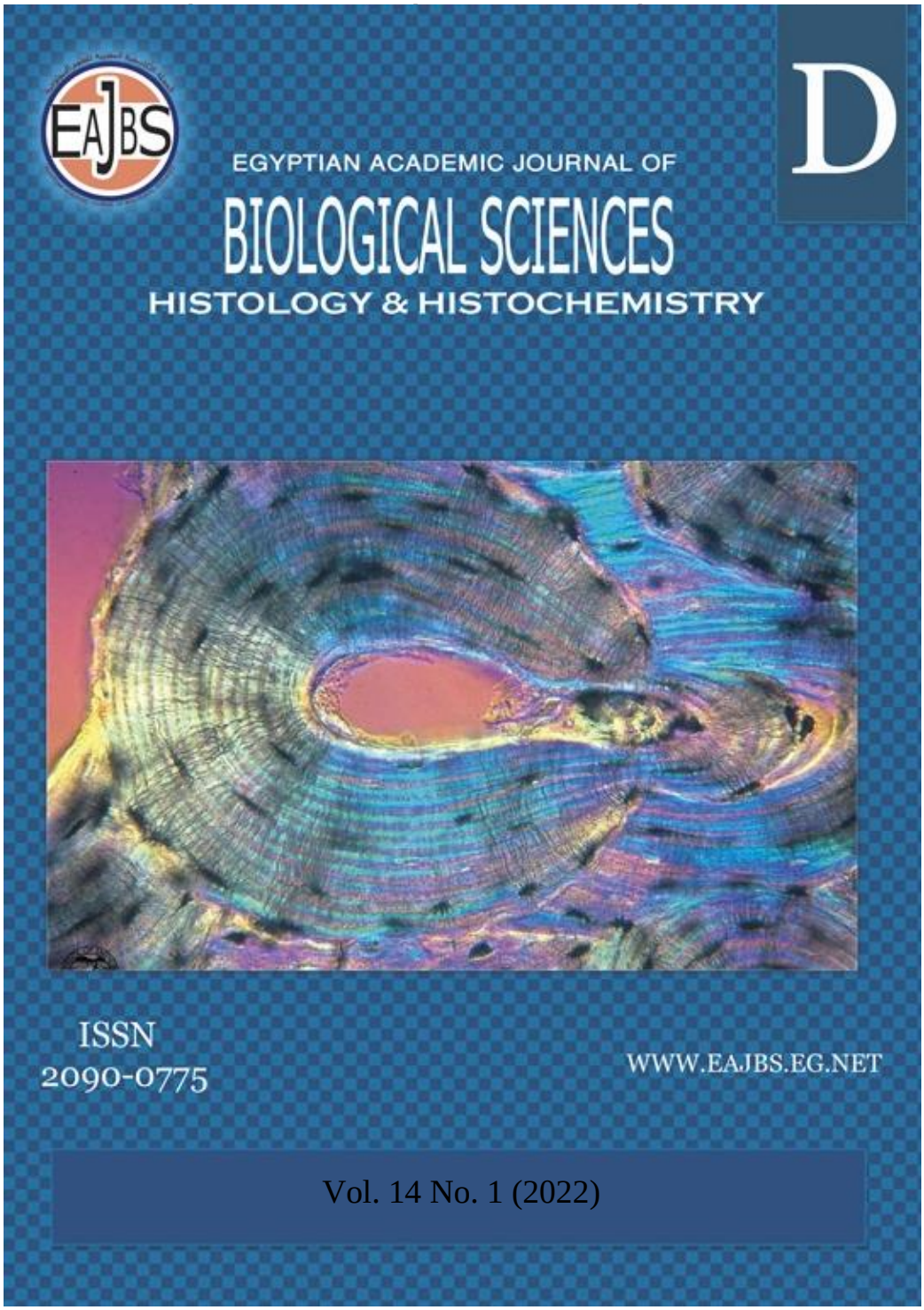

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Evaluation Effect of Transplanted Mesenchymal Stem Cell on Rat with Liver Cirrhosis

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ABSTRACT

Background/Aims: Cirrhosis is a chronic disease of the liver in which normal tissue is replaced by fibrous. Bone marrow-derived pluripotent mesenchymal stem cells (BM-MSCs) have received great interest as regenerative medicine for cirrhosis because of their advantages. The most appropriate route for transplantation of bone marrow-derived mesenchymal stem cells (BM-MSCs) in the management of cirrhosis remains poorly understood and controversial. But it has also drawn attention as a new approach to treating cirrhosis. This study aimed to evaluate the therapeutic effect of BM-MSCs on liver structure in carbon tetrachloride (CCL4)-induced cirrhosis of female rats. ***Materials and methods*:** Forty rats were randomly divided into four groups and 10 rats per group. First group: healthy control group (C), The second group: the negative control group (C.O) given olive oil intraperitoneal 0.2 ml / 100 g of body weight twice weekly for 6 weeks. The third group (CCl4-group): A mixture of CCl4 and olive oil is given (0.2ml/100g: 0.2ml/100g) of body weight twice weekly for 6 weeks. The fourth group (CCl4 + BM-MSCs): After injecting with a mixture of CCl4 and olive oil for six weeks, they left another two weeks and were given a single intravenous dose of 1 × 106 BM-MSCs. BM-MSCs were cultured and differentiated, and body weight was recorded. After the experiments, all rats were euthanized and subjected to quantification of liver function, quantitative real-time PCR (qRT-PCR) and histopathological changes. ***Results*:** The cultured BM-MSC was positive for CD90 and CD31 while it was negative for CD34. Treatment with BM-MSCs significantly increased body weight. The SRY gene and the c-JUN. gene was positive in the BM-MSCs group. Albumin was significantly increased in treated BM-MSCs while ALT, AST, ALP, total and indirect bilirubin were decreased. CCl4 significantly caused severe histological changes in the liver in addition to an increase in the number of collagen fibers compared to the control rats. BM-MSCs proved to have a powerful effect in treating cirrhosis by reducing the number of collagen fibers. ***Conclusions***: BM-MSCs proved to have a powerful effect in treating cirrhosis by reducing the number of collagen fibers.

INTRODUCTION

The liver is an important metabolic organ, so liver failure is a life-threatening state. Liver failure is accompanied by chronic fibrosis due to cirrhosis and hematoma and hepatocellular carcinoma (Koyama *et al.*, 2016).

Cirrhosis is a common liver disease that can result from a chemical injury or a viral infection in the liver. Pathologically, this disease is characterized by chronic, progressive degeneration of liver cells with nodular formation and excessive fibrosis. This may lead to severe clinical consequences, such as ascites, variceal bleeding, and encephalopathy (Friedman *et al.*, 2008 and Weber *et al.*, 2003). Hepatotoxicity is liver damage caused by chemicals. There are several chemicals that cause hepatotoxicity, for example, carbon tetrachloride, thioacetamide (TAA), galactosamine, and alcohol. CCL4 is also known for hepatic toxic actions. They reported that it causes acute liver damage like necrosis and steatosis (Bolondi and Gramantieri, 2011). Cirrhosis represents the final stage of progressive cirrhosis that is also characterized by distortion of the hepatic structure, leading to loss of liver function and development of hepatocellular carcinoma (Iredale, 2003 and Zhou *et al.*, 2016). Fibrosis is characterized by the excessive accumulation of extracellular matrix (ECM) with the formation of scar tissue enveloping the injury site in the liver as a result of imbalances in its production, deposition and erosion. ECM is a process involving activation of hepatic stellate cells (HSCs), which transform into myofibroblast-like cells (Al-Rasheed *et al.*, 2015). Moreover, activated MSCs produce inflammatory cytokines and proliferative factors that lead to increased ECM production and hepatitis (Curry, 1995). This spreading process may eventually progress to cirrhosis (Eom *et al.*, 2015). The best treatment for patients with liver fibrosis is liver transplantation, but there are obstacles such as lack of donors, complex surgeries with immune rejection and high cost (Guo *et al.*, 2016). Recent studies have shown that cell therapy is a suitable alternative treatment for cirrhosis of the liver (Rengasamy *et al.*, 2017 and Sun *et al.*, 2012). Nowadays, mesenchymal stem cells (MSCs) are found to serve as a potentially relevant therapeutic agent for the treatment of liver diseases because of their potential to differentiate into hepatocytes, suppress the pathophysiological process that is mediated by chronic inflammation. This immunosuppressive mechanism contributes to the modulation of trophic factors that are secreted and reduces tissue fibrosis, microenvironment, and ultimately tissue regeneration (Eom *et al.*, 2015 and Milosavfrjevic *et al.*, 2018). Mesenchymal stem cell type (MSCs) is an effective treatment for liver fibrosis in both animal models and humans by reducing inflammation and reshaping collagen deposition (Cho *et al.*, 2011 and Sakaida *et al.*, 2004). (MSCs) also may accelerate the liver regeneration process, reduce hepatic fibrosis, and improve liver function and survival (Hardjo *et al.*, 2009 and Higashiyama *et al.*, 2007). MSCs could control hepatic stellate cells (HSCs) activation which plays important role in causing liver failure (Berardis *et al.*, 2015). Later, it is revealed that MSCs have vast extensive secretion mechanisms for anti-inflammatory, chemokine, cytokines, and growth factors (GFs), which indirectly regulate the immune system (Jang *et al.*, 2015). In the liver, hepatocyte growth factor (HGF) can stimulate liver cell proliferation, prevent liver apoptosis, and thus promote liver regeneration, after injury (Wang *et al.*, 2012). Different MSCs treatments can mitigate negative outcomes of injury and disease by the interaction between MSC and different target cells (Atta et al., 2009 and Sotiropoulou et al.,2006) and it may secrete soluble factors (Wang *et al.*, 2012). Although many other studies have found that CSCs alleviate liver failure by interacting with various cells associated with inflammation, achieving immunosuppression and promoting survival (English, 2012 and Di Nicola, 2002). However, the differences

between MSCs therapies for liver failure have not been fully explored. The mechanism by which CSCs are repaired is also unclear, and its results appear to be reversible (Le Blanc *et al.,* 2008) and controversial (Fang *et al.,* 2005). In addition, in vivo studies confirmed that MSCs injected through a peripheral vein have ant fibrotic and anti-inflammatory functions. In 2000, the presence of Y chromosome-positive cells in the liver with chronic inflammation in autopsied women who received curative bone marrow transplants from male donors indicated the presence of pluripotent stem cells among the bone marrow cells. Since then, the focus has been on bone marrow stem cells as a source of cells for liver regeneration therapies (Hemmann *et al.,* 2007 and Zhao *et al.,* 2005). This work aims to evaluate the effect of stem cells as a novel therapeutic agent for the structure and functional restoration of liver fibrosis using a single model of CCL4-induced fibrosis. In general, the mechanisms underlying this beneficial effect are not well understood and may include MSCs ability to differentiate into hepatocyte-like cells (Chen *et al.,* 2004).

This study was conducted to evaluate the effect of intravenously introduced MSCs on experimentally induced liver fibrosis in adult female albino rats of CCL4-induced cirrhosis, and the underlying mechanism by which mesenchymal stem cells ameliorate cirrhosis.

MATERIALS AND METHODS

Chemicals and reagents:

CCl4 was obtained from (Merck, Germany). All other chemicals were obtained from certified sources and were of analytical grade.

Experimental Animals:

Forty female Sprague-Dawley rats (9-10 weeks old, weighing (180-200g mean body weight) were purchased from an animal house at the Faculty of Medicine, Assute University, Egypt. They were housed in plastic cages and were given food and water *ad libitum* and maintained at room temperature (25 ^{0}c) with a 12/12 h light/dark cycle throughout the period of the experiment. The animals were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the Research Ethics Committee of Faculty of Veterinary Medicine, South Valley University, Egypt (Code: 13/23.05.2021).

Liver Fibrosis Induction:

To induce liver fibrosis, 0.2 mL CCl4/100 g body weight of 40 ml/L CCl4 (Sigma Corp., St. Louis, MO) liquefied in olive oil (1:1) (Sigma) was injected subcutaneously twice weekly for 6 weeks. Liver cirrhosis was detected in histopathological examination of rat liver samples.

Experimental Design:

Forty female rats were randomly divided into four groups 10 rats per group. Group I: served as control giving without any sign (C). Group II: served as the negative control group (C.O), injected with olive oil (0.2 ml/100 g body weight) twice a week for six weeks. Group III (CCl4-group): given mixture of CCl4 and olive oil (0.2 mL: 0.2mL/100 g body weight) twice a week for six weeks to induce liver injury. Group IV (CCl4+ BM-MSCs): after rats were injected with a mixture of CCl4 and olive oil for six weeks, they were injected with a single intravenous dose of 1× 106 BM-MSCs. Animal weights were recorded every day and animal behaviors were checked daily. After the experiments, all rats were euthanized with diethyl ether and then sacrificed two weeks after BM-MSCs transplantation, and subjected for a histological, histopathological, quantitative real-time reverse transcription-polymerase chain reaction.

BM-MSC Preparation, Isolation, And Culture:

Bone marrow was extracted from male rat bones. The removed tissues were incubated for cell culture in 25 cm^2 flasks containing Dulbecco's

modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA). Incubations for 15 min at 37 °C would be performed in a water bath in which the flasks were shaken at 120 r/min After 10 and 15 minutes, respectively. The flasks were vigorously mixed for 10 seconds, after which the contents of the flasks were filtered through a nylon screen (250 μm pore size) to collect any remaining undisclosed tissue. The cell suspension was centrifuged at about 300 g for 3 minutes. When a homogeneous cell suspension was achieved, the suspended cells were centrifuged at 1200 rpm for 7 min, and 3 ml of culture medium were added to the cell pellets and distilled. Cells were cultured in 25 cm^2 flasks with 5 ml DMEM and maintained at 37 °C in a humidified 5% CO atmosphere. The culture media was changed every two days. The cells reached approximately 90% confluence. The mesenchyme group was isolated based on its ability to adhere to the bottom of the flask and the MSCs were observed under the inverted microscope.

MSCs Will Be Characterized Using Flow Cytometer Cd90, Cd31 and Cd34:

Immunophenotyping of BM-MSCs was performed with antibodies against rat antigens CD31 (Integrin b1 chain; Ha2/5; ABC), CD34, and CD90 (Thy-1/Thy-1.1-FITC), and their isotope controls (IgG2aj; FITC**)** (Bayati *et al.*, 2018).

Estimation of Liver Function Biomarkers:

Rats were anesthetized and blood was taken from the posterior orbital vein to measure serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) according to the treatment method (Belfield *et al.*, 1971). Serum alkaline phosphatase (ALP) levels, total direct bilirubin and albumin were measured using kits available according to the methodology followed (Doumas *et al.*, 1997).

Quantitative Real-Time PCR (qRT-PCR:

RNA was extracted from liver samples using the RNA Simple Mini Kit (Invitrogen). Reverse transcription was performed using the SMART_PCR cDNA synthesis kit (Clontech Inc., Palo Alto, CA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed in duplicate in 25-μL reaction mixtures containing 1-μL cDNA template, SYBR Green PCR Master Mix, and 10 pmol of each primer Master Mix.

To confirm the presence of male-derived BM-MSCs in the liver of female recipient rats, PCR analysis of the male-specific Sry gene was carried out and to detect the proliferation, apoptosis and autophagy, c-JUN gene of the liver was assessed. The presence of DNA in all tissues was assessed by analysis of the “house-keeping” gene GAPDH. Primer sequences for Sry gene (Forward) 5-CAT CGA AGG GTT AAA GTG CC-3, (Reverse) 5-ATA GTG TGT AGG TTG TTG TC-3). Primer sequences for c-JUN gene (Forward) 5-CGG GCT GTT CAT CTG TTT GT-3, (Reverse) 5-CCG GGA CTT GTG AGC TTC TT-3. Primer sequences for GAPDH (Forward) 5-GCA TCT TCT TGT GCA GTG CC- 3, (Reverse) 5- ACC AGC TTC CCA TTC TCA GC- 3. Reactions were performed in an I Cycler iQ (Bio-Rad) (Dorn *et al.*, 2014). The data are expressed as mean ± SE from at least three separate experiments. At the end of the reactions, the analysis of the results of the real-time PCR reaction was done with the aid of Applied Biosystem Step One software using Comparative Ct (ΔΔCt) method (Livak *et al.*, 2001).

Histopathological Assessment:

For histological and histopathological examinations, pieces of liver were fixed in 10% neutral buffered formalin with pH 7.2, dried in ascending series of alcohols, cleaned in cedarwood oil, and embedded in paraffin wax. 5 μm paraffin sections were prepared and the following stains were used.

1. Harris hematoxylin and eosin stain (Gabe, 1976).
2. Masson's trichrome stain (Gomori, 1950).

Statistical Analysis:

Collected data were organized, tabulated, and analyzed by Prism software statistical computer package version 6 (Graph Pad Software, San Diego, CA). The mean and standard deviation (SD) were calculated; one-way analysis of variance (ANOVA) was used to examine differences among the groups. Significance was set at $P < 0.05$.

RESULTS

Identification and Characterization of BM-MSCs by Flow Cytometric Analysis Based on Cell Surface Marker Expression:

Immediately after isolation on culture day 0, BM-MSCs appeared circularly and were in suspension. After 1 day of differentiation, cells began to adhere to a thin spindle shape. BM-MSCs were differentiated into different passages: In the first passage (P1), some cells appeared spindle-shaped; In the second passage (P2), the cells formed small colonies; And in the third passage (P3), the cells had fibrous appearances.

The expression of MSCs surface markers was determined by a flow cytometer to ensure their identification and purity. MSCs (cell suspension) were stained with antibodies specific for CD90 FITC, CD31 for MSCs, and CD34 for hematopoietic cells. MSCs were uniformly negative for CD 34 (Figs. 1a& 2a), and positive for CD90 and CD31 (Fig 1b,2b).

PCR Detection of Male Derived BM-MSCs (SRY gene):

After 4 weeks of treatment for male-derived BM-MSCs, the SRY gene was positive using PCR analysis in the liver of the CCL4-inducible recipient female while it was negative in the other three female groups not treated with male-derived BM-MSCs (Fig 2c).

PCR Detection of c-JUN Gene Expression:

The mean c-JUN gene expression in the liver was significantly decreased at CCL4 group when compared to the control group and C.O group while After 4 weeks of BM-MSCs treatment c-JUN gene expression significantly increased at CCL4+SCs group when compared to CCL4 group at $p < 0.05$ (Fig. 2d).

The Effect of MSCs on Weight Changes and Survival Rate:

Rats were weighed once weekly during the treatment period. Animals in the CCL4-treated group suffered from a relative weight loss than those of normal rats (C, C.O) by 13.95%. In contrast, weight gain was observed in the MSCs group than in those of CCL4 group by 20.39% (Fig. 3). With regards to survival rats, two rats of the CCL4-treated group died at weeks 7 and 8, whereas the mortality rate was zero in the other groups.

Evolution of Liver Function by Serum Biomarkers Assessment:

Liver function biomarkers were used to assay both synthetic and secretory functions of the liver. In the present study, hepatic injury caused by CCL4 was indicated by significant elevation of serum ALT, AST, ALP, total bilirubin, and indirect bilirubin to 149.5%, 187.6%, 98.6%, 1216% and 1849.5% respectively, in rats exposed to CCl4 when comparing with the control rats. In contrast, the level of albumin was markedly decreased in CCL4 rats by 4.7% compared with the control group. On the other hand, administration of MSCs decreased the high level of ALT, AST, ALP, total bilirubin, and indirect bilirubin to 20.7%, 50%, 30%, 49.9% and 64.9% respectively, whereas albumin level was significantly increased to 15.7%. (Fig. 4a).

Liver Macroscopy and Histopathological Assessment:

After euthanization, the macroscopic structure of the liver was evaluated. The livers obtained from the control groups exhibited a smooth surface and a bright red color (Fig. 5a)

while those of the CCL4-treated group were characterized by a flattened, fractured surface, and were of faintly-red color (Fig. 5b). Where those of the MSCs-treatments group resulted in an improvement in the macroscopic condition of the liver as compared with the CCL4-treated group (Fig. 5c). In parallel, the histopathological study of liver structure (H&E staining) and the extent of tissue fibrosis due to collagen fiber deposition in the ECM (classic Masson's Trichrome staining) were performed. H&E staining of the sections from the control (Figs. 6a&b and negative (oil) controls (6c) showed the typical cellular architecture, contains round single vesicular nuclei hepatocytes within the central vein, non-inflammation cells in the periportal areas were observed as well as regular lobular pattern. On the other hand liver of the CCL4-positive control group showed portal inflammation with dilated blood vessels, necrosis and vacuolation of hepatocytes. In addition to, binuclear hepatocytes and fatty changes, distorted lobular architecture with inflammatory cell infiltration and hepatocytes degeneration were observed also (Figs. 6d&e). In the MSC_S-treatment group, cellular architecture appeared most similar to the control group, with the exception of an infrequent detection of bi-nucleated hepatocytes. Importantly, a significant reduction of portal inflammation and marked improvement of the lobular structural pattern with normal tissue and decrease of the severity of histopathological changes induced by CCL4 (Fig. 6f).

Masson's trichrome was used to assess liver fibrosis. The control (fig7a) and negative (oil) control (Fig. 7b) revealed view collagen proliferation around the portal area, in contrast, the CCL4 group demonstrated collagen that was detected surrounding the portal area and central veins. Collagen also divided the hepatic parenchyma into label (fig 7c). In the MSC_S-treatment group, fibrous expansion around the portal areas was reduced, as well as improvement in hepatic fibrosis in comparison with the CCL4 group (Fig. 7d), In addition, the analysis result of histopathological fibrosis confirmed that cirrhosis was markedly reduced by MSC_S treatment, compared to the positive control group.

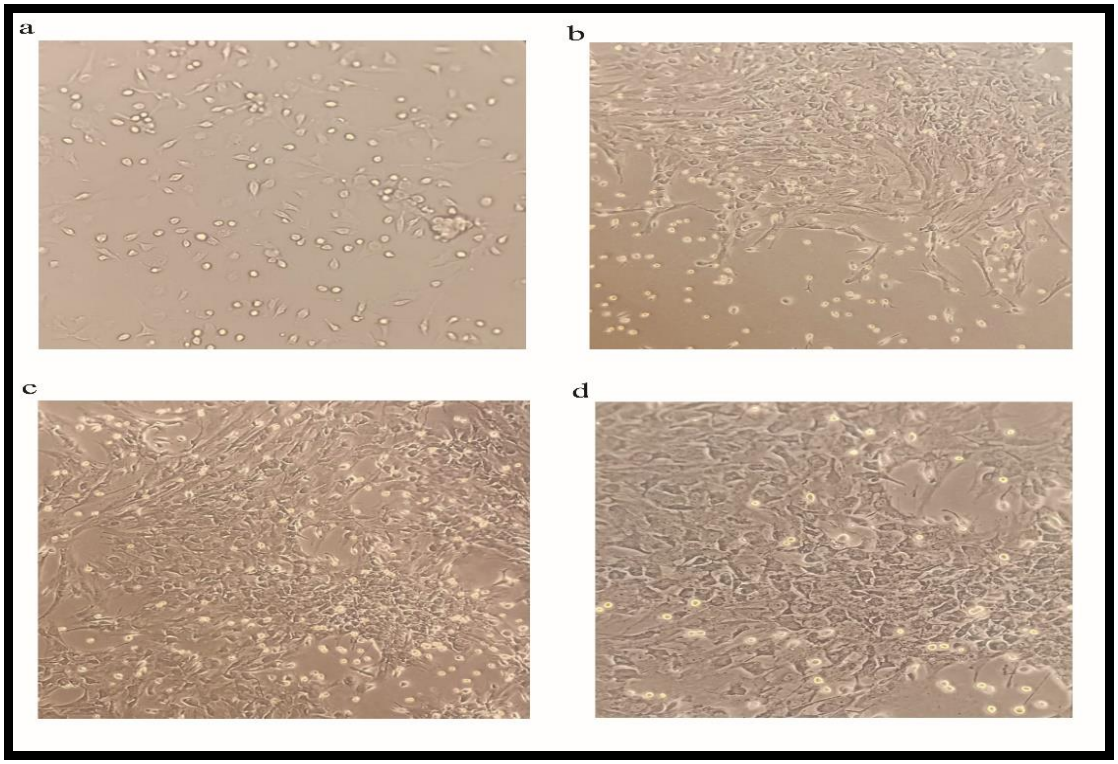

Fig. 1a, b,c,d: Bone Marrow-Mesenchymal Stem Cells (BM-MSCs).

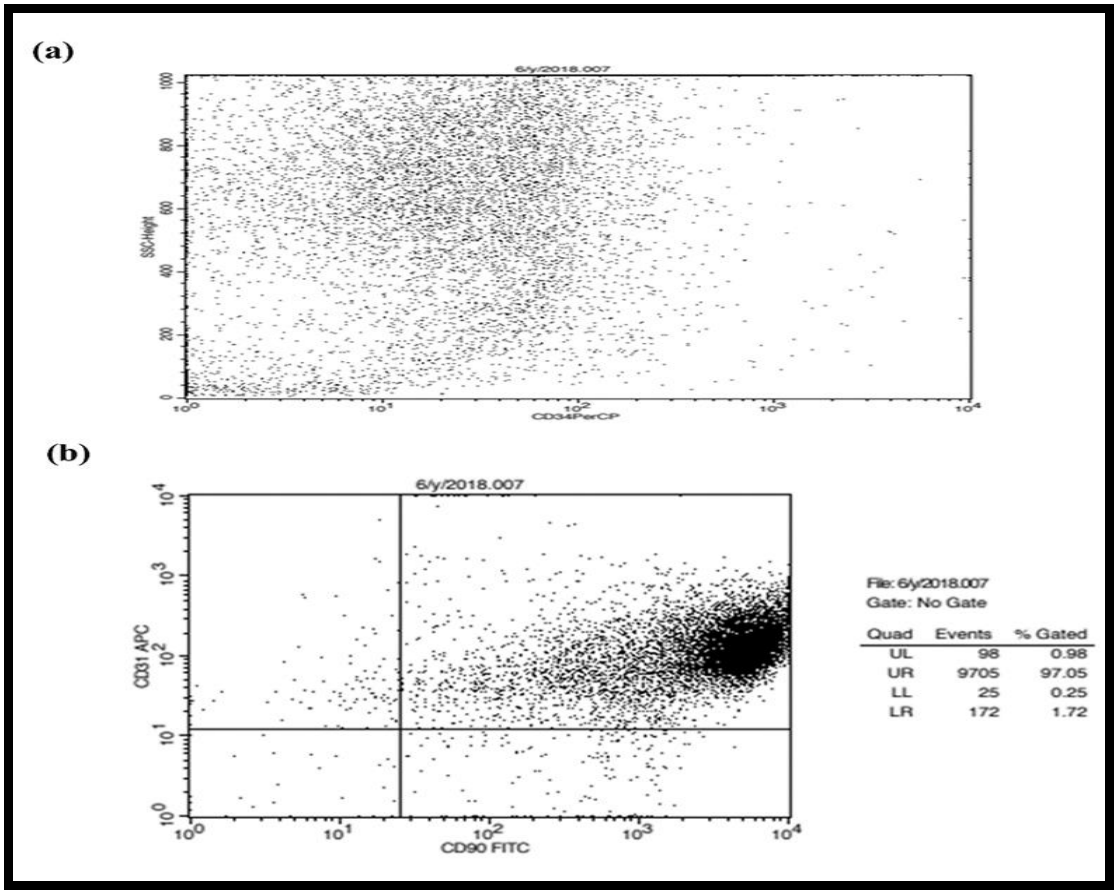

Fig 2 a,b: A diagram of flow cytometry dot plot of MSCs isolated from rat bone marrow, (a) shows that cells are negative for cd34. (b) Cd90 FITC is represented on X axis, and CD31 ABC is represented on Y axis, cells are positive for Cd90 and CD31.

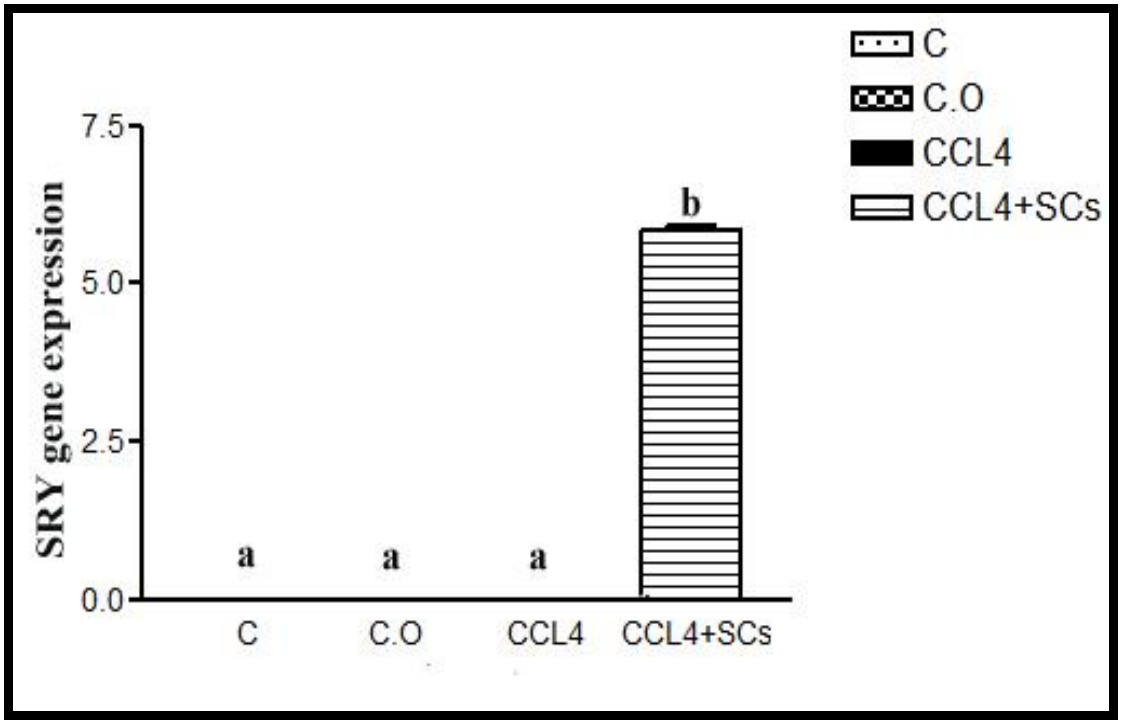

Fig. 2c: Quantitative RT-PCR was used to evaluate the SRY gene expression. Data represent fold change relative to SRY expression after normalization to GAPDH. The mean values are given as mean ± SEM. Values in the same column with unlike superscript signs are significantly different at $P < 0.01$.

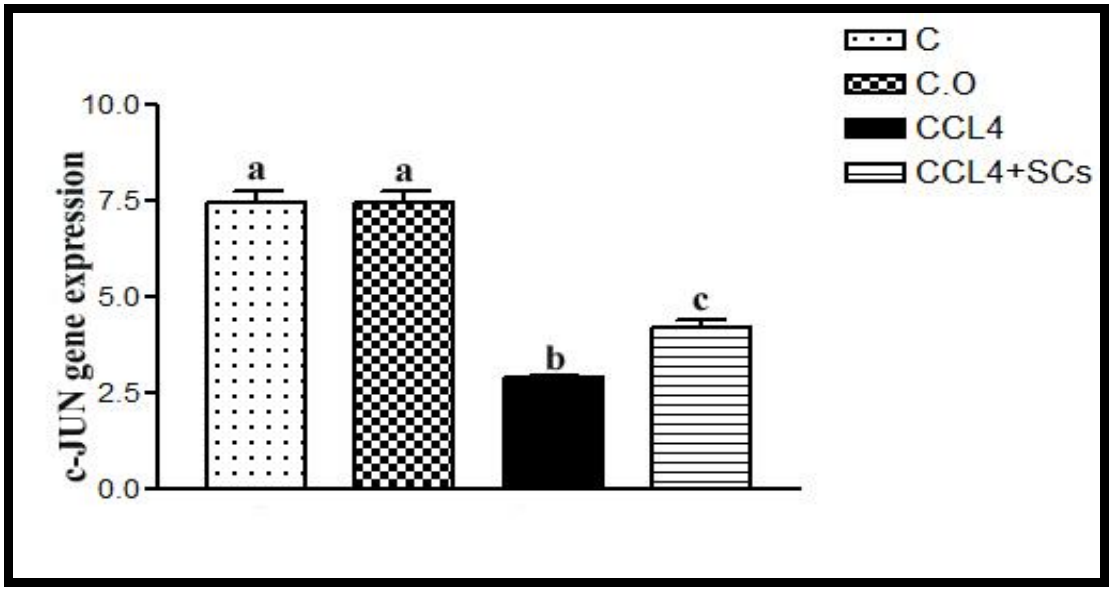

Fig. 2d: Quantitative RT-PCR was used to evaluate the c-JUN gene expression. Data represent fold change relative to c-JUN expression after normalization to GAPDH. The mean values are given as mean ± SEM. Values in the same column with unlike superscript signs are significantly different at $P < 0.01$.

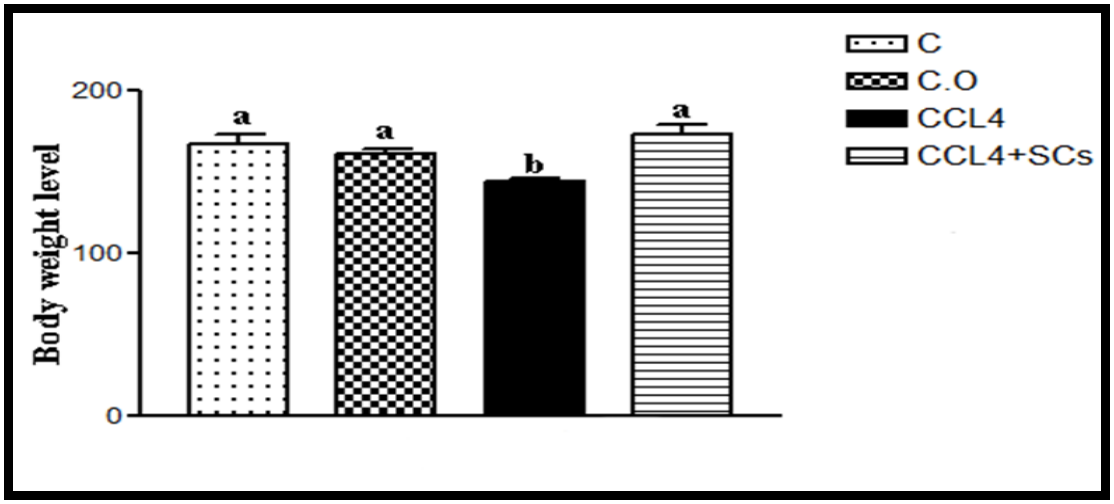

Fig. 3: Bodyweight. The effects of different treatments on body weight value of female rats. The mean values are given as mean ± SEM. Values in the same column with unlike superscript signs are significantly different at $P < 0.01$.

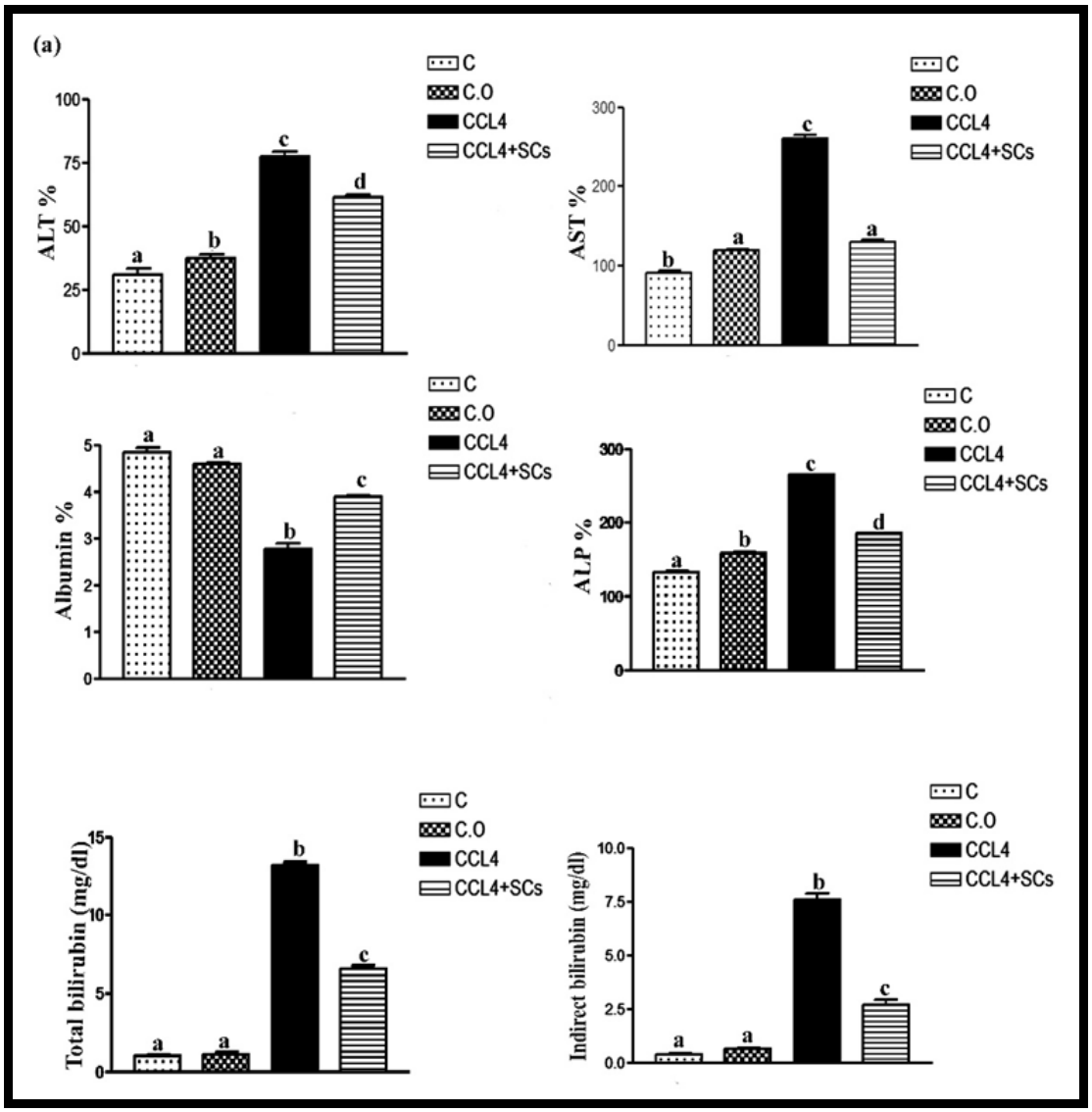

Fig. 4 a: ALT, AST, ALP, albumin, and bilirubin (total and indirect bilirubin). The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and ALP, alkaline phosphatase albumin, and bilirubin (total and indirect) in different rat groups were estimated as described in the method section. Data are mean± S.E.M. Values in the same column with unlike superscript letters are significantly different ($P<0.05$).

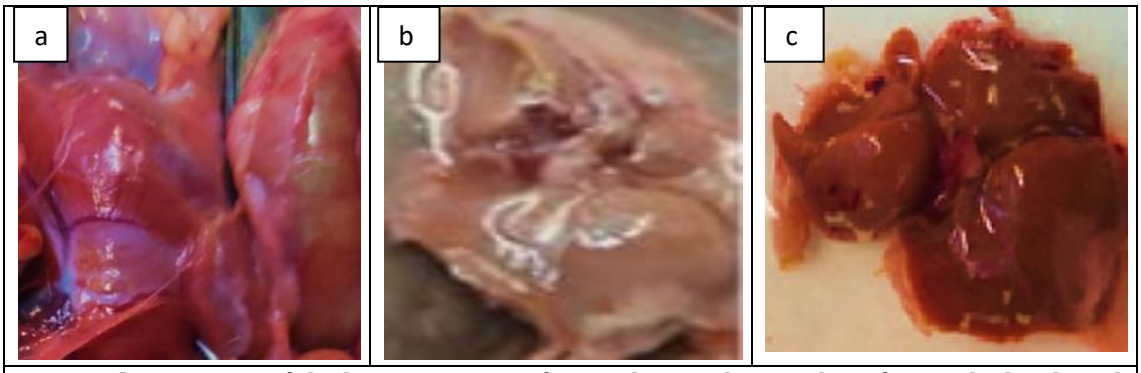

Fig. 5 a,b,c: Images of the liver specimen of control rat with smooth surface and a bright red color (a). While in the CCL4-treated rat, the livers appear with a fractured surface and were of faintly-red color (b). Where's the MSCs-treatment rat resulted in an improvement in the macroscopic condition of the liver (c).

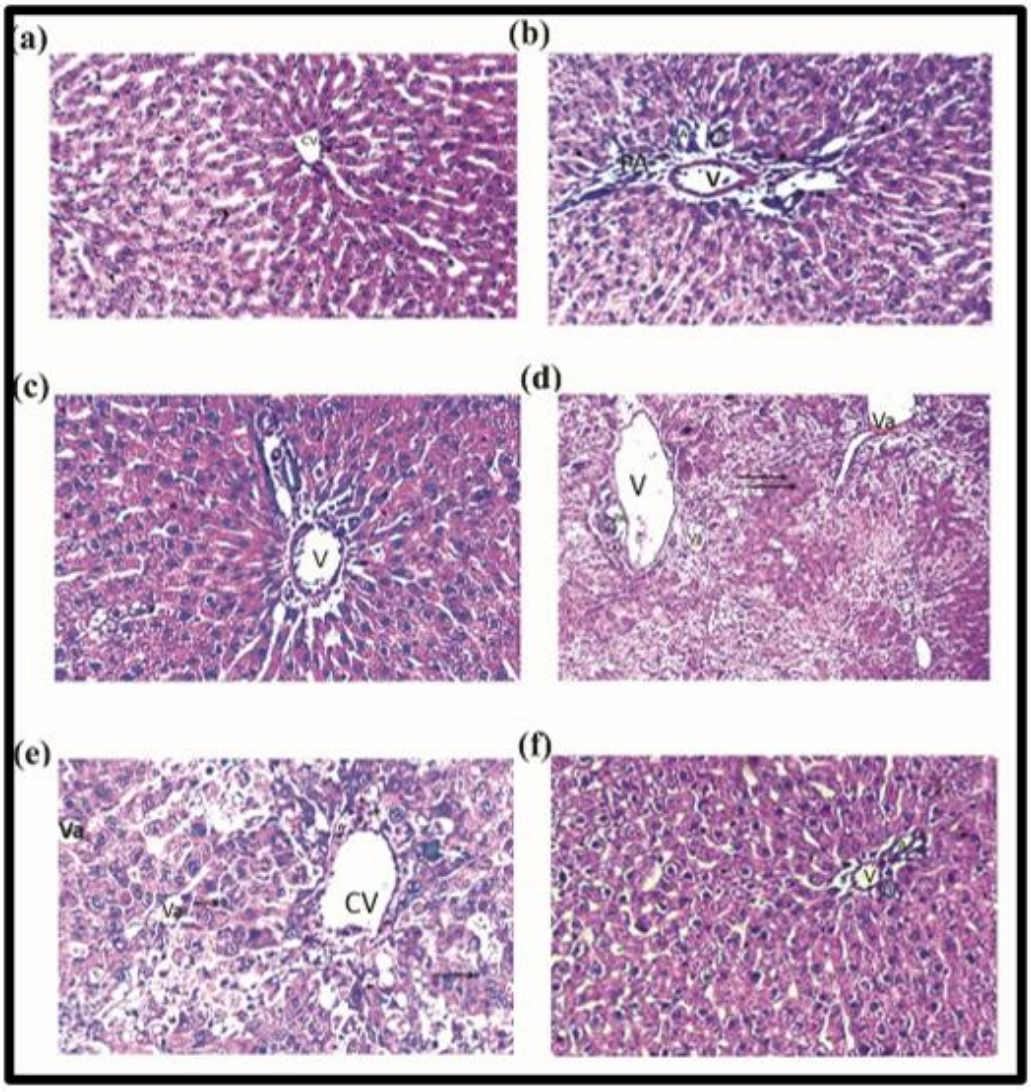

Fig. 6 a,b,c,d,e,f: Photomicrographs of liver tissue stained with Haematoxylin and Eosin (Fig a,b) control and (Fig c) negative oil group, showing normal central vein (CV) and portal area (PA) with branched of hepatic vein (V), hepatic artery (A) and bile duct (B). Most of the hepatocytes were within normal limits (Fig d,e) CCL4-group showed severe histological changes including distorted lobular architecture with dilated veins, fatty changes and portal inflammation (Fig e) MSCs-group showing more or less normal liver architecture with the normal portal area and mild degeneration of hepatocytes. (H&E, x200).

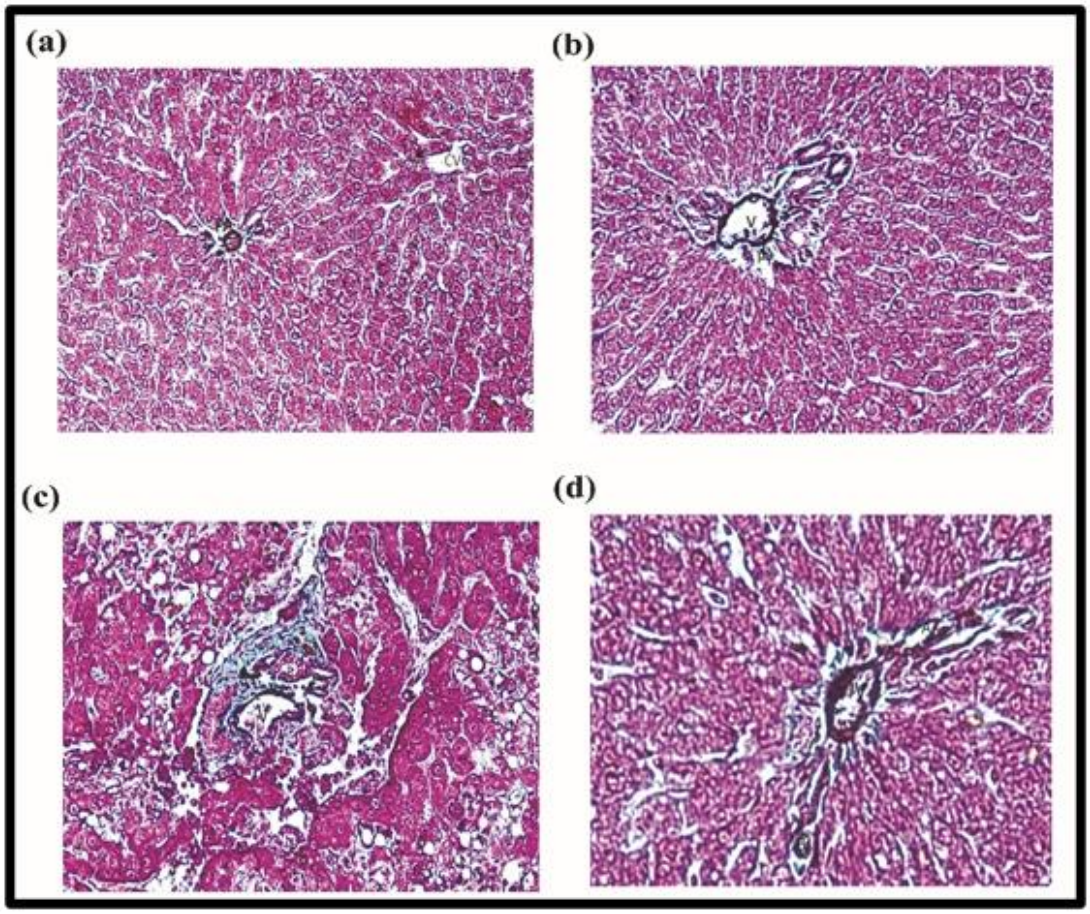

Fig7. a,b,c,d: Photomicrographs of liver tissue stained with Masson trichrome (Fig a) control and (Fig b) control negative oil group showing a minute sheet of portal collagen fibers, (Fig c) CCL4-group showing marked portal collagen fibers and also intra-lobular collagen fibers deposition. (Fig d) MSCs group showing mild portal collagen fibers deposition (MTC-x200).

DISCUSSION

This study aims to evaluate the effect of BM-MSCs on hepatic fibrosis and their ability to restore normal liver architecture in an experimental CCL4 model of liver fibrosis in female rats. MSCs are an attractive candidate for liver regeneration/repair, and the boon marrow is a predominant source of MSCs. In the current study, the presence of male donor cells was confirmed in the affected female liver. Although one limitation of the quality or quantity of MSCs grafting to repair liver fibrosis is unclear (Hemmann *et al.*, 2007), the fact that MSCs have anti-fibrotic effects in the affected liver is clearly proven. Evidence from in vitro (Rengasamy *et al.*, 2017) and in vivo studies (Aithal *et al.*, 2018) Clinical studies have indicated that MSCs have the ability to promote fibrous matrix degradation and the production of secreted factors that stimulate the regeneration of endogenous parenchymal cells, This suggests that MSCs may be ideally suited for treating liver diseases involving fibrosis (Amer *et al.*, 2011 and Kim *et al.*, 2010), including chronic hepatitis C and B and alcoholic liver disease (Tanimoto *et al.*, 2013 and Wang *et al.*, 2012).

Currently, several studies have confirmed that BM-MSCs can reverse liver fibrosis, but the precise mechanism of treatment remains controversial. Several reports have indicated that BM-MSCs can reduce cirrhosis through degradation collagen deposition via secreting the matrix metalloproteinase (Wang *et al.*, 2012 and Rabani *et al.*, 2010). Other studies indicate that the anti-fibrotic effects of BM-MSCs are mainly via increased

anti-inflammatory factors (Truong *et al.,* 2016). Mesenchymal stem cells could improve liver microcirculation to a certain extent and reduced the degree of fibrosis (Ahmed *et al.,* 2014). These results are consistent with the present findings, thus soluble factors secreted by BM-MSCs are the primary route of action during fibrosis.

The present study demonstrated the equivalent therapeutic efficacy of BM-MSC (intravenous) transplantation to restore liver function. Moreover, this hepatic functional recovery was combined with liver parenchymal healing and hepatocyte regeneration, as evidenced by histopathological evaluations of hepatic tissues. Liver functional recovery by tail vein injection of BM-MSCs has also been observed in some studies (Amin *et al.,* 2012). Thus, MSCs may play an inhibiting role in the process of HSCs transition from a quiescent state to an activated state.

In the present study, rats were injected with CCL4 and sacrificed after 6 weeks and when compared with the control group showed a highly significant increase in the activities of ALT, AST and ALP. It also showed a significant decrease in the levels of albumin, globulin and total protein. These findings were in complete agreement with many results (Atta *et al.,* 2009, Rabani *et al.,* 2010, Amin *et al.,* 2012 and English *et al.,* 2012).

The elevated liver enzymes such as ALT, AST, and ALP in intoxicated rats could be attributed to the necrosis of hepatocytes that result in the leakage of transaminase (Low *et al.,* 2004). The total protein and albumin levels were depressed in hepatotoxic conditions due to disturbance in carbohydrates, protein and lipids metabolism (Aziz *et al.,* 2007). In the group of rats treated with MSCs and sacrificed after 6 weeks, we detected a significant reduction in the activities of ALT, AST, and ALP. Meanwhile significant elevation in the levels of protein albumin, globulin when compared with CCL4 group.

Our results were in complete agreement with many authors who reported that the rats that received BM-MSCs infusions by tail vein showed better results for the biochemical parameters (Geng *et al.,* 2010, Rabani *et al.,* 2010, El-Khayat *et al.,* 2013 and Quintanilha *et al.,* 2014). MSCs can protect hepatocytes by reducing CCL4-induced ROS damage. The microscopically finding in CCL4-group revealed a severe degree of hydropic degeneration and fatty changes. Moreover, focal areas of necrosis and apoptotic changes with mononuclear leucocytes infiltration were also observed in the hepatic parenchyma. These results were in complete agreement with other results (Madani *et al.,* 2008, Buko *et al.,* 2014 and Rui *et al.,* 2014). These results recorded that these findings may be attributed to the metabolism of CCL4 due to the oxidation process which induced oxidative stress in the hepatic cells responsible for the changes in cell permeability, increase the intracellular concentration of Ca++, increase in nuclear volume, enlargement of nucleoli and also inhibits mitochondrial activity leads to cell death and severely affecting hepatic cells which are located in the previous acinus region as previously mentioned (Bigoniya *et al.,* 2009). The heavy proliferation of fibrous connective tissues that form fibrous bridges connecting portal regions led to the formation of a pseudo cleavage that separates the hepatic lobe from the other lobules. These findings were agreed with other results (Hessin *et al.,* 2015), meanwhile, after MSCs treatment there were thin strands of fibrous connective tissue between the hepatic lobules (Rabani *et al.,* 2010, Volarevic *et al.,* 2014 and Mansour *et al.,* 2015). All found that MSCs could reduce the proliferation of stellate cells and collagen synthesis and promote hepatic stellate cell apoptosis through the secretion of HGF and NGF, Thus, this leads to a significant decrease in collagen deposition. From the

histological point, the present work revealed that the portal areas showed severe congestion of the portal blood vessels with mild vacuities, multiple thrombosis and perivascular edema as well as perivascular mononuclear leucocyte infiltration. Also, the bile duct in the portal areas showed hyperplasia of their epithelial cell lining with the formation of newly formed bile ductules, besides, inter acinar mononuclear leukocyte infiltration. Additionally, the severe proliferation of the bile ductules epithelium with multiple formations of consulted cell mass gives the picture of cholangiocarcinoma. These results agreed with previous results (Al-Bader *et al.,* 2000, David *et al.,* 2002 and Ling *et al.,* 2013). Meanwhile, after MSCs treatment, the bile duct showed a mild degree of enlargement with fewer newly formed bile ducts and less myelofibrosis. Multiple focal regenerations were seen for some areas of the hepatic parenchyma diffuse in the hepatic tissue. These results are in agreement with others (Hwang *et al.,* 2012), which indicated that the managed MSCs, first underwent transient differentiation into hepatic oval cells and then into hepatocyte-like cells. During this process, inflammation was reduced, damaged liver cells repaired, and fibrosis resolved, resulting in an overall improvement in liver function.

Hepatocytes also showed a neoplastic change characteristic of hepatocellular carcinoma. The neoplastic cells were polygonal, with variable borders, an abundance of eosinophilic granulocytes, a distinct vacuolated cytoplasm, as well as a vesicular nucleus with one or two prominent purple nuclei. These results were in agreement with other authors (Newell *et al.,* 2008) who reported that hepatocellular carcinoma globally arises secondarily from inflammation and fibrosis.

Our results for PCR detection of male-derived BM-MSCs (SRY gene). It was positive in the livers of female rats treated with male-derived BM-MSCs. These results indicated that male-derived BM-MSCs were able to degrade the liver of CCL4-induced rats in agreement with the previous finding (Fang *et al.,* 2004 and Yue *et al.,* 2020).

Also, our results of PCR detection of c-JUN gene expression in the livers of the CCL4 + SCs group in comparison with the other groups indicate that c-Jun promotes cell cycle progression. In addition, c-June drives cell proliferation by regulating p53, It also regulates the migration of different types of cells such as tumor cells during metastasis and epithelial cells during embryonic development in agreement with some findings (Tarek *et al.,* 2014).

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ARABIC SUMMARY

تقييم تأثير الخلايا الجزعيه الوسيطه المزروعة على الفئران المصابة بتليف الكبد

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مقدمه: تليف الكبد هو مرض مزمن في الكبد حيث يتم استبدال الأنسجة الطبيعية بنسيج ليفي, ولقد حظيت الخلايا الجذعية الوسيطه متعددة القدرات المشتقة من نخاع العظم (BM-MSCs) باهتمام كبير باعتبارها الطب التجديدي لتليف الكبد بسبب مزاياها. لا يزال الطريق الأنسب لزرع الخلايا الجذعية الوسيطة المشتقة من نخاع العظم (BM-MSCs) في كيفية علاج تليف الكبد غير مفهومًا ومثيرًا للجدل لكنها لفتت الانتباه أيضًا كنهج جديد لعلاج تليف الكبد.

الهدف من الدراسه: هدفت هذه الدراسة إلى تقييم التأثير العلاجي لـ BM-MSCs على بنية الكبد في تليف الكبد الناجم عن حقن رابع كلوريد الكربون (CCL4) في إناث الجرذان.

المواد والطرق المستخدمه: تم تقسيم أربعين فأر عشوائياً إلى أربع مجموعات 10 فئران لكل مجموعة. المجموعة الأولى: المجموعة الضابطه, المجموعه الثانيه: المجموعه الضابطه السلبيه, اعطيت زيت الزيتون عن طريق حقن 0.2 مل / 100 جرام من وزن الجسم مرتين في الأسبوع لمدة 6 أسابيع وذلك داخل الغشاء البريتونى للبطن, المجموعة الثالثة: اعطيت خليط من كلا من CCl4 وزيت الزيتون بنسبة 0.2 : 0.2 مل / 100 جم من وزن الجسم مرتين أسبوعياً لمدة 6 أسابيع, المجموعة الرابعة : بعد الحقن بمزيج CCl4 وزيت الزيتون لمدة 6 أسابيع، اعطيت هذه المجموعه جرعة واحدة في الوريد الزيلى 1 × 106وتركت لمدة اسبوعين. تم تمييز الخلايا الجزعيه والتأكد من وجودها وايضا تم تسجيل وزن الفئران. بعد التجارب، تم القتل الرحيم لجميع الفئران وتم تقدير كلا من وظائف الكبد والمؤشرات الحيوية للإجهاد التأكسدي، وتحليل الوسترن بلوت، وPCR في الوقت الحقيقي الكمي (qRT-PCR) والتغيرات النسيجية المرضية.

النتائج: كان BM-MSC المستزرعه إيجابيًا لـ CD90 وCD31 بينما كان سالبًا لـ CD34. أدى العلاج باستخدام BM-MSCs إلى زيادة وزن الجسم بشكل ملحوظ. كان جين SRY إيجابيًا في مجموعة BM-MSCs وجين c-JUN أيضا. زاد الألبومين، بشكل ملحوظ في المجموعه المعالجه بالخلايا الجزعيه, بينما انخفض ALT ، AST، ALP ، البيليروبين الكلي وغير المباشر ، والعلامات الحيوية للكبد فى نفس المجموعه. تسبب CCl4 بشكل ملحوظ في حدوث تغيرات نسيجية حادة في الكبد وزيادة كمية ألياف الكولاجين في الكبد مقارنة بالفئران الضابطة. أثبت BM-MSCs أن له تأثيرًا قويًا في علاج تليف الكبد عن طريق تقليل كمية ألياف الكولاجين وتحسن ملحوظ فى البناء التركيبى للكبد وأيضا إختفاء معظم االتغيرات النسيجيه المرضيه للكبد.

الخلاصه: يمكن أن يؤدي الحقن الوريدي لـ BM-MSCs إلى استعادة بنية الكبد ووظيفته في نموذج الفئران التى اصيبت بتليف الكبدي الناجم عن CCL4 عن طريق تخفيف سمية CCL4.