ORIGINAL ARTICLE

Therapeutic Potential of Human Bone Marrow Derived Mesenchymal Stromal Cells in Combination with Silymarin against Carbon Tetrachloride Induced Liver Cirrhosis in Wistar Rats

Ashwini P. Aithal[1], Laxminarayana K. Bairy[2], Raviraja N. Seetharam[3], Naveen Kumar[1]*

[1]Department of Anatomy, Melaka Manipal Medical College (Manipal Campus), Manipal University, Manipal-576104 (Karnataka) India, [2]Department of Pharmacology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE, [3]Stempeutics Research Pvt. Ltd., Manipal-576104 (Karnataka), India

Abstract

Background: Self-renewal, active proliferation *in vitro*, abundant sources for isolation, and a high differentiation capacity, make mesenchymal stem cells to be potentially therapeutic for liver cirrhosis. *Aim and Objectives:* To evaluate the therapeutic potential of the combination of human Bone Marrow derived Mesenchymal Stromal Cells (BM-MSC) and silymarin in Carbon tetrachloride (CCl_4) induced liver cirrhosis in Wistar rats. *Material and Methods:* This was an experimental study. Liver cirrhosis was induced in adult female Wistar rats using CCl_4. Sixty rats were randomly divided into 6 groups: Group 1 (Normal Control Group), Group 2 (received only CCl_4), Group 3 (CCl_4+low dose BM-MSCs), Group 4 (CCl_4+high dose BM-MSCs), Group 5 (CCl_4+ silymarin), Group 6 (CCl_4+BM-MSCs+silymarin). On day 0 and at the end of 6^{th}, 12^{th}, 18^{th}, 24^{th} and 30^{th} day after treatment, blood samples were collected for liver enzyme estimations. After 30 days of treatment, the rats were sacrificed; livers were excised and used for antioxidant analysis and histopathological studies. *Results:* Liver enzyme analysis and histopathological studies indicated that combination of BM-MSCs and silymarin was effective in treating liver cirrhosis. Furthermore, oxidative stress was attenuated in the group which received combination treatment of BM-MSCs and silymarin. *Conclusion:* Evidence of this study showed that combination treatment of BM-MSCs and silymarin was beneficial and support the potential of using MSCs transplantation as an effective treatment modality for liver cirrhosis.

Keywords: Liver Cirrhosis, Mesenchymal Stromal Cells, Transplantation, Carbon tetrachloride, Silymarin

Introduction:

Liver cirrhosis is a condition in which the liver slowly deteriorates and malfunctions due to chronic injury. Excessive accumulation of extracellular matrix characterizes it, with the formation of scar tissue encapsulating the area of damage. It is considered as one of the leading causes of morbidity and mortality worldwide. The prognosis of patients with the disease is poor. Although liver transplantation is regarded as a safe alternative treatment, it is expensive and there are limited available donor livers for hundreds of millions of patients worldwide [1, 2]. So, it is imperative to investigate appropriate therapies for the disease by different treatments.

Mesenchymal Stromal Cells (MSCs) are multipotent adult cells that were first isolated and characterized from bone marrow. MSCs are considered to be involved in many key events related to hematopoiesis, immune cell generation

and activation, immunomodulation, and immune tolerance [3]. Studies have established that Bone Marrow derived MSCs (BM-MSCs) could engraft injured tissues such as lung, liver, heart, brain, and recover its function [4] as it possesses certain characteristics like self-renewal, multipotency, proliferation, secretion of paracrine factors, and hence considered as valuable source for transplantation. Adult hematopoietic and non-hematopoietic stem cells have been shown to differentiate into hepatocyte-like cells [5].

From the view of clinical practice, it is essential to select a suitable model of liver cirrhosis close to human disease for evaluating the therapeutic effect of MSCs. Carbon tetrachloride (CCl_4) is one of the oldest and most widely used toxins for experimental induction of liver cirrhosis in laboratory animals [6].

The goal of cell-based treatments is to use an approach which aims to replace, repair, or enhance the function of a cell type, tissue or organ. There are many controversies on the primary mechanisms through which cell-based therapy affects liver tissue repair. Silymarin is a standard drug used in the treatment of various ailments especially liver and gall bladder problems [7] and has hepatoprotective and anti-hepatotoxic effect. The possibility of using human BM-MSCs in combination with silymarin to repair liver damage has not yet been evaluated. We hypothesized that a combination treatment of BM-MSCs and silymarin would augment the antifibrotic effects when compared to single administration of BM-MSCs. Hence, the main objective of this study is to evaluate the therapeutic potential of BM-MSCs transplantation in combination with silymarin on liver enzyme levels, enzymatic and non-enzymatic antioxidant biochemical parameters and liver cellular architecture against CCl_4 induced liver cirrhosis in Wistar rats.

Material and Methods:

Drugs and Reagents

Silymarin was procured from Sigma Chemical Inc. (USA), Thiobarbituric acid (TBA), Trichloroacetic Acid (TCA), 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) and Reduced Glutathione (GSH) were procured from Hi-Media Laboratories, India. CCl_4 and other chemicals were obtained from Merck Chemicals, Mumbai (India). All reagents were of analytical grade and stored in a refrigerator at +4˚C. The reagents were equilibrated at room temperature for 30 minutes before the start of analysis.

Experimental Animals:

Female Wistar albino rats (4–5 months old), weighing 140-160 g was selected for this study. Animals were bred locally in the central animal house of Manipal University, Manipal, Karnataka, India. Ratswere housed in separate polypropylene cages, maintained under standard conditions with temperature (22–24^{0}C), 12-h light/12-h dark cycle and relative air humidity 40-60%. Rats had continuous access to regular calorie standard rat pellet diet and tap water. The rats were acclimatized to the laboratory conditions for one week before the start of the experiment. The experiment was done after approval from the Institutional Animal Ethics Committee (IAEC/KMC/20/2014) and conducted according to the ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Isolation and culture of BM-MSCs:

Bone marrow aspirates were obtained from healthy adult volunteers after signed informed consent and under institutional ethical guidelines. BM-MSCs were isolated and expanded using a method previously reported by Pal *et al*. [8]. Freshly-thawed BM-MSCs suspended in Plasmalyte A was used for injecting into the rats.

Experimental Design:

This was an experimental study in which 60 rats were used. The rats were randomly divided into six groups containing ten rats in each group.

CCl_4 Induced Liver Cirrhosis Model:

Rats were injected with CCL_4 (1ml/kg of body weight) thrice weekly for 28 days intraperitoneally (i. p).

Experimental Groups:

Group 1: (Normal control group) ten rats which did not receive CCl_4. Fifty rats were injected CCl_4 to induce liver cirrhosis as scheduled above and then divided into following groups;

Group 2: (CCl_4 trt group) which served as disease model and was sacrificed immediately at the end of 28 days.

Group 3: (CCl_4 + low dose BM-MSCs group) injected with single dose of 3.25 million human equivalent dose of BM-MSCs/kg body weight (b wt), intravenously.

Group 4: (CCl_4 + high dose BM-MSCs group) injected with single dose of 9.75 million human equivalent dose of BM-MSCs/kg b wt, intravenously.

Group 5: (CCl_4 + silymarin group) received silymarin at the dose of 100 ml/kg b wt, orally.

Group 6: (CCl_4 + silymarin + BM-MSCs group), received silymarin (100 ml/kg b wt) and BM-MSCs (9.75 million MSCs/kg b wt). The cell number used in this study was chosen based on dose optimization performed in previous studies. Both the doses of MSCs were formulated in 0.5 ml Plasmalyte A and injected intravenously into the tail vein of the rat after disease model confirmation. Cells were administered slowly and carefully to avoid embolism. The total study duration was sixty days.

Liver Enzyme Analysis:

On day 0 (baseline) and at the end of 6^{th}, 12^{th}, 18^{th}, 24^{th} and 30^{th} day after treatments, the animals were anesthetized with ketamine (80 mg/kg; i.p.) following a 12 h fast. Blood samples were collected in small centrifuge tubes by orbital puncture. The blood was allowed to clot and then centrifuged for 10 minutes at 3000 rpm. Serum was separated and used immediately for liver enzyme estimations. Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), direct bilirubin, albumin and total protein levels in serum were estimated using commercially available kits obtained from Agappe Diagnostics Ltd.

Antioxidant Analysis:

After thirty days of treatment, the rats were kept on overnight fasting and sacrificed by administering an overdose of ketamine. Livers were excised immediately and washed with ice-cold saline to remove as much blood as possible. Liver homogenates (10% w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10000 rpm for 30 minutes using a Remi refrigerated centrifuge. The resulting supernatant was used for estimation of malondialdehyde level, reduced glutathione (according to the method described by Satyam *et al*.)[9] and superoxide

dismutase activity. All these biochemical antioxidant parameters were estimated in triplicate manner, and optical density was read for reagent and a sample blank.

Determination of Malondialdehyde (MDA) Level

To 20 µl liver homogenate sample, 200 µl 0.67% TBA and 100 µl 20% TCA was added and incubated at 100˚C for 20 minutes. Then, it was centrifuged at 12000 rpm for 5 minutes, and 100 µl of supernatant was transferred to 96-wells of micro test plate. The optical density of supernatant was read at 540 nm by using iMark Microplate ELISA reader, Bio Rad laboratories.

Determination of Superoxide Dismutase (SOD) Activity

SOD activity was measured by using EpiQuik Superoxide dismutase activity assay kit, obtained from Epigentex Inc. NY. The test was performed according to the instructions provided in the kit, and optical density was read at 470 nm by using iMark Microplate ELISA reader, BioRad laboratories.

Determination of Reduced Glutathione (GSH) Level

A mixture of 100 µl of liver tissue homogenate and 100 µl of 5% TCA solution was centrifuged at 5000 rpm for 5 minutes. Then, 25 µl of tissue supernatant, 150 µl sodium phosphate buffer (PBS 0.2 M, pH 8.0) and 25 µl DTNB (0.6mM) was added together in 96-wells of micro test plate and incubated for 10 minutes at room temperature, and absorbance was read at 412 nm by using iMark Microplate ELISA reader, Bio Rad laboratories.

Histopathological Examination

The liver tissue samples taken from the rats of each group were fixed in 10% formalin, cut and dehydrated in ascending grades of alcohol, defatted in xylene, and embedded in paraffin 24 hours after block preparation, paraffin sections were obtained on clean glass slides using microtome. The sections were stained with Hematoxylin and Eosin (H&E) stain and observed for histopathological changes under a light microscope. Relevant microphotographs were taken.

Statistical Analysis

Using Statistical Package for the Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, USA), normally distributed data were expressed as mean ± standard deviation and analyzed by repeated measures ANOVA, one-way analysis of variance (ANOVA), followed by post hoc Tukey's test. A level of $p < 0.05$ was considered to be statistically significant.

Results:

Effectof BM-MSCs on Liver Enzyme Levels:

Repeated measures ANOVA showed that there was a significant difference in the liver enzyme levels of all the groups at different points of time i.e. at day 0, 6^{th}, 12^{th}, 18^{th}, 24^{th} and 30^{th} day ($p<0.001$). In CCl_4 intoxicated rats, serum levels of AST, ALT, ALP, direct bilirubin was increased significantly ($p<0.001$), albumin and total protein levels in the serum decreased significantly when compared to the normal control group ($p<0.001$) indicating the injury caused by CCl_4 intoxication. It was observed that the treatment with two doses of BM-MSCs, silymarin and silymarin+BM-MSCs combination was effective when compared to the CCl_4 treated group ($p<0.001$). It was also observed that the combination of silymarin and BM-MSCs treatment significantly altered the liver enzyme levels when compared to other treatments ($p<0.01$) (Fig. 1, 2, 3). These results showed that BM-MSC transplantation could facilitate liver function recovery.

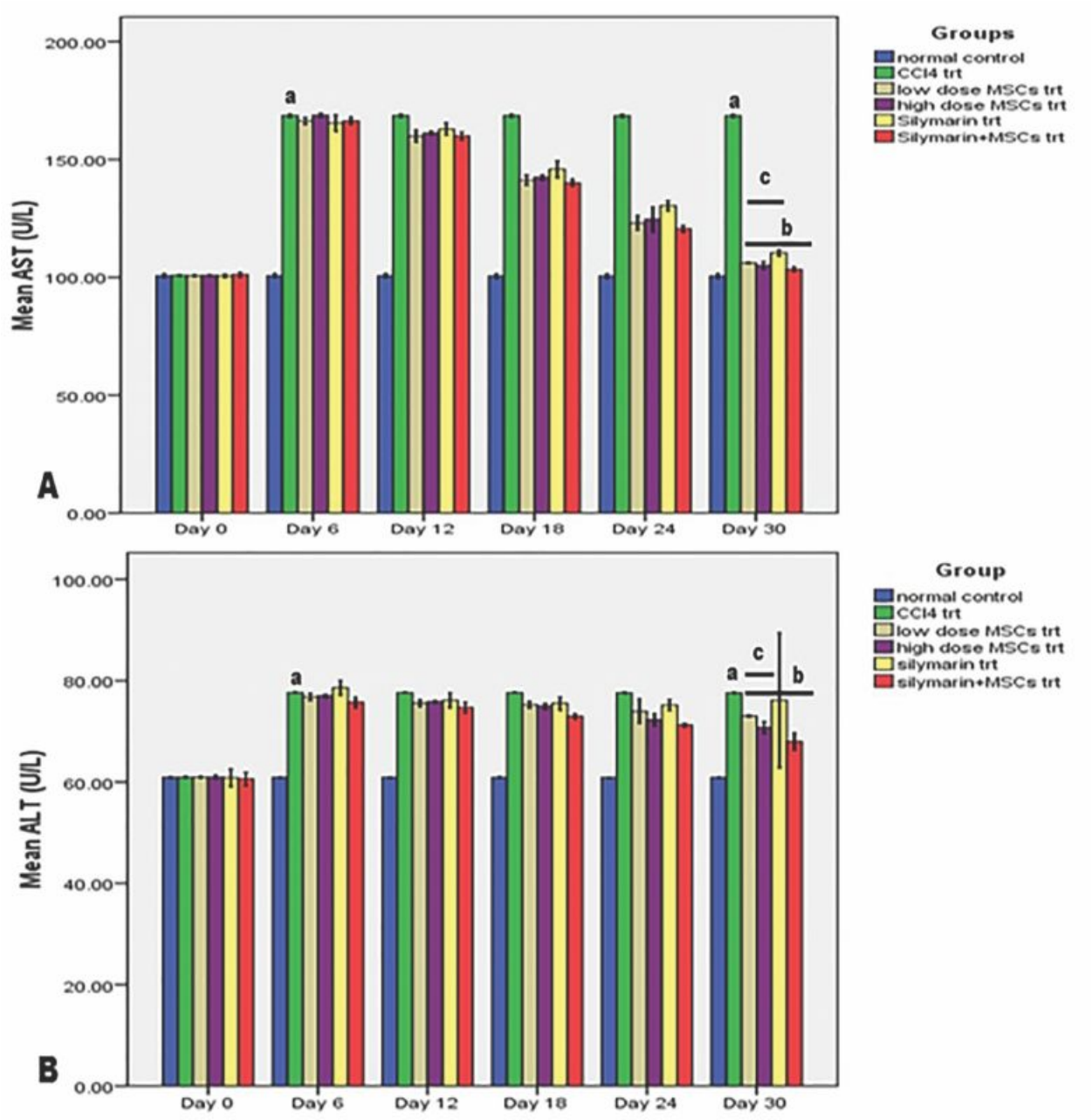

Fig. 1: Effects of BM-MSCs on the Serum Concentration of AST and ALT in CCl_4-induced Liver Cirrhosis in Rats.

Data were expressed as mean ± Standard Deviation (SD). AST: Aspartate transaminase, ALT: Alanine transaminase. Analysis between groups was done using repeated measures ANOVA followed by Tukey's post hoc test. [a] *normal control v/s CCl_4trt ($p<0.001$);* [b] *CCl_4 v/s treatments ($p<0.001$),* [c] *BM-MSC+ silymarin v/s other treatments ($p<0.01$).*

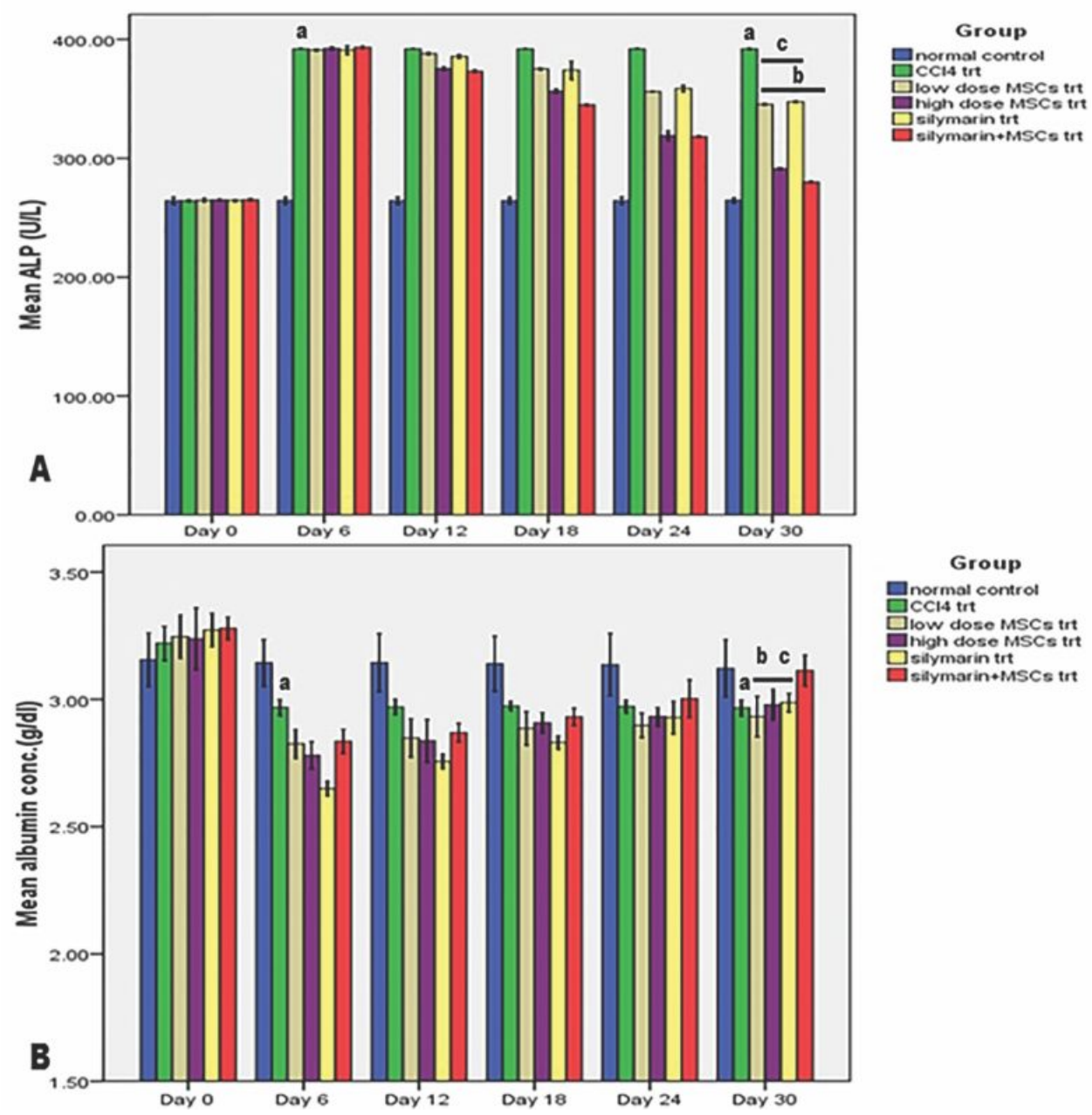

Fig. 2: Effects of BM-MSCs on the Serum Concentration of ALP and Albumin in CCl_4-induced Liver Cirrhosis in Rats.

Data were expressed as mean ± Standard Deviation (SD). ALP: Alkaline phosphatase. Analysis between groups was done using repeated measures ANOVA followed by Tukey's post hoc test. [a] normal control v/s CCl_4trt ($p<0.001$); [b] CCl_4 v/s treatments ($p<0.001$), [c] BM-MSC+ silymarin v/s other treatments ($p<0.01$).

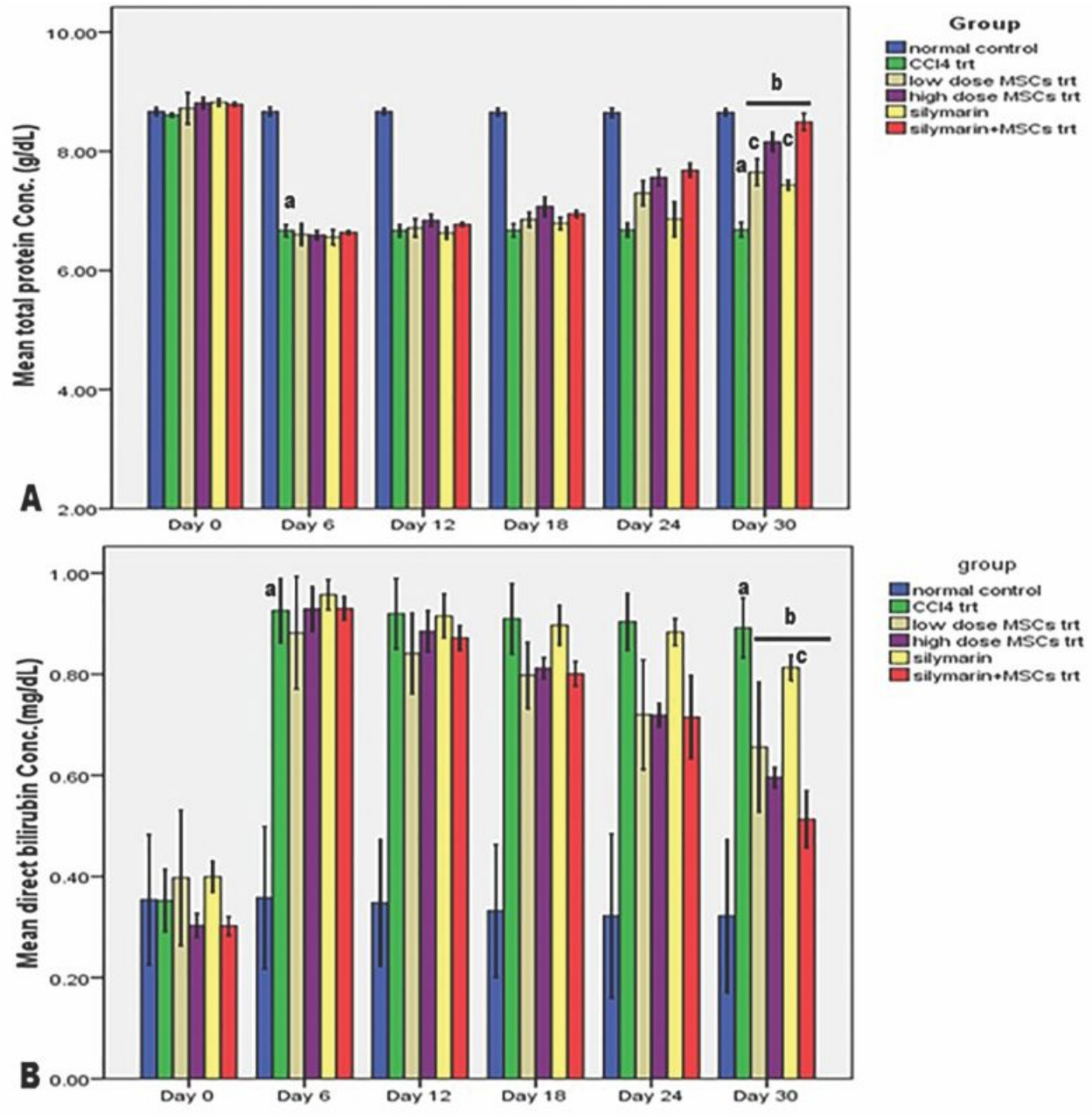

Fig. 3: Effects of BM-MSCs on the Serum Concentration of Total Protein and Direct Bilirubin in CCl_4-induced Liver Cirrhosis in Rats.

Data were expressed as mean ± Standard Deviation (SD). Analysis between groups was done using repeated measures ANOVA followed by Tukey's post hoc test. [a] normal control v/s CCl_4trt ($p<0.001$); [b] CCl_4 v/s treatments ($p<0.001$), [c] BM-MSC+ silymarin v/s other treatments ($p<0.01$).

Effect of BM-MSCs on Biochemical Antioxidant Parameters:

Antioxidant analysis revealed that there was a significant increase in liver tissue MDA levels, a significant decrease in reduced glutathione levels in CCl_4 treated group when compared with the normal control group ($p<0.001$) (Table 1).

A significant decrease in superoxide dismutase enzyme activity was seen in CCl_4 treated group when compared with the normal control group ($p<0.001$) (Table 2).

Treatment with two doses of BM-MSCs, silymarin and combination of BM-MSCs and silymarin significantly reversed CCl_4 induced alterations in the measured antioxidant parameters ($p<0.001$). The combination of BM-MSCs and silymarin treatment showed a better reduction in liver peroxidation levels compared to other treatments, which was not statistically significant in GSH (except silymarin and low dose BM-MSCs trt) and MDA (except low dose BM-MSCs trt) but, statistically significant difference was observed in superoxide dismutase enzyme levels ($p<0.001$) (Table 2).

Histopathological Study:

Histopathological examination of liver sections of the normal control group showed normal cellular architecture with distinct hepatic cells radiating from central vein, prominent and narrow sinusoidal spaces. Disarrangement of normal hepatic cells with centrilobular necrosis, fatty degeneration, vacuolization, and homogeneous cytoplasm of hepatocytes with the absence of few nuclei was observed in CCl_4 intoxicated animals. The liver sections of the rats treated with two doses of BM-MSCs and silymarin followed by CCl_4 intoxication showed a sign of protection as it was evident by the absence of necrosis and

Table 1: Effect of BM-MSCs on Reduced Glutathione (GSH), and Malondialdehyde levels (MDA).

Groups (n=10)	GSH µmoles/mg	MDA nmoles/mg
Normal Control	2.43±0.05	0.34±0.03
CCl_4trt	0.26±0.06^{x}	0.66±0.04^{x}
Low dose BM-MSCs trt	1.21±0.04$^{x\,a}$	0.45±0.07$^{xx\,a}$
High dose BM-MSCs trt	2.07±0.33^{xx}	0.39±0.04^{xx}
Silymarin trt	1.53±0.94$^{xx\,a}$	0.51±0.21$^{xx\,a}$
BM-MSCs + Silymarin trt	2.21±0.49^{xx}	0.36±0.18^{xx}

Values expressed as mean± standard deviation. Post hoc tukeys's test indicated significant difference between: Normal control v/s CCl_4trt,^{x} $p< 0.001$; CCl_4trt v/s treatment, ^{xx} $p< 0.001$; BM-MSCs+ Silymarin trt v/s other treatments, ^{a} $p<0.001$.

Table 2: Effect of BM-MSCs on Superoxide Dismutase (SOD) Enzyme Activity

Groups (n=10)	SOD Unit/min/ml
Normal Control	0.55±0.37
CCl_4trt	0.39±0.02^{x}
Low dose BM-MSCs trt	0.77±0.03$^{xx\,a}$
High dose BM-MSCs trt	0.65±0.02$^{xx\,a}$
Silymarin trt	0.72±0.03$^{xx\,a}$
BM-MSCs +Silymarin trt	0.53±0.27^{xx}

Values expressed as mean± standard deviation. Post hoc tukeys's test indicated significant difference between: Normal control v/s CCl4 trt, ^{xx} $p< 0.001$; CCl4 trt v/s treatments, ^{xx} $p< 0.001$; BM-MSCs + Silymarin trt v/s other treatments, ^{a} $p<0.001$.

vacuoles. However, a moderate amount of fibrous connective tissue, wider sinusoidal spaces were still present. The combination of BM-MSCs and silymarin treatment showed very uniform and normal hepatocytes with a prominent nucleus, reduced sinusoidal spaces, comparable to sections of normal control rats (Fig. 4).

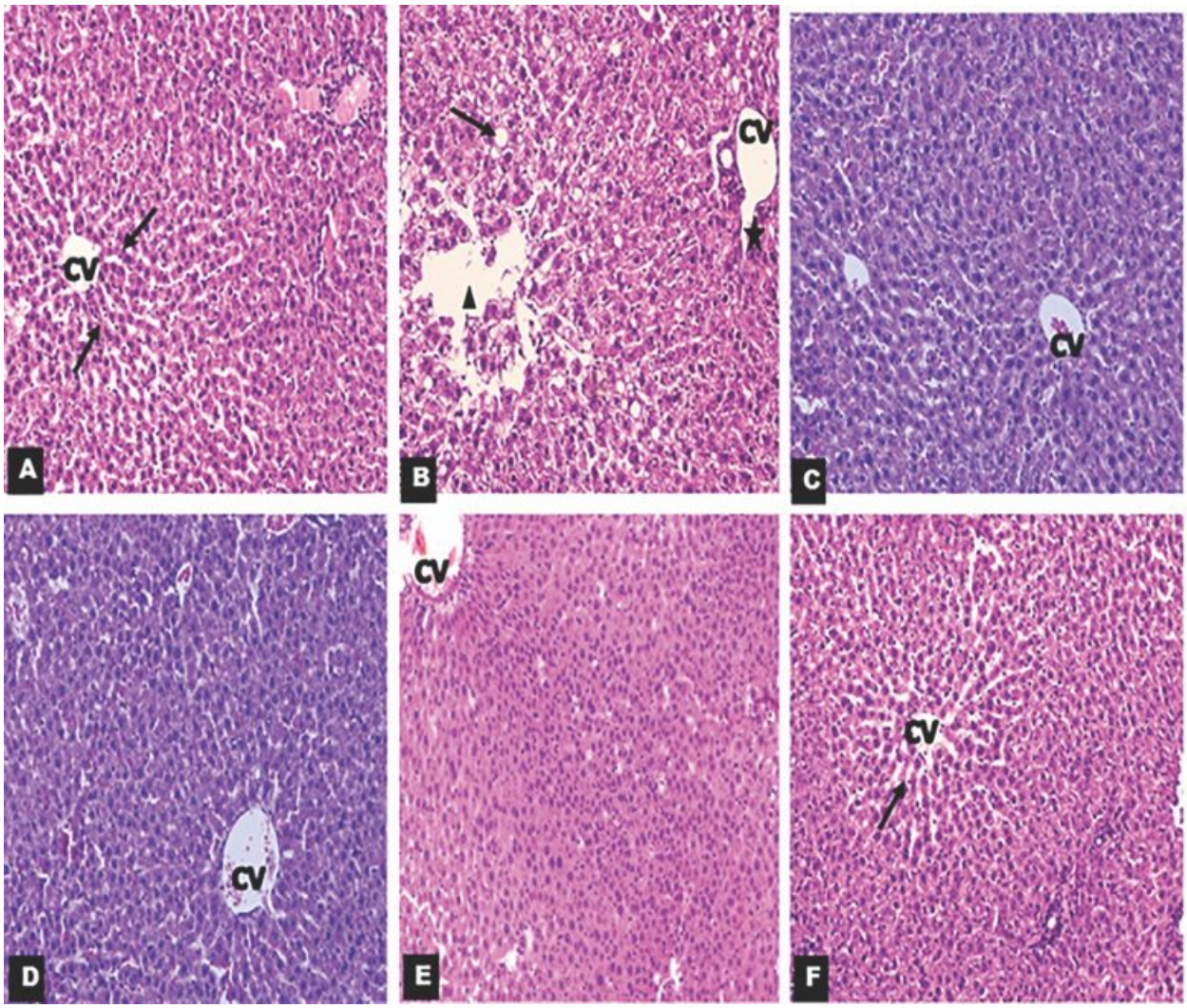

Fig. 4: Photomicrographs of H&EStained Liver Sections (10x); (A) Normal Control: Showing Radiating Cords of Hepatocytes from the Central Vein (CV). The Hepatocytes have Central, Rounded, Vesicular Nuclei (↑) and Acidophilic Cytoplasm; (B) CCl_4 Treated (Disease Model): Most of the Hepatocytes are Vacuolated (↑) with Disarrangement of Hepatocytes (▲) and Necrosis (★); (C) Low Dose BM-MSCs Treated; (D) High Dose BM-MSCs Treated; (E) Silymarin Treated; (F) BM-MSCs+ Silymarin Treated Group: showing Well Defined Central Vein and Normal Hepatocytes with Prominent Nucleus (↑).

Discussion:

The purpose of this study was to explore the hepatoprotective effect of human BM-MSCs in hepatic damage caused by CCl_4. CCl_4-induced liver cirrhosis is a classical experimental model. CCl_4 usually produces free radicals that activate a cascade of reactions leading to the development of liver cirrhosis. CCl_4 is converted to free radicals by cytochrome P450 which exerts its effects through lipid peroxidation and leads to subsequent tissue damage [10, 11]. In the present study, recovery of liver cirrhosis in CCl_4 treated rats was not observed within thirty days of withdrawal of CCl_4, which indicates that the disease model was stable. Administration of CCl_4 to normal rats increased the serum levels of AST, ALT, ALP, direct bilirubin and decreased the albumin, total protein concentration. Although serum enzyme levels are not a direct measure of hepatic injury, they show the status of the liver. The enzymes releasing out from damaged liver cells into circulating blood represent the damage caused to hepatic cells. AST, ALT and ALP serum levels in animals treated with a combination of BM-MSCs and silymarin, improved more efficiently than low dose, high dose BM-MSCs and silymarin treated groups. Similarly, liver function markers (direct bilirubin, albumin, total protein) levels changed positively in BM-MSCs and silymarin combination treated groups. This is a definite indication of its hepatoprotective action which proved that the combination treatment was more effective than BM-MSCs and silymarin treatments alone. Comparable studies in the past have shown that MSCs facilitate recovery from chemically induced liver damage and help in decreasing liver cirrhosis in rat model [12-14]. Studies have demonstrated that transplantation of single dose of MSCs through intravenous injection is effective enough to protect the liver against fibrosis and has been shown to give best results [15]. Hence in this study also a single infusion of BM-MSCs was given intravenously.

MSCs secrete numerous factors which enhance antioxidant defenses, inhibit oxidation factors, and reduce necrosis of hepatocytes [16]. Glutathione, which is present in all tissues, especially the liver, provides the reduction capacity for most reactions and plays a substantial role in the detoxification of hydrogen peroxide, other peroxides and free radicals [17]. It has been reported that when the intensity of stress increases, GSH concentrations usually decline and redox state becomes more oxidized, leading to deterioration of the system[18]. In the present study, the hepatic content of GSH was found to be decreased significantly in the CCl_4 intoxicated rats compared to the control group which indicated lipid peroxidation. Hepatic GSH content was significantly increased in the treated groups. But the combination of BM-MSCs and silymarin significantly restored the level of GSH compared to other treatments.

SOD is one of the main cellular defense enzymes that dismutase superoxide radical to H_2O_2 and oxygen [19]. The reduction in the activities of SOD observed in this study succeeding the administration of CCl_4, suggests oxidative stress in the toxic control group. The increase in SOD activities found in combination treatment of BM-MSCs and silymarin represent a cytoprotective response in the injured liver.

MDA which is a secondary product of lipid peroxidation, is used as an indicator of tissue damage [20]. Elevated levels of MDA resulting

after CCl_4 administration have been well documented in various organs mainly in the liver [21, 22]. Increased levels of MDA in the present study in CCl_4 treated rats indicated higher O_2 free radical production. Treatment with the combination of BM-MSCs and silymarin significantly reduced the MDA levels. MSCs would have prevented the formation of free radicals by interfering with cytochrome P-450. Reduced MDA levels might be attributed to immune modulation of MSCs which inhibits inflammatory cell proliferation, thereby exerting anti-inflammatory effects [23, 24]. We consider that these essential functions of BM-MSCs are responsible for the liver injury recovery after transplantation. These results suggest the synergistic action of BM-MSCs with silymarin in preventing oxidative stress and their potential role in strengthening antioxidant defense mechanism.

The histopathological appearance of rat liver treated with CCl_4 showed disarrangement of hepatocytes, homogenous hepatic cytoplasm with the absence of few nuclei, necrosis and fatty changes. Liver tissue of CCl_4 intoxicated rats treated with a combination of BM-MSCs and silymarin exhibited a good progress with the disappearance of fatty changes and necrosis. Findings from the present study demonstrate the efficacy of BM-MSCs and its synergism with silymarin to express significant hepatoprotective effect against liver cirrhosis induced by CCl_4.

Conclusion:

The combination of BM-MSCs and silymarin treatment showed significant hepatoprotective activity confirmed by estimating the liver enzymes and antioxidant analysis. Further, the results were supported by histopathological studies indicating the therapeutic effect of BM-MSCs. Although MSCs are considered a potentially relevant therapeutic tool for the treatment of liver diseases, further studies *in vitro* as well *in vivo* are needed to achieve a better understanding of the benefical effects of MSCs as a therapeutic agent.

References

1. Iredale JP. Cirrhosis: new research provides a basis for rational and targeted treatments. *BMJ* 2003; 327: 143-47.
2. Lee DS, Gil WH, Lee HH, Lee KW, Lee SK, Kim SJ, *et al*. Factors affecting graft survival after living donor liver transplantation. *Transplant Proc* 2004; 36: 2255-56.
3. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. *Cell* 1997; 88(3):287-98.
4. Wen GM, Li HW, Xiao QZ, Cheng ZG, Zhang XM, Li Y, *et al*. Studies on differentiation potential of human bone marrow mesenchymal stem cells into hematopoietic cells in vivo. *Zhongguo Bingli Shengli Zazhi* 2003; 19: 157-62.
5. Aurich I, Mueller LP, Aurich H, Luetzkendorf J, Tisljar K, Dollinger MM, *et al*. Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. *Gut* 2007; 56:405-15.
6. Tsukamoto H, Matsuoka M, French SW. Experimental models of hepatic fibrosis: a review. *Seminar in Liver Disease* 1990;10:56-65.
7. Ahmed AF, Mahmoud MF, Ouf MA, El-Fathaah EA. Aminoguanidine potentiates the hepatoprotective effect of silymarin in CCl4 treated rats. *Ann Hepatol* 2011; 10(2):207-15.
8. Pal R, Hanwate M, Jan M, Totey S. Phenotypic and functional comparison of optimum culture conditions for upscaling of bone marrow-derived mesenchymal stem cells. *J Tissue Eng Regen Med* 2009; 3:163-74.
9. Satyam SM, Bairy KL, Pirasanthan R, Vaishnav R. Grape seed extract and zinc containing nutritional food supplement decreases the oxidative stress induced by carbon tetrachloride in rats. *Int J Pharm Pharm Sci* 2013; 5(Suppl 4): 626-31.

10. Janakat S, Al-Merie H. Optimization of the dose and route of injection, and characterisation of the time course of carbon tetrachloride-induced hepatotoxicity in the rat. *J Pharmacol Toxicol Methods* 2002; 48:41-4.
11. Bruckner JV, Ramanathan R, Lee KM, Muralidhara S. Mechanisms of circadian rhythmicity of carbon tetrachloride hepatotoxicity. *J Pharmacol Exp Ther* 2002; 300:273-81.
12. Zhao DC, Lei JX, Chen R, Yu WH, Zhang XM, Li SN, *et al*. Bone marrow-derived mesenchymal stem cells protect against experimental liver fibrosis in rats. *World J Gastroenterol* 2005; 11(22):3431-40.
13. Abdel Aziz MT, Atta HM, Mahfouz S, Fouad HH, Roshdy NK, Ahmed HH, *et al*. Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis. *Clin Biochem* 2007; 40(12):893-9.
14. Tsai PC, Fu TW, Chen YM, Ko TL, Chen TH, Shih YH, *et al*. The therapeutic potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis. *Liver Transpl* 2009; 15(5):484-95.
15. Zhao W, Li J-J, Cao D-Y, Li X, Zhang L-Y, He Y, *et al*. Intravenous injection of mesenchymal stem cells is effective in treating liver fibrosis. *WJG* 2012; 18(10):1048-58.
16. Pulavendran S, Vignesh J, Rose C. Differential anti-inflammatory and anti-fibrotic activity of transplanted mesenchymal vs. hematopoietic stem cells in carbon tetrachloride induced liver injury in mice. *Int Immunopharmacol* 2010; 10(4):513-19.
17. Meister A, Larsson A. Glutathione synthetase deficiency and other disorders of the g-glutamyl cycle. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic bases of inherited disease, 6th ed. New York: McGraw-Hill, 1989:855-868.
18. Tausz T, Sircelj H, Grill D. The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid? *J Exp Bot* 2004; 55:1955-62.
19. Jimoh FO, Babalola SA, Yakubu MT. Assessment of the antioxidant potential of Cnidoscolouschayamansa. *Pharm Biol* 2009; 47:903-909.
20. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 1979; 95:351-358.
21. Lee KJ, Jeong HG. Protective effect of *Platycodix radix* on carbon tetrachloride-induced hepatotoxicity. *Food Chem Toxicol* 2002; 40:517-25.
22. Shahjahan M, Sabitha KE, Jainu M, Shyamala Devi CS. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. *Ind J Med Res* 2004; 120:194-198.
23. PuglisiMA, TesoriV, LattanziW, Piscaglia AC, Gasbarrini GB, D'Ugo DM, *et al*. Therapeutic implications of mesenchymal stem cells in liver injury. *J Biomed Biotech* 2011; 2011, Article ID 860578, 8 pages.
24. VolarevicV, NurkovicJ, ArsenijevicN,StojkovicM. Concise review: therapeutic potential of mesenchymal stem cells for the treatment of acute liver failure and cirrhosis. *Stem Cells* 2014; 32(11): 2818-23.

[*] ***Author for Correspondence:*** *Dr. Laxminarayana K. Bairy, Department of Pharmacology, RAK College of Medical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE. Email: klbairy@gmail.com.*