Mesenchymal Stromal Cells Suppress Hepatic Fibrosis Via Modulating the Expression of Fibronectin and Integrin and Inhibiting DNA Fragmentation in Rats

Type
Research paper

Keywords
fibronectin, liver fibrosis, fibrogenesis, integrin-β1, Multipotent mesenchymal stromal stem cells

Abstract
Introduction
Liver fibrosis is currently the 11th most common cause of death worldwide. Because of self-renewal, available sources for isolation, and high differentiation properties, multipotent mesenchymal stromal stem cells are suggested to be potential tool for treatment of liver fibrosis. In this study, we examined the anti-fibrotic and anti-inflammatory activity of bone marrow-derived multipotent mesenchymal stromal stem cells (MSCs) on liver fibrosis induced by carbon tetrachloride on rats relative to silymarin as a standard drug.

Material and methods
This study was performed on 40 male Sprague Dawley rats divided into 4 groups of ten rats each: Group 1 served as controls, Group 2 served as CCl4 (diseased) group, Group 3 served as silymarin treated group and Group 4 served as MSCs treated group. Liver fibrosis was assessed by determination of liver markers and fibrogenesis related genes together with the anti-inflammatory markers in the liver tissue. DNA fragmentation was assessed by Comet assay.

Results
MSCs treatment reduced all liver fibrosis markers as well as the oxidative stress and inflammatory markers. Additionally, MSCs reduced the expression of integrins and fibronectin compared with the control group as well as decreasing DNA fragmentation.

Conclusions
Treatment by MSCs significantly ameliorates liver fibrosis in rats. This amelioration was a result of acting on both the anti-inflammatory and anti-fibrotic activity of hepatocytes.
Mesenchymal Stromal Cells Suppress Hepatic Fibrosis Via Modulating the Expression of Fibronectin and Integrin and Inhibiting DNA Fragmentation in Rats

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Abstract

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Methods: This study was performed on 40 male Sprague Dawley rats divided into 4 groups of ten rats each: Group 1 served as controls, Group 2 served as CCl4 (diseased) group, Group 3 served as silymarin treated group, and Group 4 served as MSCs treated group. Liver fibrosis was assessed by determination of liver markers and fibrogenesis-related genes together with the anti-inflammatory markers in the liver tissue. DNA fragmentation was assessed by Comet assay.

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Keywords: Multipotent mesenchymal stromal stem cells; liver fibrosis; fibrogenesis; fibronectin; integrin-β1
1 **Background**

Epidemiological data shows that liver diseases gradually became an increasing health burden worldwide contributing to 2 million of the world’s deaths every year [1]. Hepatic fibrosis is characterized by the activation of hepatic stellate cells (HSC), the accumulation of extracellular matrix (ECM) proteins inside the liver parenchyma leading to a chronic injury and eventually loss of liver functions [2]. Over the last decades, the gold-standard treatment of liver cirrhosis was orthotopic liver transplantation. Although considered as a substitution that can promote liver function, this treatment is limited due to the scarcity of donors, the cost of the operation, and the need for lifelong immunosuppression [3–5].

There is an emerging role for multipotent mesenchymal stromal stem cells (MSCs) in regenerative medicine due to its high proliferation rate [6–9], its ability to produce several active molecules that are capable of inhibiting inflammation and stimulate the recovery of injured cells, and its capability to differentiate into HSCs with the successful expression of hepatic specific markers. Recently, many studies have shown that MSCs injected in rat models have anti-inflammatory and anti-fibrotic functions improving liver damage [6].

A known model for cirrhosis and liver damage is the CCl₄ model, where it is metabolized inside the body by CYP2E1 to release highly reactive free radicals. These reactive free radicals interact with different cellular macromolecules especially membrane lipids forming lipid peroxides [10]. This study highlights recent findings to assess the therapeutic effects of MSCs in the treatment of liver fibrosis in a rat model of liver fibrosis induced by CCl₄ by evaluating changes in liver histopathology, liver function markers, fibronectin and integrin-β1 gene expression, and DNA integrity in comparison to silymarin as a standard drug.

2 **Methods**

2.1 **Animals**
Sprague Dawley male albino rats weighing 180-220g were purchased from the Nile Center of Experimental Research, Mansoura, Egypt. The animals were maintained at constant temperature (22°C). Food and water were given ad libitum throughout the experimental period. The study was approved by the Ethical Committee of the MSA (Bp7/EC7/2018F).

2.2 Experimental Design

The animals were randomly divided into four groups (n=10) as follows:

Group 1: served as the control group receiving olive oil (2 ml/kg single oral dose) twice per week for 8 consecutive weeks.

Groups 2: served as a positive control (liver fibrosis). The rats received orally 2 ml/kg CCl₄ (20% olive oil dilution) twice per week for 8 consecutive weeks for the induction of liver fibrosis [11]. CCl₄ was used in this study for liver fibrosis induction because this model closely resemble that of human [12].

Group 3: served as a standard treatment group receiving 100mg/kg silymarin (SigmaAldrich #S0292) orally daily along with 2 ml/kg CCl₄ (20% olive oil dilution) orally twice per week for 8 consecutive weeks.

Group 4: served as MSC-treated group (MSCs-group). These were treated with 1x10⁶ MSCs suspended in 0.5 ml PBS, single-dose i.v. along with 2 ml/kg CCl₄ (20% olive oil dilution) orally twice per week for 8 consecutive weeks. MSCs were injected 4-weeks after starting the CCl₄ administration [13].

2.3 MSCs Isolation and Identification

Six-week aged Sprague Dawley male albino rats were euthanized with thiopental. MSCs were isolated from the bone marrow of the tibiae and femurs after being carefully dissected [14]. MSCs were isolated from the bone marrow of the tibiae and femurs after being carefully dissected [13]. Bone marrow was carefully flushed with Dulbecco’s modified Eagle’s medium. Nucleated cells were completely isolated by a gradient (Ficoll/Paque from Pharmacia) and then suspended in 1% penicillin-streptomycin culture.
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(Sigma-Aldrich, St. Louis, MO, USA). The isolated cells were cultured for 12 -14 days at 37°C and 5%
humidified CO₂. Cells are then harvested by trypsinization and incubated in a culture flask.

2.4 Assessment of Liver Fibrosis

2.4.1 Liver Function Tests

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed using
(Biodiagnostics, CA, USA) and serum albumin was assayed using (QuantiChrom BCG Albumin Assay Kit,
Bioassay Systems).

2.4.2 Determinations of Oxidative stress and Inflammation Markers

The liver was weighed and a fraction was homogenized in ice-cold phosphate-buffered saline (PBS)
to prepare a 20% solution. Lipid peroxidation marker expressed as malondialdehyde (MDA) and
Glutathione (GSH) as an antioxidant marker were assayed using the dithio-binitrobenzoic acid method [15]
and the thiobarbituric acid reactive substances (TBARS) method [16].

For the anti-inflammatory markers, a fraction of the liver was carefully homogenized in a hypotonic
lysis buffer using a protease inhibitor. Interleukin-10 (IL-10), an anti-inflammatory marker, and tumor
necrosis factor (TNF-α), a pro-inflammatory marker, were determined using ELISA kits provided by
MyBiosource, Inc. (San Diego., USA)

2.4.3 Expression of Fibronectin and Integrins-β1

RNA was extracted for examination from the liver according to the manufacturer’s instructions
using RNA Extraction Kit (iNtrON Biotechnology, Korea). Fibrotic gene expression for fibronectin and
integrin-β1 was determined using quantitative reverse-transcription polymerase chain reaction (RT-PCR)
(Brilliant II QRT-PCR Master Mix Kit, 1-Step, Agilent, CA, USA) using the following primers.
Results were compared with that of the control group using the "2-ΔΔCt" method.

### 2.5 Western Blotting

The ReadyPrep TM protein extraction kit; Bio-Rad Inc (Catalog #163-2086) was employed according to manufacturer instructions to the homogenized liver tissues. Bradford Protein Assay Kit (SK3041) provided by Bio basic Inc (Markham Ontario L3R 8T4 Canada) was used for protein quantification in all samples. 20 μg protein of each sample was then loaded with an equal volume of 2x Laemmli sample buffer and separated using a polyacrylamide gel electrophoresis. The blot was then run for 7 min at 25 V to allow protein bands transfer from the gel to a PVDF membrane using BioRad Trans-Blot Turbo. Fibronectin (catalog NBP1-84468, Novus Biologicals) and β1 integrin (catalog# 4706, Cell signaling Technology, USA) primary antibodies were incubated overnight against the blotted target protein at 4°C. Finally, incubation was done in the HRP-conjugated secondary antibody (Goat anti-rabbit IgG- HRP-1mg Goat mab -Novus Biologicals) solution against the blotted target protein for 1hr at room temperature. The chemiluminescent substrate (ClarityTM Western ECL substrate Bio-Rad cat#170-5060) was applied to the blot. Image analysis software was used to read the band intensity of the target proteins against β-actin by protein normalization on the ChemiDoc MP imager.

### 2.6 Histopathological Assessment
2.6.1 Hematoxylin and Eosin Staining

After fixation of liver specimens in 10% formaldehyde in PBS, liver tissues were then dehydrated, embedded into paraffin and sections were made at a thickness of 5μm. These sections were carefully stained with hematoxylin and eosin (H&E) for histopathology [17].

2.6.2 Metavir Scoring System for Fibrosis Assessment

The hepatic fibrosis was determined using the Metavir scoring system [17]. The fibrosis score is determined using a five-point scale (0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis).

2.7 Immunohistochemistry

Briefly, liver tissue sections were cut into 5μm sections and subjected to deparaffinization, dehydration and heat-induced antigen retrieval then endogenous peroxidase and protein blocking steps. After washing, liver tissue sections were incubated with primary antibodies (anti-CD68, Santa Cruz Biotechnology Inc.) overnight at 4°C in a humid chamber with a dilution ratio of 1:200 in PBS. HRP-labelled secondary antibodies (Abcam, UK) was applied for 2 hours after washing. Finally, DAB-substrate kit was then used for color development and Meyers hematoxylin was used as counter stain. Negative control slides were obtained by escaping the primary antibody step. Positive expression was quantified as area percent (%).

2.8 Comet Assay

The extent of DNA damage was accessed using the comet assay under alkaline conditions. Comet tail length was measured firstly by the fluorescence microscopy and then secondly it is analyzed using the CaspLab Comet Assay Software v1.2.3 (Tritek Corporation, Summerduck, VA). From each group, ten cells are analyzed.
2.9 Statistical Analysis

All data are expressed as mean ± SD. The difference between all groups was determined by GraphPad Prism 6, using the one-way ANOVA test and Tukey's Kramer Multiple Comparison Test at P-value < 0.05

3 Results

3.1 MSCs Improved Liver Function Tests

After induction of liver fibrosis with CCl₄, both ALT (U/L) and AST (U/L) levels were increased by 1.7 and 2.5-folds in the serum while the albumin concentration decreased. Silymarin administration enhanced the liver function tests and decreased the level of both enzymes as well as enhancing the synthesis of albumin. On comparing the MSCs group with silymarin, although both decreased the liver enzymes and increased the albumin synthesis, MSCs were able to reduce the more liver-specific ALT significantly more than silymarin (Table 1) indicating a higher healing power.

3.2 MSCs Posses Antioxidative and Anti-inflammatory Properties

The CCl₄ resulted in a 2-fold increase in the MDA and a nearly 3-fold decline in the level of GSH compared with control rats indicating liver damage. Treatment with silymarin decreased MDA by around 30% and enhanced GSH by more than 50%. Groups treated with MSCs showed MDA levels comparable to the silymarin group and 26% higher levels of GSH (Figure 1).

The anti-inflammatory properties of silymarin and MSCs were assessed by measuring the IL-10 and TNF-α levels. Induction of fibrosis with CCl₄ resulted in the decrease of IL-10 and the increase of the proinflammatory TNF-α. Upon treatment with silymarin, the IL-10 increased by more than 1.8-folds while MSCs increased it by 2.6-folds. On the other hand, silymarin decreased TNF-α by 25% compared to the diseased group while MSCs surpassed this to reach a 56% decrease in the levels of TNF-α (Figure 2). MSCs showed both anti-inflammatory and antioxidative stress properties.
3.3 MSCs Suppresses the Expression of Fibrogenesis-Related Genes

QRT-PCR revealed a more than 2.5-fold increase in both fibronectin and integrin-β1 expression in rats after administering CCl4 for 8 weeks showing a role for both proteins in the progression of fibrosis. These values were decreased after treatment. Silymarin decreased the expression of fibronectin by 74% and integrin-β1 by 77%. Treatment with MSCs lowered the expression of both proteins to the levels of the control group where fibronectin decreased by 32% more and integrin-β1 by 40% compared to the standard silymarin group (Figure 3).

3.4 Western Blotting Analysis

The results of the QRT-PCR were confirmed with the western blotting analysis as the expression of both genes was overexpressed in cases of fibrosis and treatment decreased their expression levels. Treatment with MSCs shows a greater decrease in the level of both fibronectin and integrin β1 (Figure 4).

3.5 Histopathological Assessment Shows Improvement after Treatment with MSCs

3.5.1 MSCs Decrease Liver Fibrosis

H&E staining revealed significant changes obtained in the histological examination between the control, untreated and treated groups. In the CCl4 group, the liver tissue shows activation of Kupffer cells and sporadic hepatocytes necrosis while both the silymarin and the MSCs groups showed slight activation of Kupffer cells and few necrosis effects of hepatocytes (Figure 5).

3.5.2 MSCs Reduces Fibrosis on the Metavir Scoring System

The fibrosis score was assessed using the Metavir scoring system. Both silymarin and MSCs had better scores than the diseased groups but MSCs had better results regarding the extent of fibrosis (Table 2). Both the H&E staining and the Metavir Scoring showed a positive impact of the MSCs on the extent of liver fibrosis.
3.6 Immunohistochemistry

Immune expression of CD68 in liver tissue is illustrated in Figure 6. The control group showed normal limited expression of CD68 cells. On the contrary, CD68 expression was significantly increased in the CCl4 group compared to the control group. Silymarin treated group exhibited mild improvement in comparison to CCl4 group. MSCs treated grouped showed the greatest reduction in CD68 positive cells compared with CCl4 group (Figure 6).

3.7 MSCs Inhibits the DNA Fragmentation

The effect of the administration of either CCl4 or CCl4 followed by treatment with silymarin or MSCs on liver DNA is shown in Figure 7. A significant more than 8-fold increase in the tail length and 3.7-fold increase in tail DNA% (tDNA%) was shown in the liver tissues of rats intoxicated with CCl4. Treatment with silymarin and MSCs significantly protected the rats’ livers from DNA damage as indicated by a decrease in tail length% and tDNA%. Silymarin decreased the tail’s length% by 24% and the tDNA% by 40% compared to the diseased group while MSCs restored both do the level of the control group as it decreased the length by 20% and the tDNA% by 35% more than the silymarin (Figure 7). Results show that the MSCs have a great protective effect on the integrity of the DNA.

Discussion

Recent studies have been focusing on the role of using MSCs in medicine for the treatment of multiple diseases as Alzheimer, lung fibrosis, corneal diseases, and digestive diseases [19–22]. In this study, injection of MSCs in rats with liver fibrosis resulted in improving liver functions and decreasing the extent of fibrosis, inhibit the expression of fibrogenic genes and decrease hepatic DNA fragmentation.

The blood samples taken from the tail were used to assess the extent of liver damage using ALT, AST, and serum albumin, and the damage was confirmed. Treatment with MSCs gradually attenuated the liver injury by reducing the CCl4-elevated serum levels of AST and ALT more than the control, diseased,
and silymarin groups. The level of albumin was elevated in the MSCs group significantly more than the silymarin group. Previous studies agreed with our study and showed elevated levels of albumin and reduced liver damage markers, showing the ability of MSCs in restoring liver functions [23]. Furthermore, the decrease in liver enzymes was accompanied by a similar decrease in the levels of TNF-α in the MSCs group compared to other groups [24]. The improvement in the MSCs group can be attributed to its ability to regenerate the liver cells after CCl₄-injury [7].

Additionally, our work shows that injection with MSCs ameliorates the levels of GSH and reduces the MDA level as well within the MSCs group compared to other groups indicating its ability to reduce oxidative stress caused by CCl₄. We believe that MSCs soluble mediators could play a role in reducing inflammation and oxidative stress which may in turn play a role in liver fibrosis [25–27]. Similar results were confirmed by other studies [24,28]. MSCs are suggested to have an essential role as an anti-inflammatory and immunomodulatory that allows MSCs to have a very important role in liver recovery [28].

Liver fibrosis is campaigned by the accumulation of extracellular matrix proteins in the liver injured tissue where hepatic stellate cells (HSC) play an important role. Upon injury, the level of transforming growth factor-b (TGF-β) increases and results in the activation of the HSC through the TGF-β/SMAD pathway [29,30]. This activation results in the production of fibronectin, an ECM protein a major profibrotic factor [29]. In the present study, MSCs treatment decreased the level of fibronectin expression compared to the silymarin group through a dual pathway. First, MSCs decrease the proliferation of HSC and promotes its apoptosis [31]. The second is through the elevation of IL-10 which in turn downregulates the TGF-β and inhibits the transcription of fibronectin [32,33]. MSCs also successfully inhibited the expression of integrin, a potent activator of TGF-β [34,35]. The decrease in the levels of both fibronectin and integrin can explain the decrease in the extent of fibrosis and the antifibrotic effect of MSCs. This also explains the reduction in DNA damage within the liver tissue as assessed by the Comet assay.
The histopathological examination is the main tool for the diagnosis of liver diseases. In our study, MSCs groups showed slight activation of Kupffer cells’ and few necrosis of hepatocytes compared to other groups by its regenerative properties. Also, we assessed stages of fibrosis through the Metavir scoring system. According to this system, a significant improvement of hepatic fibrosis was shown in the MSCs group compared to diseased and silymarin groups. Our findings were confirmed by previous studies which also indicate that MSCs inhibit the production of profibrotic factors as fibronectin and integrin which was observed in our study [36,37].

A study by Popp et al., back in 2007 [38] stated that MSCs do not contribute to liver regeneration but we believe that there were 2 reasons for this. The first is that the authors used Dipeptidyl peptidase IV as a marker for hepatic regeneration which, as the authors stated, is a very late marker of hepatic development and the second is that part of the action of MSCs is mediated through soluble mediators and not only differentiation [26,27].

Our results showed that MSCs have more regenerative, anti-fibrotic, and anti-inflammatory properties than silymarin which is a standard drug in the treatment of liver fibrosis. And our findings were strongly proved by the histopathological evaluation, the METAVIR scoring system, and comet assay. MSCs can restore liver function and structure through several mechanisms which include secretion of many growth factors and cytokines that help to reduce inflammation, fibrogenesis, and repair the injured hepatic cells [39].

Conclusion

MSCs treatment ameliorates all liver functions in this rat model of CCl₄-induced liver fibrosis which was clearly shown by histopathological assessment and comet assay. Furthermore, MSCs treatment decreased the expression of fibrogenesis, inflammation and restore the liver function significantly more than silymarin treatment. Considering safety, MSCS transfusion is a potential tool for treatment of liver fibrosis.

Abbreviations
Mesenchymal stromal stem cells: MSCs
Extracellular matrix: ECM
Transforming growth factor-β: TGF-β
Hepatic stellate cells: HSC
Interleukin-10: IL-10
Tumor necrosis factor: TNF-α
Malondialdehyde: MDA
Thiobarbituric acid reactive substances: TBARS

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Consent for publication: We affirm that the submission represents original work that has not been published previously. Also, we confirm that each author has seen and approved the contents of the submitted manuscript.
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Code availability: Not applicable
Author Contributions:
AMF: Model development and treatment procedures, SM: Stem cell preparation, qPCR and DNA fragmentation assay, and AM: Inflammatory mediators, data analysis, and writing. all Authors read and approved the manuscript.

Signature on behalf of all authors

[Signature]

Preprint
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Figure legends

Figure 1: (A) Effect of therapy on MDA levels in the studied groups. Treatment decreased liver peroxidation compared to the diseased group. There was no significant difference between silymarin and MSCs (B) Effect of therapy on GSH levels in the studied groups. Both treatments enhanced GSH synthesis with the MSCs group showing better antioxidant results. Each value represents the mean of 10 experiments ± SD. a: significant difference versus the control group; b: significant difference versus the CCl₄ group, c: significant difference versus the silymarin group.

Figure 2: (A) Effect of therapy on the IL-10 levels in the studied groups. Treatment increased the expression of the anti-inflammatory cytokine compared to the diseased group. The expression of IL-10 in the MSCs group was significantly higher than that of the silymarin group. (B) Effect of therapy on the TNF-α levels in the studied groups. Both treatments decreased TNF-α levels showing anti-inflammatory properties. MSCs were 17-fold more potent than silymarin. Each value
represents the mean of 10 experiments ± SD. a: significant difference versus the control group; b: significant difference versus the CCl_4 group, c: significant difference versus the silymarin group.

Figure 3: (A) Effect of therapy on the expression levels of fibronectin in the studied groups. The fibronectin expression was enhanced by the administration of CCl_4 and treatment decreased its expression compared to the diseased group. MSCs treatment decreased the expression levels to that of the control group. (B) Effect of therapy on the expression levels of integrin-β1 in the studied groups. Integrin-β1 expression was enhanced by CCl_4. Treatment with silymarin significantly decreased its expression but MSCs treatment decreased the expression levels to that of the control group. Each value represents the mean of 10 experiments ± SD. a: significant difference versus the control group; b: significant difference versus the CCl_4 group, c: significant difference versus the silymarin group.

Figure 4: Western blotting of fibronectin and integrin. Administration of CCL_4 enhanced the expression of both fibronectin and integrin by more than 2.5-folds compared to the control group. Treatment with silymarin or MSCs resulted in a decrease in the level of both genes by around 1.9-folds with silymarin and 2.2-folds with MSCs.

Figure 5: H&E stain (x400) (A) Liver of control rat showing no histopathological changes (B) Liver of CCl_4 intoxicated rat showing Kupffer cells activation and necrosis of sporadic hepatocytes. (C) Liver of CCl_4 intoxicated rat treated with silymarin showing some Kupffer cells activation and decreased necrosis of sporadic hepatocytes (D) Liver of CCl_4 intoxicated rat treated with MSCs showing slightly Kupffer cells’ activation and decreased necrosis of sporadic hepatocytes.

Figure 6: Immunostaining of CD68 in liver tissue. (A) control group, (B) CCl_4 group, (C) Silymarin treated group and (D) MSCs treated group. (E) Quantification of CD68 positive cells as area % of expression. Value presented as means ± SE significant difference was
considered at P<0.05. a: significant difference versus the control group; b: significant difference versus the CCl₄ group, c: significant difference versus the silymarin group.

Figure 7: Effect of treatments on the integrity of hepatocytes’ DNA. A: % Tail DNA. Both minimized DNA degradation but MSCs were 2.4-folds more protective. B: % Tail length. Both minimized DNA degradation but MSCs were 1.9-folds more protective. C: Comets from hepatocytes: a: control, b: CCl₄, c: silymarin treated, and d: MSCs treated. Each value represents the mean of 10 experiments ± SD. a: significant difference versus the control group; b: significant difference versus the CCl₄ group, c: significant difference versus the silymarin group.
Table 1: Effect of MSCs therapy on liver function enzymes and albumin in the studied groups.

| Group   | ALT (U/L)     | AST (U/L)     | Albumin (g/dL) |
|---------|--------------|--------------|---------------|
| Control | 37.59 ± 3.56 | 38.42 ± 3.59 | 3.91 ± 0.18   |
| CCl4    | 75.38 a ± 5.28 | 145.1 a ± 16.12 | 1.9 a ± 0.24   |
| Silymarin | 53.8 a, b ± 3.89 | 57.11 a, b ± 4.49 | 3.18 b ± 0.28   |
| MSCs    | 47.12 a, b, c ± 5.09 | 49.54 a, b ± 3.43 | 3.27 b ± 0.25   |

Each value represents the mean of 10 experiments ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey’s multiple comparison post hoc test; a: significant difference versus the control group; b: significant difference versus the CCl4 group; c: significant difference versus the silymarin group.
The fibrosis score was assessed on a five-point scale using Metavir scoring system where F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis.

| Fibrous score | Normal | CCl4 | Silymarin | MSCs |
|---------------|--------|------|-----------|------|
| F0            | 9      | 0    | 0         | 2    |
| F1            | 1      | 2    | 4         | 6    |
| F2            | 0      | 5    | 5         | 2    |
| F3            | 0      | 2    | 1         | 0    |
| F4            | 0      | 1    | 0         | 0    |
Figure 1: (A) Effect of therapy on MDA levels in the studied groups. Treatment decreased liver peroxidation compared to the diseased group. There was no significant difference between silymarin and MSCs (B) Effect of therapy on GSH levels in the studied groups. Both treatments enhanced GSH synthesis with MSCs group showing better antioxidant results.
Each value represents the mean of 10 experiments ± SD. *: significant difference versus the control group; †: significant difference versus the CCl4 group; ‡: significant difference versus the silymarin group.
Figure 2: (A) Effect of therapy on the IL-10 levels in the studied groups. Treatment increased the expression of the anti-inflammatory cytokine compared to the diseased group. The expression of IL-10 in the MSCs group was significantly higher than that of the silymarin group. (B) Effect of therapy on the TNF-α levels in the studied groups. Both treatments decreased TNF-α levels showing anti-inflammatory properties. MSCs was 17-folds more potent than silymarin.

Each value represents the mean of 10 experiments ± SD. *: significant difference versus the control group; #: significant difference versus the CCl4 group; &: significant difference versus the silymarin group.
Figure 5: (A) Effect of therapy on the expression levels of fibronectin in the studied groups. The fibronectin expression was enhanced by the prolonged administration of CCL4 and treatment decreased its expression compared to the diseased group. MSCs treatment decreased the expression levels to that of the control group.

(B) Effect of therapy on the expression levels of integrin-β1 in the studied groups. Integrin-β1 expression was enhanced by CCL4. Treatment with silymarin significantly decreased its expression but MSCs treatment decreased the expression levels to that of the control group.

Each value represents the mean of 10 experiments ± SD. *: significant difference versus the control group; †: significant difference versus the CCL4 group; ‡: significant difference versus the silymarin group.
Figure 5: H&E stain (x400) (A) Liver of control rat showing no histopathological changes (B) Liver of CCl₄ intoxicated rat showing Kupffer cells activation and necrosis of sporadic hepatocytes. (C) Liver of CCl₄ intoxicated rat treated with silymarin showing some Kupffer cells activation and few necrosis of sporadic hepatocytes (D) Liver of CCl₄ intoxicated rat treated with MSCs showing slighty Kupffer cells activation and few necrosis of sporadic hepatocytes.
