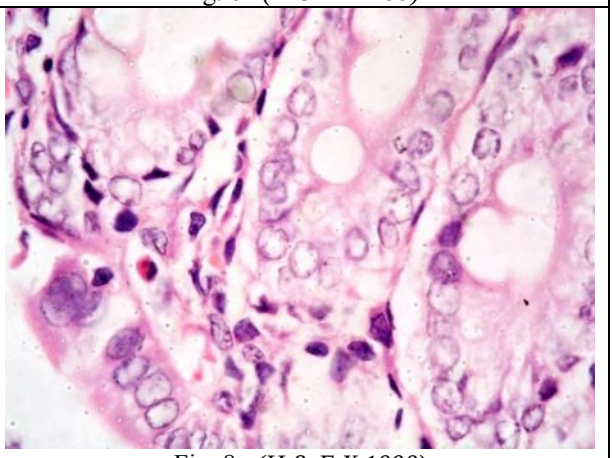


Fig. 8 *(H & E X 1000)*

Group 2 ***(DSS-induced colitis group):*** showing degenerated crypts lined with degenerated cells with pyknotic nuclei and decrease number of Goblets cells together with heavy inflammatory cellular infiltrate.

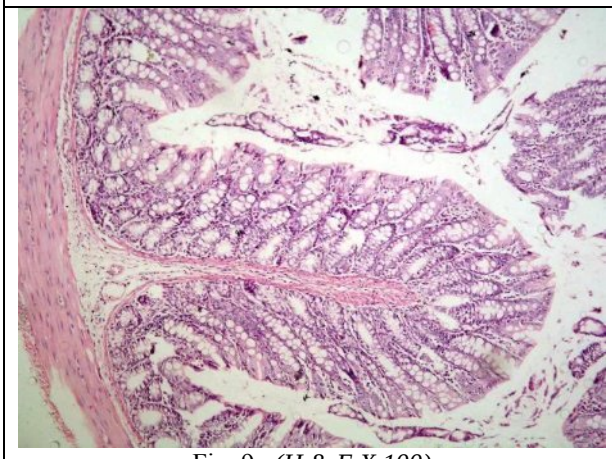


Fig. 9 *(H & E X 100)*

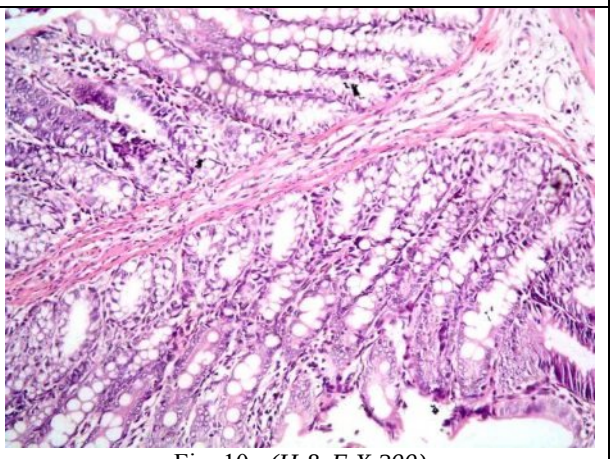


Fig. 10 *(H & E X 200)*

Group 3 ***(MSCs treated group):*** showing proliferating crypts, lined with regular columnar cells together with increased number of Goblets cells and a marked reduction in the inflammatory infiltrate.

4.**Discussion**

IBD is an autoimmune disease characterized by an inflammation of the gastrointestinal tract. It is associated with increased abnormal T cell activity. Owing to their immunomodulatory effects and their ability to suppress T cell, MSCs are able to alleviate adverse inflammatory reactions such as those in autoimmune disorders. In the present study, DSS-induced colitis was done to test the possible beneficial effect of infused bone marrow-derived MSCs on experimental colitis.

In the present study a decrease body weight was observed in DSS-induced colitis compared to control group. This decrease in the body weight was found to be much reduced in MSCs treated group indicating an overall improvement of the clinical condition of these rats.

Similarly the histopathological changes in DSS-induced colitis group were found to be ameliorated in MSCs treated group which showed proliferating crypts with regular lining and increased Goblets cells denoting reparative attempts.

These findings are in accordance with Gonzalez *et al.* (2009) [26] who noticed reduction of the weight loss, diarrhea and inflammation of the colon in mice with colitis after infusion of mesenchymal stem cells, indicating valuable effects of these cells in management of colitis by inhibiting autoimmune and inflammatory responses. He *et al.* (2012) [27] also observed that bone marrow-derived MSCs significantly ameliorated the clinical and histopatholgic severity of DSS-induced colitis. The authors concluded that bone marrow-derived MSCs may exert therapeutic efficacy on DSS-induced colitis in mice through their anti-inflammatory effects which demonstrate the feasibility of using bone marrow-derived MSCs to treat IBD.

In the current work DSS-induced colitis was accompanied by a significant increase in the level of pro-inflammatory cytokines as TNF-α and IL6, compared to the control group. Amazingly these pro-inflammatory cytokines were significantly reduced in the MSCs treated group compared to DSS-induced colitis group.

On the contrary, the level of the anti-inflammatory cytokines as IL10, PGE_2 and bFGF were significantly less in DSS-treated group compared to control group. Again the level of these cytokines was higher in the MSCs treated group compared to DSS-induced colitis group.

These observations are in accordance with Zhang *et al.* (2009) [28], who suggested that the therapeutic effects of MSCs was mediated, in part, by the suppression of pro-inflammatory cytokines and the expression of anti-inflammatory cytokines at the colonic sites. The authors concluded that MSCs can function as an immunomodulatory and anti-inflammatory. Similarly, Aggarwal and Pittenger (2005) [29] who examined the immunomodulatory functions on human MSCs by coculturing them with purified subpopulations of immune cells and reported that MSCs altered cytokines secretions to induce more anti-inflammatory cytokines as IL10 and PGE2 together with decrease secretion of pro-inflammatory as TNF-α.

The present work showed that bFGF was significantly higher in MSCs treated group compared to DSS-induced colitis group. This finding was also reported by Paunovic *et al.* (2011) [30] who observed that colonic lesions were significantly reduced by bFGF treatment which enhanced proliferation of fibroblasts and epithelial and endothelial cells promoting angiogenesis. The authors stated that the molecular mechanisms of bFGF in UC healing not only involve the expected increased cell proliferation especially angiogenesis, but also encompass the reduction of inflammatory cytokines and infiltration of inflammatory cells. They hypothesized that bFGF might accelerate the healing of experimental colitis in rats and suggested that bFGF enema may be a new therapeutic option for UC. Kojima *et al.* (2007) [31] also found that administration of bFGF enema significantly improve weight loss, stool consistency, gross rectal bleeding as well as mucosal injury in colonic tissue samples. The authors supported that bFGF enema is clinically useful and safe in the treatment of inflammatory bowel disease and that BFGF enema may contribute as a novel therapy of IBD. Singh *et al.* (2010) [22] reported that MSCs accelerate the proliferation and migration of residual epithelial cells over denuded areas by releasing TGF-beta, EGF and bFGF.

The current study showed that galectins were significantly higher in MSCs treated group compared to DSS-induced colitis group suggesting that these galectins represent key mediators of the MSCs induced immunomodulatory and anti-inflammatory effects.

Our results are in agreement with Santucci *et al.* (2003) [32], who reported that administration of galectin 1 resulted in a striking improvement in the clinical and histopatholgical aspects of the disease. They noticed that galectin 1 reduced the number of activated T cells, decreased inflammatory cytokine production. The authors concluded that galectin-1 exerts immunomodulatory and protective activity in colitis and that it might be effective in the treatment of inflammatory bowel diseases. Also, Van Der Leij *et al.* (2007) [33], found that galectin 1 has potent anti-inflammatory and immunomodulatory effects and that these effects are probably related to the induction of apoptosis in activated T cells. The authors suggested

that application of galectin 1 may therefore serve as an improved treatment of chronic inflammatory diseases.

Similarly, Paclik *et al.* . (2008) [34], observed that galectin 2 treatment induced apoptosis of mucosal T cells and inhibit pro-inflammatory cytokine release, thus ameliorated acute and chronic DSS-induced colitis. Authors also found that galectin 2 is well tolerated and has no significant toxicity over a broad dose range; they suggest that it may serve as a new therapeutic agent in the treatment of inflammatory bowel disease.

Furthermore, Müller *et al.* (2006) [35], noticed that galectin 3 is expressed at comparable levels in controls and IBD patients in remission. However, it was significantly downregulated in inflamed biopsies from IBD patients. The authors reported that galectin 3 acts as immunomodulatory by inducing apoptosis of T cell.

Moreover, Paclik *et al.* (2008) [36], reported that galectin 4 reduce pro-inflammatory cytokine secretion and induce apoptosis of mucosal T-cell ameliorating intestinal mucosal inflammation. The authors concluded that galectin 4 is a novel anti-inflammatory agent that could be therapeutically effective in diseases with a disturbed T cell expansion such as inflammatory bowel disease.

The results of the present study showed that administration of MSCs ameliorate the clinical and histopathological finding in DSS-induced colitis in rats. These results also suggest that MSCs-secreted galectins represent key mediators involved in the immune-modulatory and anti-inflammatory properties of these cells. These findings must encourage further scientific researches regarding the efficacy and safety of the use of MSCs and/or of the studied galectins as new therapeutic options to alleviate inflammatory reactions, improve symptoms and fasten healing in autoimmune disorders such as IBD.

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