INTRODUCTION

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are relapsing disorders associated with inflammation within the gastrointestinal tract. They are multifactorial diseases of unknown etiology ***(Sands, 2007)***. Recently, the widely accepted cause of IBD is disturbed interaction of the host immune system with the commensal microflora and other luminal antigens, which leads to mucosal inflammation ***(Baumgart and Carding, 2007).*** The critical pathophysiological feature in IBD is impaired epithelial barrier function ***(Xavier and Podilsky, 2007).***

Ulcerative colitis involves the rectum but may affect any part of the colon or the entire colon ***(Abraham and Cho, 2009)***. Despite the improvement in the clinical management of IBD, none of the present treatment agents have a curative effect and few ''nontoxic'' options are existing to modulate the intestinal inflammation. One of these agents is Sulfasalazine which has a local anti-inflammatory effect by inhibiting prostaglandin synthesis and other mediators like leukotrienes and platelet activating factor. However, it is still challenging to find new treatment approaches for IBD that have fewer side-effects and are more effective.

Bone marrow (BM) contains pluripotent mesenchymal stem cells (MSCs) that can be safely isolated and purified. Mesenchymal stem cells lack major histocompatibility II so

they are able to escape the immune recognition or at least are hypo-immunogenic ***(Le Blanc et al., 2003).*** Mesenchymal stem cells have the ability to proliferate quickly in vitro and to differentiate along the chondrogenic, osteogenic and adipogenic lineages both in vivo and in vitro ***(Bianco et al., 2008; Jones and McGonagle, 2008).***

In the past years, it was suggested that MSCs could serve as an effective treatment agent for tissue repair ***(Brooke et al., 2007; Pa¢unescu et al., 2007).***

Recently, MSCs were observed to modulate the innate and adaptive immunity ***(Uccelli et al., 2008; Zhang et al, 2009).*** Mesenchymal stem cells may suppress the function of the immune cell populations, including dendritic cells, B cells, T cells, and natural killer cells. The immunomodulatory and anti-inflammatory effects of MSCs have been tested in many animal models and have been applied in different clinical settings. The most powerful data are from in vivo studies conducted in humans demonstrating successful management of the life-threatening graft-versus-host disease (GVHD) with MSCs ***(Rinden et al., 2006; Le Blanc et al., 2008).***

From the above information we suggest that systemic infusion of bone marrow-derived MSCs may exert therapeutic efficacy on experimentally-induced ulcerative colitis in adult albino rats through their anti-inflammatory effects.

AIM OF THE WORK

Many trials were done to obtain the best therapeutic management for ulcerative colitis. So, the aim of this study is:

- To induce an experimental animal model of ulcerative colitis.
- To evaluate the possible curative role of isolated cultured Bone marrow-derived mesenchymal stem cells (BM MSCs) in ameliorating the process of experimentally-induced ulcerative colitis in adult male albino rats.
- To compare between the effect of BM MSCs and the effect of sulfasalazine in the healing process of experimentally-induced ulcerative colitis in adult male albino rats.

HISTOLOGY OF THE COLON

The large intestine, the last part of the digestive system, is formed of the cecum, the colon, the rectum, the anal canal and the appendix. The colon is further subdivided anatomically into ascending colon, descending colon, transverse colon and sigmoid colon ***(Gartner and Hiatt, 2014).***

The colon has special features at the gross level: teniae coli, haustra coli and omental appendices. Histologically the wall of the colon is formed of the four layers characteristic of the gastrointestinal tract (GIT): Mucosa, Submucosa, Muscularis externa and Serosa ***(Ross, 2016).***

Mucosa of the colon:

It is the innermost layer that is formed of regularly arranged closely packed tubular glands called crypts extending through the full thickness of the mucosa and lined by simple columnar epithelium with underlying lamina propria and muscularis mucosa. It has no folds and no villi ***(Gartner and Hiatt, 2014).***

The mucosal surface and the crypts are lined by different epithelial cell types, mainly columnar absorptive cells and goblet cells.

The absorptive cells are tall columnar cells. The apical surface of the cells shows brush border containing microvilli that increase the surface area as much as 600 times. The lateral

surface exhibits tight junctions that are present between them and other cells of the epithelium. These tight junctions establish a barrier between the intestinal lumen and the epithelial intercellular space. The primary function of these cells is water and electrolytes reabsorption through Na^+/k^+-activated ATPase-driven transport system so they have numerous mitochondria and wide intercellular space ***(Ross, 2016).***

The goblet cells (GCs) represent unicellular mucin secreting glands with merocrine mode of secretion. They are present between the other cell types of mucosal epithelium. They have a characteristic shape with the apex containing a large accumulation of mucinogen granules and the basal portion resembles a narrow stem. They are highly polarized with basal RER and supranuclear Golgi complex. Their number is more numerous in the large intestine than the small intestine.

Mucus is formed mainly of water (normally >98%) and when dehydrated, it will shrink to a very thin structure. So, mucus is not observed on formaldehyde-fixed tissue sections and can be best preserved using Carnoy fixative based on dry methanol, dry chloroform and glacial acetic acid (Methacarn). The two mucus layers of colon can be well visualized using this fixative, although even this method results in some shrinkage and often a gap between the epithelial cells and the inner mucus layer ***(Puchtler et al., 1970).*** Mucin serves to lubricate the bowel facilitating the passage of the solid contents ***(Ross, 2016).***

In addition to mucin secretion, goblet cells also act as passages for low molecular weight soluble antigens from the gut lumen to CD103+ LP-DCs (lamina propria dendritic cells) that have tolerogenic properties. This GC function implies a key role in intestinal immune homeostasis ***(McDole et al., 2012).***

Wang et al. (2016) mentioned that Mucins produced by the gut goblet cells are believed to be an important factor for the innate defense in a number of intestinal infections, including many parasitic infections, and also have a protective role against the gut microorganisms.

The lamina propria, that is present between the crypts underlying and supporting the epithelium, is formed of loose areolar connective tissue with blood and lymphatic vessels ***(Mescher, 2010)***. It contains B-lymphocytes, T-lymphocytes, macrophages, and dendritic cells ***(Geissmann et al., 2010).***

In addition to the basic contents of lamina propria in the alimentary tract, the lamina propria of the colon shows other structures and greater development of some of the basic content:

Collagen table**,** it is a thick layer of collagen and proteoglycans lying between the basal lamina of both the epithelium and the fenestrated capillaries.

Pericryptal fibroblast sheath which is a well-developed fibroblast population that divides just before they reach to the luminal surface. Some arguments suggest that the macrophages

of the lamina propria in the large intestine may arise as a terminal differentiation of the pericryptal fibroblasts.

Gut associated lymphatic tissue (GALT) and lymphatic vessels ***(Ross, 2016).***

Muscularis Mucosa, it is formed of inner circular and outer longitudinal smooth muscle fibers ***(Gartner and Hiatt, 2014).***

Submucosa:

It is the second inner layer beneath the mucosa. It is formed of a dense irregular connective tissue containing nerve plexus, blood and lymphatic vessels ***(Ross, 2016).***

Muscularis Externa:

It consists of inner circular and outer longitudinal smooth muscle fibers. The outer longitudinal smooth muscle layer is modified into teniae coli. Bundles of muscle from the teniae coli penetrate the inner circular layer at irregular intervals; these discontinuities allow segments of the colon to contract separately, leading to the formation of haustra coli. Auerbach's plexus is present in the thin connective tissue between the two muscle layers with blood and lymphatic vessels ***(Ross, 2016).***

Serosa:

The colon possesses both serosa and adventitia. The serosa shows small, fat filled pouches called appendices epiploicae ***(Gartner and Hiatt, 2014).*** The serosa is a serous

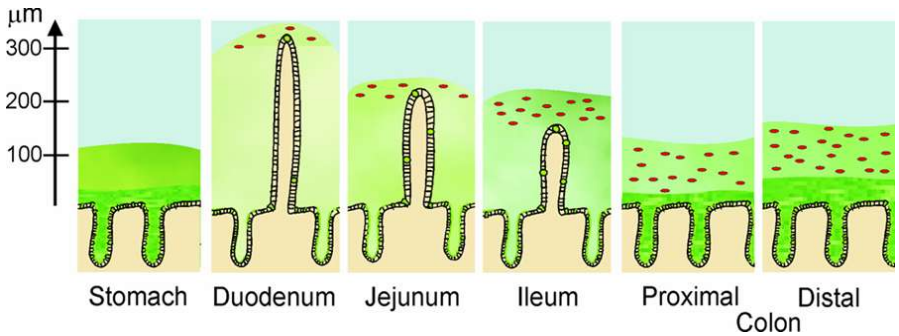
membrane that is formed of small amount of connective tissue covered by a layer of simple squamous epithelium called mesothelium ***(Ross, 2016).***

Intestinal immune response

Intestine is the main site of bacterial colonization, with more than 1000 prevalent bacterial species identified ***(Lievin-Le Moal and Servin, 2006).*** It has been estimated that the GIT houses several trillion microbes with a total weight of about 2kg ***(Gartner and Hiatt, 2014).***

Mucins; produced and secreted by GCs, are large glycoproteins with highly polymeric protein backbone structure, linked to numerous oligosaccharide side-chains that contribute to the formation of gel-like structure forming the mucus layers ***(Andrianifahanana et al., 2006)***

There are two types of mucus organization in the GIT. The two-layered system with an inner and an outer mucus layer that is present in glandular stomach and colon and the single layered one that is found in the small intestine ***(Ermund et al., 2013).***



(Pelaseyed et al., 2014)

The mucous layer facilitates the elimination of intestinal contents and protects against physical and chemical injury caused by ingested food, microbes and the microbial products ***(Hollingsworth and Swanson, 2004).***

The mucous layers serve as the front line of innate host defense mechanism. They play an important role in the hosting of the commensal intestinal microorganisms and the protection from colonization and invasion by the pathogenic organisms. The commensal bacteria, trapped in the mucus layer, which fail to reach the epithelial cell surface, are eliminated by peristaltic movement ***(Dharmani et al., 2009).***

The thickness of mucus layers is preserved by a balance between synthesis, secretion, and degradation, modified by the microbial glycosidases and proteases and the mechanical shear forces of peristalsis. The maintenance of the intestinal mucosal homeostasis is mediated by the balanced and dynamic interactions between mucus layers, intestinal epithelial cells, microorganisms and host immune defense ***(McGuckin et al., 2009).***

In most gut infections, the synthesis and secretion goblet cells and mucin occur mostly during the acute phase. However, chronic infection leads to the depletion of goblet cells and qualitative and quantitative change in mucus layers due both to altered production and secretion of mucins and to microbial glycosidases and proteases ***(Dharmani et al., 2009)***

Gut associated lymphatic tissue (GALT):

The lymphatic tissue in the lamina propria of GIT is represented by: diffuse lymphatic tissue (B cells and T cells), Lymphatic nodules and eosinophils, macrophages (MΦ), dendritic cells, plasma cells and sometimes neutrophils ***(Ross, 2016).***

They act as an integrated immunologic barrier. They play an important role in the protection against antigenic substance and micro-organisms invasion that could enter from the lumen of GIT when the mucus and epithelial cell lining fail ***(Gill et al., 2010).***

Goblet cells pass the luminal antigens to the lamina propria dendritic cells of the tolerogenic $CD103^{+}$type ***(McDole et al., 2012)***. The immune system develops tolerances, a process that should be well balanced in order to trigger convenient responses to the harmful organisms as well as tolerate the commensal bacteria. Then DCs take up and present antigens to T-cells to develop an action or tolerance ***(Pelaseyed et al., 2014).***

Resident intestinal MΦs clear the organisms that penetrate the epithelial cell barrier without strong stimulation to the immune response by phagocytosis ***(Bain et al., 2013).*** Other MΦs are attracted upon inflammation. The DCs and MΦs have important roles in determining the outcome of any

challenge that is highly dependent on their interactions with the epithelial cells ***(Gill et al., 2010).***

GALT is more developed in large intestine. Large Lymphatic nodules distort the spacing between the crypts extending into the submucosa. This extensive development of the immune system reflects the large amount of pathogens and noxious end products of metabolites normally present in the lumen. Small lymphatic vessels at the bases of the crypts drain into the lymphatic network in the muscularis mucosa ***(Ross, 2016).***

ULCERATIVE COLITIS

Idiopathic Inflammatory Bowel Disease (IBD) includes two major forms: Ulcerative colitis (UC) and Crohn's disease (CD). They have been recognized as distinct disease entities for over a century.

Wilks (1859) first described ulcerative colitis in a patient who died after several months of diarrhea and fever. In addition, ***Wilks and Moxon (1875)*** reported a case of ulceration and inflammation of the entire colon in a young patient who had had severe bloody diarrhea. This was an early instance of ulcerative colitis**.**

Epidemiologic studies done in the Baltimore area during the 1960s documented the rising incidence of ulcerative colitis during the first half of the 20th century ***(Calkins and Mendeloff, 1986).***

Ulcerative colitis occurs with different frequencies around the world. The countries reporting for the highest incidence of UC are the United States, the United Kingdom and Sweden. No accurate registry or cohort of patients had ever studied the exact prevalence of UC in populations of Middle East and Africa. In Mediterranean countries, the prevalence of UC was estimated at 5/100000 in urban areas ***(Tezel et al., 2003).***

The initial pathologic description of ulcerative colitis was the diffuse mucosal/ submucosal involvement, beginning

in the rectum and recto-sigmoid region, and advancing proximally to involve the entire colon in a diffuse inflammation of the mucous membrane with chronic inflammatory cells, vascular congestion, goblet cell depletion, and crypt abscesses ***(Morson, 1975).***

There was a debate about the etiology of ulcerative colitis and several theories were proposed while the exact etiology is unknown.

Theories of etiology:

Buie and Bargen (1933) suggested vascular thrombotic phenomena as the patholgical basis for ulcerative colitis. ***Warren and Sommers (1949)*** suggested an etiologic agent in the fecal stream. Meanwhile, ***Warren and Sommers (1954)*** described ulcerative colitis as an inflammatory necrosis of arteries, veins, or both, leading to vascular occlusions and infarction of the colon in some patients.

During the past decades many attempts have been made to know the most popular theories concerning the etiology of ulcerative colitis which can be listed as follows:

1. Infection:

Bacterial causes of ulcerative colitis attracted attention during the early 20th century when bacterial origins of intestinal disease were first being identified, including bacillus coli, streptococci, and B.Colicommunis ***(Hurst, 1935)***.

***De Dombal et al., (1969)*,** mentioned that, in 1924, Bargen JA claimed to have isolated a diplococcus from the stools of patients suffering from ulcerative colitis and even produced a vaccine against this diplococcus, which was claimed to be effective in such patients.

Later on these claims were not entirely justified. Bargen's 'diplococcus' was shown to be a harmless type of enterococcus found in the stools of large numbers of the general population; and no real evidence was strong to suggest that the vaccine was effective in preventing attacks of colitis. Since that date other authors have postulated that a number of organisms might be partly responsible for ulcerative colitis, including parasites, fungi and various viruses ***(Frakin, 1937)***. No sooner had the diplococcus controversy subsided, than claims were made on behalf of other organisms. ***Fradkin (1937)*** asserted that Entamoeba histolytica was the causal agent.

In1941, it was reported that a bacterium, known as 'spherophorus necrophorus', was involved in the etiology of ulcerative colitis ***(Dragstedt et al., 1941)***. On the other hand, ***Henderson et al. (1942)*** implicated a fungus; Histoplasma capsulatum, and with the surge of interest in viruses, ulcerative colitis was soon added to the list of 'viral' diseases.

***De Dombal et al. (1969)*,** mentioned that neither bacteria nor parasites are the primary offenders in the disease, although they may play an important role as secondary invaders. Bacon's

argument was based on the failure of investigators to isolate any one specific organism consistently from patients with ulcerative colitis, together with the fact that antibiotic therapy has been unsuccessful alone in bringing about a permanent cure**.**

Moreover, ***Shaw et al. (2011)*** suggested that patients with IBD, who received antibiotic therapy 2 to 5 years before the diagnosis, were more subjected to develop the disease as a risk factor for its etiology. They also observed that there was a dose-dependent relation between the number of antibiotic relief, and the risk of ulcerative colitis.

2. ***Mucolytic Enzymes:***

Meyer et al. (1947) suggested that ulcerative colitis might be caused by the destruction of the mucus epithelial cells lining the surface of the colon by enzymes that are normally found in gastrointestinal tract thus make the colon more liable for invasion by bacteria and other agents. It was shown that the lysozyme titre in the stools of colitic patients was higher than that in the stools of normal control patients, and also that the lysozyme titre in individual colitic patients varied directly in association with the clinical course of the disease**.** This hypothesis fell into disrepute when it was shown that the rise in the lysozyme titre could be produced by diathermy of the rectal mucosa ***(Hiatt et al., 1952)*** and lysozyme was incapable of dissolving or digesting human mucus ***(Glass et al., 1950).***