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The Ameliorating Effect of Adipose Tissue Stem Cells on Liver Function in Experimental Rats with Liver Fibrosis

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ABSTRACT

Background: As mesenchymal stem cells have anti-inflammatory and immunomodulating properties and can be a therapeutic option in regenerative medicine, this study was undertaken to determine ameliorating effect of adipose tissue stem cells (AdSCs) on liver function in experimental rats with liver fibrosis.

Methods: Thirty-two rats were randomly divided into four equal groups including control group 1 receiving just 1 mL/kg of distilled water, twice a week for 8 weeks (intraperitoneally: IP). Control group 2 received 1 mL/kg of olive oil, similarly. Sham group was treated with CCl_4 (1 mL/kg) dissolved in 1 mL of olive oil to induce liver injury, identically. In control and sham groups, blood samples were collected at the beginning of week 8. Finally, the experimental group after induction of liver injury, was injected with $2×10^6$ AdSCs in the tail vein at the beginning of week 8, while a blood sample was provided after 3 and 8 weeks following cell transplantation to determine liver function.

Results: AdSC were spindle shape, positive for osteogenic and adipogenic inductions and expressed mesenchymal and lacked hematopoietic markers. Following cell transplantation, an improvement in albumin, total protein, and direct and indirect bilirubin were noticed denoting to repairing effect of AdSCs and treatment of liver injury.

Conclusion: AdSCs improved liver function and acted as a promoting factor for liver regeneration. So they can be helpful for ameliorating hepatic injuries. These findings can be beneficial for cell therapy and can open a new era for researchers trying to improve mesenchymal stem cell therapeutic outcomes.

Introduction

Liver cirrhosis can be considered as the latest stage of some chronic liver conditions that have common properties of necro-inflammation, fibrosis, and regenerative nodules changing the natural structure of the liver by reducing its functional mass and altering the vascular structure (1). Cirrhosis results from the production of extracellular matrix (ECM) under chronic injury (2). In the first stage, liver cells are damaged by an injury leading to the activation and recruitment of Kupffer cells and other inflammatory cells. After that, hepatic stellate cells (HSCs) are activated (3) that have an important role in liver fibrosis and cirrhosis (4). Activation of these cells leads to the destruction of liver structures, hepatic innervation, and decreased liver function due to the accumulation of collagen and other extracellular matrix components (5). In fact, extracellular matrix proteins, serum levels of fibrogenic cytokines, and degradation products are substantially increased in advanced fibrosis that can cause cirrhosis subsequently (6). It is noticeable that bleeding, liver failure or encephalopathy and hepatocellular carcinoma (HCC) (7) can be considered as the complication of liver cirrhosis.

Studies show that chronic hepatitis B, alcoholic liver disease, chronic hepatitis C, and nonalcoholic fatty liver disease (NAFLD) are known as primary etiologies for cirrhosis (8). In most developed countries, alcoholic liver disease and hepatitis C virus (HCV) and nonalcoholic steatohepatitis (NASH) are the most common reasons for cirrhosis (9), while chronic hepatitis B is considered as a main cause in Asia and sub-Saharan Africa (10). Autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hemochromatosis, Wilson disease, alpha-1 antitrypsin deficiency, Budd-Chiari syndrome, drug-induced liver cirrhosis, and chronic right-sided heart failure are other causes of cirrhosis (11). Healthy diet, avoidance of alcohol consumption, hepatitis C virus (HBV) and HCV vaccinations, controlling weight and early treatment of factors such as hypotension, dehydration and infections are general management to prevent chronic liver disease (11).

Currently, there is no improved cure for liver cirrhosis. The only way is management of symptoms and etiology such as ursodeoxycholic acid and obeticholic acid in primary biliary cholangitis, antiviral medicine in viral hepatitis, steroids and immunosuppressant agents in autoimmune hepatitis, copper chelation in Wilson disease, iron chelation and phlebotomy in hemochromatosis (12). If the liver is seriously damaged, liver transplant can be considered as only treatment option which is costly (13).

Mesenchymal Stem cells are immature cells with extensive ability of self-renewal and high potential of differentiation into specific cells like adipocytes, osteocytes and chondrocytes under special physiological circumstances (14). These cells are isolated from different tissues of human body like dental pulp (15), Wharton's jelly (16), bone marrow (17) and adipose tissue (18). For cell therapy, adipose tissue has been known as a main source due to several reasons such as abundance, accessibility, high proliferation and rich source of MSCs, while the isolation of stem cells from bone marrow is painful with low number of mesenchymal stem cells (19). This study aimed to investigate the effect of adipose tissue-derived stem cells on liver function tests in experimental rats.

Materials and Methods

Thirty two male Sprague-Dawley rats (200±20 g) were purchased from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Science, Shiraz, Iran. They were kept under 12 h light and 12 h dark cycle with a controlled temperature between 20 °C and 22 °C. All rats had access to food and water *ad libitum*. They were kept, treated and euthanized based on the instructions of the Animal Care Committee of Shiraz University of Medical Sciences, Shiraz, Iran and according to laws and regulations of Iran Veterinary Organization defined for working with laboratory animals. The study was approved by Arsanjan Azad University Ethics Committee too.

Hepatic injury was created by intraperitoneal (IP) injection twice a week for eight weeks with 1 mL/kg carbon tetrachloride (CCl_4) diluted in olive oil 1:1 (v/v). Adipose tissue stem cells (AdSCs) were preconditioned for 24 h in normal culture medium. AdSCs (2×10^6 cells/1 mL of phosphate buffered saline: PBS) were injected via tail vein at the beginning of week 8. The rats were randomly divided into four equal groups of 8 rats including the control group 1 receiving just 1 mL/kg of distilled water, twice a week for 8 weeks (IP). The control group 2 received 1 mL/kg of olive oil, twice a week for 8 weeks (IP). The sham group (group 3) was treated with CCl_4 (1 mL/kg) dissolved in 1 mL of olive oil twice a week for 8 weeks (IP) to induce liver injury. In control and sham groups, blood samples were collected at the beginning of week 8. Finally, the experimental groups (group 4 and 5) that received CCl_4 (1 mL/kg) dissolved in 1 mL of olive oil (IP, twice a week for 8 weeks) for induction of liver injury, was further injected with 2×10^6 AdSCs in the tail vein at the beginning of week 8, while a blood sample was provided after 3 and 8 weeks

following cell transplantation and considered as experimental groups 1 and 2, respectively.

The rats were anesthetized to collect blood samples under general anesthesia using a mixture of 10% ketamine (Alfasam, Netherlands) and 2% xylasine (Alfasam, Netherlands). Blood samples were transferred into a chelate tube, and later placed in a centrifuge at 2000 rpm for 15 minutes. According to the manufacturer's instructions, serum direct bilirubin, total bilirubin, albumin, and total protein were estimated using commercially available kits (Abcam, Tokyo, Japan) (20).

Rats were anesthetized using a mixture of 10% ketamine (Alfasam, Netherlands) and 2% xylasine (Alfasam, Netherlands) and euthanized by cervical dislocation. Under sterile condition, adipose tissue was isolated from abdominal and pelvic regions and transferred into falcon tubes containing Dulbecco's modified Eagle's medium (DMEM, Gibco, Waltham, USA). The adipose tissue was further washed three times in sterile PBS and then chopped into small pieces by a sterile blade. The mechanically cut adipose tissue was later transferred into a 15 mL falcon tube containing 5 mL of DMEM and underwent centrifugation at 200 g for 10 min.

After removal of the supernatant, the remaining pellet was treated with 1.5 mL of 0.2% collagenase type II (Gibco, USA) at 37 °C for 40 min and later put in incubator with 5% CO_2 at 37 °C and saturated humidity. A total of 5 mL of DMEM was added to the falcon tube, and was centrifuged at 200 g for 7 min. The supernatant was taken out, and the remaining cell pellet was re-suspended in 1 mL of culture media containing 88% DMEM, 10% FBS, 1% penicillin-streptomycin, and 1% non-essential amino acids (Sigma-Aldrich, USA). They were later transferred into a culture flask already containing 4 mL of culture media and placed in an incubator with 5% CO_2 at 37 °C and saturated humidity. The media change was carried out every 3 days until the cells were 80% confluent. Sub-culturing of cells was conducted at 80% confluence by subjecting cells with 0.25% (w/v) trypsin-EDTA (Gibco, USA) until passage 3.

The cells were characterized morphologically using an inverted microscope (Nikon, Tokyo, Japan), to assess to be spindle shape, while the images were taken by a digital camera (Olympus, Tokyo, Japan) for all passages. They were also investigated for osteogenic and adipogenic differentiation properties for further characterization tests to verify their mesenchymal potential. RT-PCR technique was applied to assess the presence of mesenchymal markers such as CD73 and CD90 and hematopoietic markers of CD34 and CD45.

For osteogenic differentiation potential, AdSCs were put in 6-well plates to reach 80% confluence. The culture medium was altered to osteogenic medium containing complete culture medium supplemented with 15% FBS, 50 µM ascorbic acid (Merck, Germany), 100 nM dexamethasone (Sigma-Aldrich, USA), and 10 mM glycerol 3-phosohate (Merck, Germany) for 21 days, while media change occurred every 3 days. After 3 weeks, the cells were subjected to 10% formalin for 20 min, and, following three washes with deionized water, they were stained with 1.4% Alizarin Red solution (solved in deionized water at pH of 4.1, Sigma-Aldrich, USA). The differentiated cells appeared in red color denoting to calcium deposit and calcification in differentiation process.

For adipogenic induction potential, the AdSCs were transferred in 6-well plates with complete culture medium and after 80% confluence, media changed was replaced with adipogenic medium consisted of complete culture media supplemented with 15% FBS, 100 µM ascorbic acid, 100 nM dexamethasone, and 200 µM indomethacin (Sigma-Aldrich, USA) for 21 days. The cells were further fixed using 10% formalin for 20 min and after 3 washes with deionized water, they were stained with 0.5% Oil Red-O (Sigma-Aldrich, USA) solved in 2-propanol solution (Merck, Germany) for 2 h. The differentiated cells appeared in red color revealing presence of oil droplets.

The RT-PCR investigated the gene expression of 208 bp cluster of differentiation (CD) 73 (forward primer of TGCATCGATATGGCCAGTCC and reverse primer of AATCCATCCCCACC GTTGAC) and 177 bp CD90 (forward primer of GACCCAGGACGGAGCT ATTG and reverse primer of TCATGCTGGATGGGCAAGTT) being mesenchymal markers; and 257 bp CD34 (forward primer of GCCATGTGCTCACACATCA and reverse primer of CAAACACTCGGGCCTAACCT) and 450 bp CD45 (forward primer of CCAAGA GTGGCTCAGAAGGG and reverse primer of CTGGGCTCATGGGACCATTT) being hematopoietic markers. RNA extraction kit (Cinna Gen Inc., Tehran, Iran) assessed the total RNA based on the manufacturer's protocol. The first-strand cDNA was synthesized by Revert Aid™ first strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, USA). The PCR thermal cycler (Veriti Thermal Cycler, Thermo Fisher Scientific, Waltham, USA) determined all PCR runs as: 1 cycle at 94 °C for 3 min, 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and 1 cycle at 72 °C for 10 min. In the last step, electrophoresis visualized the bands using DNA safe stain in 1.5% agarose gel medium and gel

documentation system (UVtec, Cambridge, UK).

To assess liver function, the whole blood of sacrificed rats was provided and kept at room temperature for 2 h and centrifuged at 4000 rpm for 10 min to get serum. The obtained sera were assayed for biochemical biomarkers of total proteins, albumin and direct and total bilirubin. They were assessed according to the manufacturer's instructions, using commercially available kits (Abcam, Tokyo, Japan). All biochemical variables were evaluated using tail venous blood samples. Similar instrument was utilized for the measurement of all indicators [20].

The normality of serum data distribution was confirmed using the Kolmogorov-Smirnov test. And after that, Tukey test was used for comparing the differences between the mean values of the control, sham and experimental groups. Additionally, One-way analysis of variances in SPSS statistical package for Windows (version 11.5, Chicago, IL, USA) was used in this study for comparing the differences between the mean values of the control, sham and experimental groups. P<0.05 was considered as a significant level.

Results

Regarding morphological characterization, the AdSCs were adhered to culture flasks and displayed a fibroblast-like spindle-shaped morphology in all passages (Figure 1A). For osteogenic differentiation potential, cells in osteogenic medium revealed calcium deposits displayed in red color by Alizarin Red staining after three weeks (Figure 1B). Considering adipogenic differentiation property, cells stained by Oil Red-O also denoted to intracellular lipid droplets in red color (Figure 1C). In RT-PCR, the cells were positive for gene expression of mesenchymal markers (CD73 and CD90) and negative for gene expression of hematopoietic markers (CD34 and CD45, Figure 1D).

Regarding liver function test (LFT) to assess total protein level, Table 1 demonstrates that in 3rd group (sham group) receiving CCl_4, a significant decrease in total protein level happened in comparison to the control groups ($p=0.0001$). In group 5 (experimental group 2) after 8 weeks of cell transplantation, an increase in total protein level was noticed and the difference was significant with sham group ($p=0.01$). In group 4 (experimental group 1) after 3 weeks of cell therapy, no significant difference was visible when compared to sham group ($p>0.05$). There was a significant difference for total protein level between group 4 (experimental group 1) and control groups ($p=0.05$, $p=0.05$, respectively). These findings reveal the healing impact of AdSCs in treatment and repair of hepatic injuries.

As Table 2 presents, a significant decrease in albumin level occurred in sham group receiving CCl_4 (group 3), when compared to control groups ($p=0.05$). In group 5 (experimental group 2) after 8 weeks of stem cell therapy, a significant increase in albumin level was seen in comparison to sham group ($p=0.05$); but this difference was not significant when group 5 was compared with control groups ($p>0.05$).
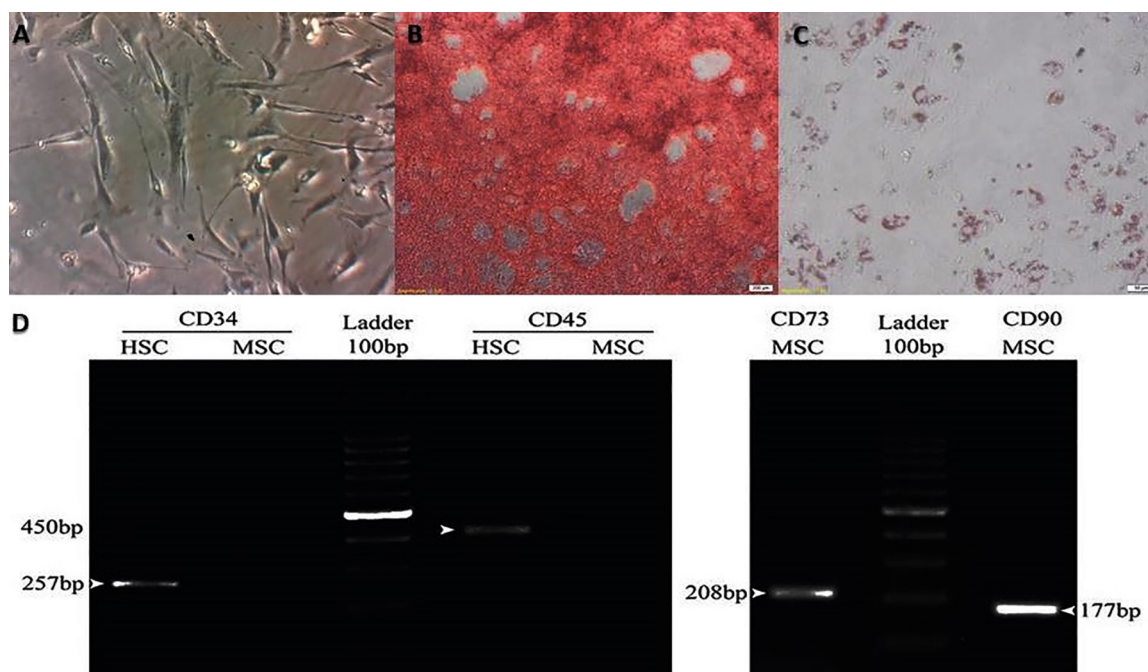

Figure 1: Characterization of rat adipose tissue stem cells. (A): Spindle shape cell morphology at 3rd passage, (B): Osteogenic induction in red color, (C): Adipogenic induction in red color, (D): RT PCR revealing presence of mesenchymal (MSC) markers (CD73 and CD90) and absence of hematopoietic (HSC) markers (CD34 and CD45).

Table 1: Comparison of mean total protein level between all groups.

Group	Total protein (g/dL) Mean±SD	Significance (2-tailed)				
		Group 1	Group 2	Group 3	Group 4	Group 5
1	172.75±5.54	-	0.968	0.0001	0.05	0.931
2	161.84±11.57	0.968	-	0.0001	0.023	0.906
3	252.66±17.09	0.0001	0.0001	-	0. 521	0.005
4	203.75±19.70	0.05	0.023	0. 521	-	0.255
5	177.50±6.29	0.931	0.906	0.005	0.255	-

Table 2: Comparison of the mean albumin level among the groups.

Group	Albumin level (g/dL) Mean±SD	Significance (2-tailed)				
		Group 1	Group 2	Group 3	Group 4	Group 5
1	3.38±0.27	-	0.999	0.018	0.259	0.999
2	3.73±0.20	0.999	-	0.013	0.275	0.996
3	2.43±0.38	0.018	0.013	-	0.785	0.015
4	2.92±0.17	0.259	0.075	0. 785	-	0.223
5	3.87±0.41	0.999	0.996	0.015	0.223	-

Table 3: Comparison of the mean total bilirubin level among the groups.

Group	Total bilirubin (mg/dL) Mean±SD	Significance (2-tailed)				
		Group 1	Group 2	Group 3	Group 4	Group 5
1	0.33±0.04	-	0.999	0.013	0.622	0.999
2	0.34±0.02	0.999	-	0.009	0.698	0.999
3	0.50±0.03	0.013	0.009	-	0.313	0.013
4	0.40±0.04	0.622	0.698	0. 313	-	0.622
5	0.32±0.03	0.999	0.999	0.013	0.622	-

Table 4: Comparison of the mean direct bilirubin level between groups.

Group	Direct bilirubin (μmol/L) Mean±SD	Significance (2-tailed)				
		Group 1	Group 2	Group 3	Group 4	Group 5
1	0.16±0.01	-	0.976	0.0001	0.981	0.999
2	0.14±0.01	0.976	-	0.0001	0.751	0.976
3	0.45±0.05	0.0001	0.0001	-	0.0001	0.0001
4	0.18±0.02	0.981	0.751	0.0001	-	0.981
5	0.16±0.02	0.999	0.976	0.0001	0.981	-

In group 4 (experimental group 1) and 3 weeks following cell transplantation, the increase in albumin level was not significant when compared to sham and control groups ($p>0.05$). It seems that AdSCs had therapeutic and healing effects on liver tissue damages.

Table 3 depicts a significant increase in total bilirubin level in the sham group received CCl$_4$ (group 3) when compared to the control groups ($p=0.05$, $p=0.01$, respectively). No significant change was observed in group 4 (experimental group 1) three weeks after transplantation of AdSCs in comparison to control and sham groups ($p>0.05$). In group 5 (experimental group 2) eight weeks followed by injection of AdSCs, a significant decrease in total bilirubin level was observed when compared to sham group ($p=0.05$); but the difference with control groups was not significant ($p>0.05$). These results indicate the healing effect of AdSCs in liver injuries.

As Table 4 displays, there was a significant increase in mean concentration of direct bilirubin in the sham group receiving CCl$_4$ (group 3) in comparison to the control groups ($p=0.0001$, $p=0.0001$, respectively). In groups 4 and 5 (experimental groups 1 and 2) three and eight weeks after transplantation of stem cells, a significant decrease in direct bilirubin level was observed when compared to sham group ($p=0.0001$); while no significant difference was seen between experimental groups 1 and 2 with control groups ($p>0.05$). Therefore, amelioration of hepatic injuries has happened following cell transplantation.

Discussion
Most types of chronic liver diseases can cause

liver fibrosis and lead to liver cirrhosis. Liver transplantation is the only treatment available for liver cirrhosis; so alternative therapeutic strategies are necessary to be undertaken due to limitations in access to liver tissue for transplantation (2). Recently, promising results from preclinical and clinical studies demonstrated that mesenchymal stem cells would be a therapeutic option for liver fibrosis and cirrhosis (21). It is suggested that mesenchymal stem cells can eliminate the progression of fibrosis due to differentiation potential and their immune-modulatory properties (22).

Additionally, these cells were mentioned to be safe and their extraction is a non-invasive method and they can be utilized in treatment of acute, chronic and metabolic liver diseases (21, 22). Various mesenchymal stem cells have been proposed in liver diseases including bone marrow (21-23), umbilical cord (24), menstrual blood (25), dental pulp (26), liver (27), and adipose tissue (28). It was shown that adipose tissue stem cells are similar to mesenchymal stem cells isolated from bone marrow in terms of morphology and expression of surface markers, but they were reported to have a higher potential for passage and proliferation than bone marrow derived stem cells (23).

As they can have hepatogenic potential (27), the present study was carried out in rats experimentally induced with liver injury by carbon tetrachloride (CCl$_4$) to determine the therapeutic effect of adipose tissue stem cells in improvement of hepatic injury, while our findings have revealed the ameliorating effect of adipose tissue stem cells and were effective in treatment of experimental liver injury induced in rat model. Mesenchymal stem cells were shown to decrease hepatic fibrosis and stimulate liver regeneration and to have immunomodulatory and anti-inflammatory functions that may reduce hepatic inflammation, improve liver function, and decrease infection incidences.

Injection of mesenchymal stem cells can improve biochemical tests and lead to a survival benefit in hepatic injuries. Mesenchymal stem cells can also perform various functions, including the downregulation of proinflammatory responses (29). Li *et al.* have demonstrated that after transplantation of bone marrow stem cells, the liver synthesis (prothrombin time, albumin and ascites) and secretion functions (total bilirubin) were significantly improved compared with those of the control group (30). Luo *et al.* by using umbilical cord stem cells in liver cirrhosis illustrated that the inflammation and fibrosis scores were lower in the group receiving stem cells and showed a largely improved liver cirrhosis degree and lower Child-Pugh scores (24).

Gharbia *et al.* have shown that stem cell therapy with AdSCs could attenuate inflammation and microvesicular steatosis, reduce collagen deposits, and repair liver fibrosis (31). It seems that mesenchymal stem cells suppress hepatic fibrosis by downregulating the lnc-BIHAA1/rno-miR-667-5p signaling pathway (32). These findings are in agreement with our study results too. Sabry and her colleagues evaluated the effect of mesenchymal stem cells in treatment of experimental CCL$_4$ induced liver fibrosis in rats and suggested mesenchymal stem cells to have anti-inflammatory, anti-fibrotic, and pro-angiogenic properties that can promote the resolution of CCL4 induced liver fibrosis (33).

Khajehahmadi *et al.* and Mehrabani *et al.* demonstrated that transplantation of bone marrow stem cells can significantly lead to the regeneration of hepatic injuries and to be considered as a therapy of choice in recovery of liver fibrosis (21, 22). Zhao *et al.* assessed 23 reports of studies comparing mesenchymal stem cell-based therapy to conventional treatment in liver fibrosis and reported mesenchymal stem cell-based therapy to be safe, and to improve liver function during the first 6 months after administration (34). Suk *et al.* conducted a phase II study for treating alcoholic liver cirrhosis using bone marrow stem cells. Six months after cell administration, there were 25% (one-time cell administration) and 37% (two-time cell administrations) reductions in the fibrosis zones (35).

Nomura *et al.* in surgical implantation of human adipose derived stem cells in rats experimentally induced hepatic fibrosis showed a significant increase in serum albumin and total protein levels (28). Abdelbaset-Ismail *et al.* showed that transplantation of adipose tissue stem cells ameliorated hepatic injury in rats. They reported migration of stem cells to the injured liver. The adverse effects were significantly recovered after engraftment with stem cells evidenced by enhanced survival and body weight, improved liver function tests (36). These findings are in agreement with our study results.

Conclusion

The present study illustrated that AdSCs improved liver function and acted as a promoting factor for liver regeneration and be helpful for ameliorating hepatic toxicity and injuries. These findings can be beneficial for mesenchymal-based cell therapy applications and can open a new era for researchers trying to improve mesenchymal stem cell therapeutic outcomes.

Acknowledgement

Conflict of Interest
None declared.

References

1 Dehghani SM, Gholami S, Bahador A, et al. Morbidity and mortality of children with chronic liver diseases, who listed for liver transplantation in Iran. Pediatric *Transplantation.* 2007;11:21-23. DOI:10.1111/j.1399-3046.2006.00619.x. PMID:17239119.

2 Dehghani SM, Bahador A, Gholami S, et al. Pediatric liver transplantation in Iran: Evaluation of the first 50 cases. *Pediatr Transplant.* 2007;11:256-60. DOI:10.1111/j.1399-3046.2006.00665.x. PMID:17430479.

3 Dehghani SM, Gholami S, Bahador A, et al. Comparison of child-turcotte-pugh and pediatric end-stage liver disease scoring systems to predict morbidity and mortality of children awaiting liver transplantation. *Transplant Proc.* 2007;39:3175-7. DOI:10.1016/j.transproceed.2007.07.080. PMID:18089346.

4 Rabbani Haghighi N, Naghsh N, Mehrabani D. The comparison of pretreatment effects of boiled coffee and curcuruma longa on serum albumin as a liver indicator in male rats injected with thioacetamide. *J Fasa Univ Med Sci.* 2014;4:58-66.

5 Rabbani Haghighi N, Mehrabani D. The protective effect of boiled coffee on liver enzymes ast, alt, alp and total bilirubin in rats treated with thioacetamide. *J Babol Univ Med Sci.* 2014;16:41-9.

6 Jamhiri I, Zahri S, Khodabandeh Z, et al. Enhancing the apoptotic effect of il-24/mda-7 on the human hepatic stellate cell through rgd peptide modification. *Immunol Invest.* 2018;47:335-350. DOI:10.1080/08820139.2018.1433202. PMID:29498551.

7 Jamhiri I, Hosseini SY, Khodabandeh Z, et al. The pattern of il-24/mda-7 and its cognate receptors expression following activation of human hepatic stellate cells. *Biomed Rep.* 2017;7:173-178. DOI:10.3892/br.2017.931. PMID:28804632.

8 Mostaghni AA, Soltanian A, Mokhtari E, et al. Seroprevalence of hepatitis B virus among hemodialysis patients in Bushehr Province, Southern Iran. *Hepat Mon.* 2011;11:200-2. PMID:22087144.

9 Hosseini SY, Sabahi F, Modarresi MH, et al. Cloning and evaluation of expression of a novel overlapping region of ns3 gene of hepatitis c virus by expressing vector. *J Med Sci Pathobiol.* 2011;13: 21-32.

10 Mojiri A, Behzad-Behbahani A, Saberifirozi M, et al. Hepatitis B virus genotypes in southwest Iran: Molecular, serological and clinical outcomes. *World J Gastroenterol.* 2008;14:1510-3. DOI:10.3748/wjg.14.1510. PMID:18330939.

11 Hosseini SV, Motazedian GR, Kumar PV, et al. Iinstillation of hypertonic glucose into gall bladder of rabbit: Does it induce sclerosing cholangitis? *J Appl Anim Res.* 2006;29:73-76. DOI:10.1080/09712119.2006.9706575.

12 Yavarian M, Saberfiroozi M, Amirizadeh S, et al. Prevalence of the HFE gene mutation in the liver transplanted and primary hemochromatosis patients in the southern Iran. *Iran Red Crescent Med J.* 2010;12:22-26.

13 Theocharidou E, Adebayo D. Challenges in liver transplantation in the context of a major pandemic. *World J Transplant.* 2022;12:347-358. DOI:10.5500/wjt.v12.i11.347. PMID:36437846.

14 Jalali E, Rabiee M. Adipose tissue derived mesenchymal stem cells. *IIOAB J.* 2016;7:112-118.

15 Zare S, Mehrabani D, Jalli R, et al. MRI-Tracking of Dental Pulp Stem Cells In Vitro and In Vivo Using Dextran-Coated Superparamagnetic Iron Oxide Nanoparticles. *J Clin Med.* 2019;8:1418. DOI:10.3390/jcm8091418. PMID:31505807.

16 Mehrabani D, Nazempour M, Mehdinavaz-Aghdam R, et al. MRI tracking of human Wharton's jelly stem cells seeded onto acellular dermal matrix labeled with superparamagnetic iron oxide nanoparticles in burn wounds. *Burns Trauma.* 2022;10:tkac018. DOI:10.1093/burnst/tkac018. PMID:36380853.

17 Khodabandeh Z, Mehrabani D, Dehghani F, et al. Spinal cord injury repair using mesenchymal stem cells derived from bone marrow in mice: A stereological study. *Acta Histochem.* 2021;123:151720. DOI:10.1016/j.acthis.2021.151720. PMID:34083065.

18 Mehrabani D, Rabiee M, Tamadon A, et al. The growth kinetic, differentiation properties, karyotyping, and characterization of adipose tissue-derived stem cells in hamster. *Comp Clin Pathol.* 2016;25:1017-1022. DOI:10.1007/s00580-016-2300-7.

19 Iravani K, Mehrabani D, Doostkam A, et al. Use of Magnetic Resonance Imaging to Assess the Regenerative Effects of Adipose Tissue-Derived Mesenchymal Stem Cells in a Rabbit

Cartilaginous Laryngeal Defect Model. *Curr Ther Res Clin Exp.* 2022;97:100682. DOI:10.1016/j.curtheres.2022.100682. PMID:35959231.

20 Fouzi M, Razmi N, Mehrabani D. The effect of Citrullus colocynthis on serum lipid profile and hepatic histology in CCl 4-induced liver injury rat model. *Int J Nutr Sci.* 2020;5:1-6. DOI:10.30476/IJNS.2020.88244.1094.

21 Khajehahmadi Z, Mehraban D, Ashraf MJ, et al. Healing effect of conditioned media from bone marrow-derived stem cells in thioacetamide-induced liver fibrosis of rat. *J Med Sci.* 2016;16:7-15. DOI:10.3923/jms.2016.7.15.

22 Mehrabani D, Khajehahmadi Z, Tajik P, et al. Regenerative Effect of Bone Marrow-derived Mesenchymal Stem Cells in Thioacetamide-induced Liver Fibrosis of Rats. *Arch Razi Inst.* 2019;74:279-286. DOI:10.22092/ari.2018.110029.1120. PMID:31592593.

23 Oyagi S, Hirose M, Kojima M, et al. Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCl4-injured rats. *J Hepatol.* 2006;44:742-8. DOI:10.1016/j.jhep.2005.10.026. PMID:16469408..

24 Luo L, Lai C, Feng T, et al. Umbilical cord blood-derived mesenchymal stem cells transplantation decreases incidence of liver cancer in end-stage liver disease patients: a retrospective analysis over 5 years. *Am J Transl Res.* 2022;14:5848-5858. PMID:36105061.

25 Chen L, Zhang C, Chen L, et al. Human menstrual blood-derived stem cells ameliorate liver fibrosis in mice by targeting hepatic stellate cells via paracrine mediators. *Stem Cells Transl Med.* 2017;6:272–84. DOI:10.5966/sctm.2015-0265.

26 Hirata M, Ishigami M, Matsushita Y, et al. Multifaceted therapeutic benefits of factors derived from dental pulp stem cells for mouse liver fibrosis. *Stem Cells Transl Med.* 2016;5:1416–24. DOI:10.5966/sctm.2015-0353.

27 Lee J, Choi J, Kang S, et al. Hepatogenic Potential and Liver Regeneration Effect of Human Liver-derived Mesenchymal-Like Stem Cells. *Cells.* 2020;9:1521. DOI:10.3390/cells9061521. PMID:32580448.

28 Nomura M, George J, Hashizume C, et al. Surgical implantation of human adipose derived stem cells attenuates experimentally induced hepatic fibrosis in rats. *Mol Med.* 2022;28:143. DOI:10.1186/s10020-022-00566-6. PMID:36447136.

29 Nevens F, van der Merwe S. Mesenchymal Stem Cell Transplantation in Liver Diseases. *Semin Liver Dis.* 2022;42:283-292. DOI:10.1055/s-0042-1755328. PMID:36049782.

30 Li L, Si Y, Cheng M, et al. Therapeutic effect of autologous bone marrow cells injected into the liver under the guidance of B-ultrasound in the treatment of HBV-related decompensated liver cirrhosis. *Exp Ther Med.* 2022;24:633. DOI:10.3892/etm.2022.11570. PMID:36160909.

31 Gharbia S, Nazarie SR, Dinescu S, et al. Adipose-Derived Stem Cells (ADSCs) Supplemented with Hepatocyte Growth Factor (HGF) Attenuate Hepatic Stellate Cell Activation and Liver Fibrosis by Inhibiting the TGF-β/Smad Signaling Pathway in Chemical-Induced Liver Fibrosis Associated with Diabetes. *Cells.* 2022;11:3338. DOI:10.3390/cells11213338. PMID:36359733.

32 Feng Y, Li Y, Xu M, et al. Bone marrow mesenchymal stem cells inhibit hepatic fibrosis via the AABR07028795.2/rno-miR-667-5p axis. *Stem Cell Res Ther.* 2022;13:375. DOI:10.1186/s13287-022-03069-7. PMID:35902883.

33 Sabry D, Mohamed A, Monir M, et al. The Effect of Mesenchymal Stem Cells Derived Microvesicles on the Treatment of Experimental CCL4 Induced Liver Fibrosis in Rats. *Int J Stem Cells.* 2019;12:400-409. DOI:10.15283/ijsc18143. PMID:31474025.

34 Zhao L, Chen S, Shi X, et al. A pooled analysis of mesenchymal stem cell-based therapy for liver disease. *Stem Cell Res Ther.* 2018;9:72. doi:10.1186/s13287-018-0816-2.

35 Suk KT, Yoon JH, Kim MY, et al. Transplantation with autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: phase 2 trial. *Hepatology.* 2016;64:2185-2197. DOI:10.1002/hep.28693.

36 Abdelbaset-Ismail A, Tharwat A, Ahmed AE, et al. Transplantation of adipose-derived mesenchymal stem cells ameliorates acute hepatic injury caused by nonsteroidal anti-inflammatory drug diclofenac sodium in female rats. *Biomed Pharmacother.* 2022;155:113805. DOI:10.1016/j.biopha.2022.113805. PMID:36271578.