Therapeutic Effects of Bone Marrow-Derived Mesenchymal Stem Cells Against Isoniazid and Rifampicin-Induced Hepatorenal Toxicity in Rats

Short title: Stem cells against hepatorenal toxicity

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Abstract: Tuberculosis is a dangerous disease and its death toll is increasing aggressively. Isoniazid and rifampicin lead to fatal hepatorenal toxicity. The emerging field of stem cell therapy has raised great hope for improving the treatment of liver diseases. Toxicity was induced by gastric intubation of isoniazid and rifampicin (200 mg/kg for 30 days). Rat bone marrow mesenchymal stem cells (MSCs) were injected intraperitoneally into rats received isoniazid and rifampicin at the 30th day. Results revealed that Serum liver enzymes and lipid profile, liver and kidney thiobarbituric acid reactive substance, nitric oxide levels and DNA fragmentation, exhibited by COMET assay, were significantly elevated by isoniazid and rifampicin associated with reduction of liver and kidney glutathione levels and antioxidant enzymes activities, catalase and superoxide dismutase. MSCs exert its therapeutic activity through hepatocytes regeneration leading to inhibiting the production of free radicals, induction of antioxidant enzymes and improving non-enzymatic thiol antioxidant levels. Based on this study, it might be concluded that MSCs possess hepatorenal protective behavior and may be hopeful therapeutic regimen against drug-induced toxicity.
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Key words: isoniazid; rifampicin; mesenchymal stem cells; antioxidant enzymes; DNA damage.

Introduction

Mycobacterium Tuberculosis (TB) infection is one of the fatal communication diseases and spread easily amongst people. Out of 1.86 billion people (about 32% of people worldwide) estimated to be infected with TB; an estimated 1.3 billion infected people were living in developing countries **(Saad *et al*., 2010; Santhosh *et al*., 2007)**. The preferred regimen recommended by **WHO, (2010)** for successful treatment of TB involves two phases, 1) an initial (or intensive) phase which is a daily treatment with isoniazid (INH), rifampicin (RIF), pyrazinamide and ethambutol for 2 months, followed by 2) a continuation phase of 4-6 months of INH and RIF. However, this regimen often causes serious adverse drug reactions, which may result in discontinuing the scheduled treatment. Liver and kidney dysfunction possess a major problem for effective completion of the course of anti-TB chemotherapy **(Palanisamy and Manian. 2012; WHO, 2010)**.

Liver, the key organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics. By virtue of its unique vascular and metabolic features, liver is exposed to absorbed drugs and xenobiotics in concentrated form. Drug-metabolizing enzymes detoxify many xenobiotics but bioactivate or increase the toxicity of others. In case of bioactivation, the liver is the first organ exposed to the damaging effects of the newly formed toxic substance **(Mintra *et al*., 1998)**.

Nephropathy is one of the important microvascular complications of anti-TB therapy, especially RIF **(Rekha *et al*., 2005)**. RIF-induced acute renal failure is sometimes encountered in the treatment of TB. RIF often elicits a liver and renal toxicity response. A severe response to RIF typically occurs when the drug is readministered **(Rekha *et al*., 2005)**.

Isoniazid (isonicotinic acid hydrazide), introduced in 1950s, is still the most active agent against TB, and is used for both treatment and prophylaxis **(Girling, 1978)**. INH and RIF combination are widely used for the treatment of TB; since 1968; they kill more than 99% of *tubercule bacilli* within two months of initiation of therapy, leading to reducing the treatment course from 18 months to 6 months **(Saad *et al*., 2010)**.

The most frequent and most serious adverse effect of anti-TB treatments is hepatotoxicity. The incidence of hepatotoxicity during standard multidrug TB treatment has been variably reported as between 2% and 28%. The administration of INH-RIF combination produces many metabolic and morphological aberrations in the liver due to the fact that the liver is the main detoxifying site for these anti-TB drugs **(Saad *et al*., 2010; Santhosh *et al*., 2007)**.

The emerging field of stem cell therapy has raised great hope for improving the treatment of liver diseases, because it has the potential to promote liver repair and regeneration with fewer complications **(Li *et al*., 2013)**. Stem cells are capable of self-renewal and can differentiate into specialized cell types such as

osteoblasts, adipocytes, chondrocytes **(Bruder *et al*., 1997)**, astrocytes, oligodendrocytes, neurons **(Chopp *et al*., 2000)** and hepatocytes **(Abdel Aziz *et al*., 2007; Sato *et al*., 2005)**. Bone marrow (BM) contains at least two types of stem cells, hematopoietic and non-hematopoietic stem cells, usually called mesenchymal stem cells (MSCs). MSCs are of great interest because they are easily isolated from a small aspirate of BM and readily generate single-cell-derived colonies, which can be expanded through as many as 50 population doublings in about 10 weeks **(Abdel Aziz *et al*., 2007)**. MSCs may offer a potential therapy for liver diseases, because of their capacity to differentiate into hepatocytes *in vitro* **(Lee *et al*., 2004)** and *in vivo* **(Abdel Aziz *et al*., 2007; Sato *et al*., 2005)**.

In light of the reported hepatotoxic effects of the anti-TB drugs, INH and RIF, this work was proposed to experimentally study the potential protective effects of MSCs against the hazardous hepatorenal effects of INH-RIF combination. Secondly to assess the interplay between the cellular antioxidant defense mechanism, nitric oxide, lipid profile parameters and INH and RIF-induced toxicity in rats. Moreover, as a result of the regenerative properties of stem cells, the study will shed more light on the possibility to use stem cells as a treatment regimen against drug-induced hepatorenal damage.

Materials and Methods

Animals

The experimental animals used in this study were adult male albino rats, weighing (200 ± 20g). The animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt. Animals were housed in groups of four per plastic cage, allowed to adjust to the new environment for two weeks before starting the experiment for adaptation. The rats were housed at 23±2°C and 55±5% humidity with 12 h light/dark cycle. Animals were fed on normal laboratory chow and given tap water *ad libitum* throughout the experimental work.

Preparation of BM-derived MSC

Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque(Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37 °C in 5% humidified CO_2 for 12–14days as primary culture or upon formation of large colonies. When large colonies developed (80–90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25% trypsin in1 mM EDTA (GIBCO/BRL) for 5 min at 37 °C. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50 cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures **(Abdel Aziz *et al*., 2007)**.

Experimental protocol

Twenty four adult male albino rats, weighing 200±20 grams, were randomized into three groups each containing eight rats:

Group (I): Control: animals receiving a daily intragastric intubation (i.g.) of distilled deionized water for 30 days.

Group (II): Hepatotoxicity **(HT)** group: animals receiving a daily i.g. intubation of INH (200mg/kg body weight) and RIF (200mg/kg body wt.) for 30 days **(Kumar *et al.*, 2010; Santhosh *et al*., 2007)**. Both were dissolved in distilled deionized water.

Group (III): Mesenchymal stem cells group **(HT + MSC)**: animals receiving INH and RIF for 30 days, and rat bone marrow mesenchymal stem cells (BM-MSC) were injected intraperitoneally (in a volume of 0.5ml/rat) containing 1×10^6 cells at the 30^{th} day according to **Abdel Aziz *et al.* (2007)** then left for 15 days.

Determination of serum ALT, AST, albumin, total protein, urea and creatinine

All these parameters were determined in serum colorimetrically using conventional laboratory methods.

Determination of serum cholesterol, triglycerides and total lipids

Serum cholesterol and triglycerides and total lipids were measured using commercial kits following the manufacturer's instructions.

Determination of liver and kidney thiobarbituric acid reactive substance (TBARS)

Lipid peroxides concentration is determined by analyzing the thiobarbituric acid-reactive substances (TBARS) as malondialdehyde (MDA) by the method of **Uchiyama and Mihara, (1978)**.

Determination of liver and kidney nitric oxide (NO), reduced glutathione (GSH) levels, superoxide dismutase (SOD; E.C.1.15.1.1) and catalase (CAT; E.C.1.11.1.6) activities

Total nitric oxide (NO) concentration was assayed in tissue according to the method of **Miranda *et al.* (2001)**, reduced GSH content was determined according to the method of **Beutler *et al.* (1963)** and the assay of SOD was carried out kinetically according to the method of **Marklund and Marklund (1974)**, while, catalase enzyme activity was determined kinetically according to **Beers and Sizer, (1952)**. The decomposition of hydrogen peroxide which catalyzed by catalase can be followed by ultraviolet spectroscopy, due to the absorbance of hydrogen

peroxide in this region at 240 nm **(Beers and Sizer, 1952)**.

Determination of liver and kidney DNA fragmentation (Comet assay)

DNA fragmentation was evaluated quantitatively according to **Singh *et al.*(1988)**, in which 1 gram of crushed samples were transferred to 1 ml phosphate buffered saline (PBS), then stirred for 5 min and filtered. Cell suspension (100 µl) was mixed with 600 µl of low-melting agarose (0.8 % in PBS). 100 µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lyses buffer (0.045 M TBE, PH 8.4, containing 2.5 % SDS) for 15 min. the slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. Staining with ethidium bromide 20 µg/ml at 4^{o}C. The observation was with the samples still humid, the DNA fragment migration patterns of 100 cells for each dose level were evaluated with a fluorescence microscope (with excitation filter 420 nm-490 nm). The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40 x increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr- stained DNA using a 40 x objective on a fluorescent microscope.

Statistical Analysis

Statistics were performed using SPSS program for Windows version 18.0 (SPSS Inc, Chicago, IL). The data were subjected to one-way ANOVA. LSD or James-Howell multiple comparison post hoc tests were performed to evaluate the significance of difference in means between various treatments groups according to the test for homogeneity. Values were presented as means ± SEM and a *P* value <0.05 were considered significant.

Results

Serum ALT and AST enzyme activities

Figure (1) shows that INH and RIF combination resulted in elevated serum activities of ALT and AST relative to normal control group and amounted to (187% and 339%, respectively). Furthermore, serum cholesterol, triglyceride and total lipid levels were significantly elevated as compared to control group (Fig. 1). Co-treatment for 2 weeks with BM-MSCs, had significantly lowered ALT and AST serum activities compared with HT combination group reaching (92%) for ALT and (124%) for AST. MSCs markedly decreased cholesterol level reaching 38.50±2.82 mg/dl and resulted in normalization of triglyceride and total lipid levels (29.71±3.00 and 857±38.43 mg/dl, respectively).

Serum albumin and total protein level and A/G ratio.

Table (1) depicts that the hepatotoxic group showed remarkable decrease in serum albumin, total protein and (A/G) ratio compared with normal control group. Co-treatment with MSCs significantly prevented these alterations and maintained these parameters levels at near normal values (93% for albumin, 92% for total protein and 106% for A/G ratio); these values were significantly different from HT group and non-significant from normal control group.

Serum urea and creatinine levels

Table (1) also demonstrates the renal function tests in the studied groups. INH and RIF treated animals showed a significant decrease in serum urea level; however, serum creatinine level was significantly elevated. Co-treatment with BM-MSCs markedly remodeled these levels, reaching to 79% for serum urea and 101% for serum creatinine of normal values, respectively. The later results were non-significant from normal control group, which reflects gradual restoration of the renal functions to near-normal values.

Hepatic TBARS, NO and GSH levels and SOD and CAT activities

As indicated in figure 2, rats administered anti-TB combination showed a remarkable elevation of liver TBARS and NO levels. Concomitant treatment with MSCs showed a significant reduction of TBARS and NO levels when compared with HT group (269±24, 24±0.55), respectively. On the other hand, HT group showed marked depletion of liver GSH content relative to the normal control group (3.7±0.38 vs 13.5±0.51 mmol/gm tissue). Co-treatment with MSCs significantly elevated GSH content compared with HT group (5.1±0.33). Furthermore, INH and RIF treatment significantly decreased the activities of hepatic antioxidant enzymes, SOD and CAT compared with normal control group (27.6±2.9 and 0.34±0.02 vs 86.8±2.9 U/mg protein and 0.81±0.04 U/mg protein, respectively). MSCs succeeded to significantly elevate SOD and CAT activities (54.1±2.5 and 0.50±0.05), respectively.

Renal TBARS, NO and GSH levels and SOD and CAT activities

As shown in figure 3. Administration of INH and RIF combination to rats resulted in a remarkable increase in kidney TBARS and NO levels (1181±45 and 33±0.97 *vs* 387±37 nmol/gm tissue and 23 ± 0.87 µmol/gm tissue, respectively). Treatment with MSCs showed a significant reduction of TBARS and NO levels (634±44 and 22±1.40), respectively, compared with HT group. On the other hand, HT group showed a significant decrease in kidney GSH content compared with the control group (1.7±0.15 vs 3.6±0.33 mmol/gm tissue). Treatment with MSCs efficiently normalized GSH level. Furthermore, INH–RIF combination resulted in a significant reduction ($P < 0.05$) in liver SOD, CAT activities. MSCs significantly elevated

SOD activity to a value exceeding the normal control level (54.6±2.6), while failed to modify kidney CAT activity (0.31±0.02).

Liver and Kidney DNA fragmentation (Comet assay)

As indicated in figure 4, 5a and 5b, a marked DNA fragmentation was observed in both liver and kidney tissues of rats treated with INH and RIF compared with normal control, as indicated by increased tail moments which amounts (547% and1191% of their normal values, respectively). MSCs succeeded to significantly decrease DNA fragmentation levels in both liver and kidney, amounting to 242% for liver and 580% for kidney compared to their normal values.

Discussion

The transient abnormalities in liver and kidney functions are common during the early stages of anti-TB therapy, but sometimes hepatotoxicity may be more serious and require a change of treatment **(Kumar *et al.*, 2010)**. The elevated liver marker enzymes activities and the reduced serum protein levels were in parallel with several studies **(Dhamal *et al*., 2012; Kumar *et al.*, 2010; Santhosh *et al*., 2007)**. Aminotransferases are originally present in high concentrations in the cytoplasm. Upon injury, these enzymes leak into the blood stream and manifest significantly elevated serum levels. Previous report in rats **(Dhamal *et al*., 2012)** suggested that the hydrazine is involved in the development of INH-induced hepatotoxicity.

Stem cell therapy has raised great hope for improving the treatment of liver diseases, as it has the potential to promote liver repair and regeneration with fewer complications. BM-MSCs are easily isolated from BM, readily cultured *in vitro* and can be used for autologous transplantation **(Sato *et al*., 2005; Schwartz *et al*., 2002)**. The potential therapeutic benefit of MSCs can only be realized through their homing efficiency to the required site. The migration of MSCs from the circulation into damaged tissues is the most crucial step bringing MSCs into play **(Li *et al*., 2013).** On the other hand, **Xiang *et al*. (2005)** reported that in rats with CCl_4-induced liver injury, the timing and number of MSCs homing to the liver is closely related to the presence of liver injury but not to the route of MSCs administration **(Xiang *et al*., 2005)**. In our work, BM-MSCs resulted in significant reduction in the serum activities of liver marker enzymes. Furthermore, MSCs caused significant elevation of serum levels of albumin and total protein. These findings were in agreement with a previous report by **(Abdel Aziz *et al*., 2007)**, who found that the autologous transplantation of BM-MSCs can differentiate into hepatocytes, restore serum albumin and total protein levels, and suppress transaminases activities in experimental models of liver injury.

Our study revealed that administration of INH and RIF significantly reduced the serum levels of urea. This result was in accordance with **Santhosh *et al.* (2007)**. Formation of urea is the mode of disposal of nitrogen. In hepatotoxic condition, due to the failure of the liver to convert amino acids and ammonia to urea, a significant decrease in urea was observed. Moreover, there is an increased catabolism of proteins, investigated by decreased serum albumin and total protein levels, coupled with the diminished ability of kidneys to excrete the nitrogenous waste. Nephropathy is one of the important microvascular complications of anti-TB therapy **(Rekha *et al*., 2005)**. The increase in serum creatinine level in INH and RIF-intoxicated rats indicates a significant degree of glomerular dysfunction. These results were in consistence with a previous study **(Rekha *et al*., 2005)**. This renal injury is usually reversible if detected early and treated appropriately **(Rekha *et al*., 2005)**.

On the other hand, BM-MSCs restored the renal functions to normal levels. The previous studies clearly showed that the captured MSCs are released from the vascular bed and redistributed to peripheral organs including the kidney **(Kunter *et al*., 2006)**. Furthermore, serum creatinine was significantly decreased, which was in agreement with **(Suzuki *et al*., 2013; Kunter *et al*., 2006)**.

In our hepatotoxicity model, free radicals formed could be attributed to either by the reaction of metabolites of INH and RIF (especially hydrazine and acetylhydrazine) with oxygen or by the interaction of superoxide radicals ($O_2^{\bullet-}$) with H_2O_2 producing hydroxyl radical ($OH^{\bullet}$), seem to initiate peroxidative degradation of membrane lipids and endoplasmic reticulum rich in poly unsaturated fatty acids. This leads to formation of lipid peroxides which in turn give products like TBARS that result in loss of integrity of cell membrane and liver and kidney tissue damage **(Kumar *et al.*, 2010)**. In the present study, liver and kidney NO levels were significantly elevated which was in accordance with **(Saad *et al*., 2010)**. NO overproduction was previously linked to drug-induced hepatotoxicity such as; acetaminophen **(Gardner *et al*., 2002)**, CCl_4 and cadmium **(Harstad and Klaassen, 2002)**. NO is a highly reactive oxidant produced by liver parenchymal and non-parenchymal cells from L-arginine via an inducible form of NO synthase enzyme (iNOS) **(Harstad and Klaassen, 2002)**. The reactive nature of NO with ROS suggests several biological pathways through which NO promote oxidative stress-induced cell injury. The released NO scavenges ($O_2^{\bullet-}$) to produce peroxynitrite anion ($ONOO^-$), a potent prooxidant and cytotoxic intermediate that is capable of damaging proteins, lipids and DNA **(Saad *et al*., 2010;**

Yue *et al*., 2009). Peroxynitrite is protonated in a short time and this reaction generates highly toxic hydroxyl radicals, which may explain the cytotoxicity associated with the elevated level of NO **(Saad *et al*., 2010**).

Both liver and kidney tissues showed depleted GSH levels, especially hepatic GSH which was aggressively depleted (27%), as the liver is the main site of drugs' detoxification. Our results were in harmony with several *in vivo* studies **(Dhamal *et al*., 2012; Palanisamy and Manian, 2012; Kumar *et al*., 2010; Saad *et al*., 2010; Santhosh *et al*., 2007)**. This was in contrary to **Rana *et al*. (2006)**, probably because they used only RIF in a lower dose (50 mg/kg IP). Depletion of GSH is known to result in enhanced LPO and excessive LPO can cause increased GSH consumption **(Palanisamy and Manian, 2012)**.

It is well established that SOD and CAT constitutes a mutually supportive team of antioxidant enzymes which provides a defense system against ROS **(Saad *et al*., 2010)**. Our study revealed that liver and kidney activities of SOD and CAT were significantly depressed in HT rats. These results were in accordance with several reports discussing the role of enzymatic antioxidant defense mechanisms against anti-TB drugs-induced liver injury **(Palanisamy and Manian, 2012; Kumar *et al*., 2010; Santhosh *et al*., 2007)**. SOD and CAT activities were significantly decreased due to the abundant production of ($O_2^{\bullet-}$) and other free radicals in rats treated with INH and RIF. Moreover, it has been reported that ($O_2^{\bullet-}$) in addition to singlet oxygen and peroxyl radicals have been shown to directly inhibit the activity of CAT. These observations manifest and explain the significant inhibition of CAT activity in the INH and RIF administered rats. Another mechanism that might be involved in the reduced activities of SOD and CAT was through their inactivation by the ROS/RNS production **(Ferna´ndez *et al*., 2006)**. At high concentration of NO, the ROS and NO react to form $ONOO^{\bullet-}$ which may react fast with Mn-center of SOD enzyme and inactivate the enzyme involving the nitration of Tyr^{34} amino acid, which is critical for SOD enzyme activity and is the most prone to peroxynitrite-mediated nitration **(Ferna´ndez *et al*., 2006)**. This assumption was ascertained by the elevated NO levels in accordance with the decreased SOD activities.

Concerning antioxidant parameters, our study showed that, BM-MSCs elevated the content of both hepatic and renal GSH as well as the enzymatic activities of SOD compared with HT group. Also, a significant elevation of CAT activity was observed in liver tissue only but not kidney tissue. These results were in accordance with previous investigations **(Burra *et al*., 2012; Kuo *et al*., 2008)**. On the other hand, **Fang *et al*. (2013)** demonstrated that induced pluripotent stem cells potentially elevated the activities of SOD and CAT and decreased the levels of ROS in the retina damaged by ischemia reperfusion injury. Furthermore, the antioxidant properties of adipose tissue derived-MSCs were previously demonstrated *in vitro* by **Kim *et al*. (2008)**. Moreover, stem cells were previously demonstrated to have antioxidant properties in cardiovascular diseases **(Yao *et al*., 2006)**.

In a recent study **Burra *et al*. (2012)**, they found that CAT activity was significantly elevated after 8 days of transplanting MSCs into CCl_4-intoxicated mice. Furthermore, CAT activity, measured in MSCs transplanted mice without CCl_4-induced damage, was higher compared with placebo group. From our results and the previously mentioned study, we could speculate that MSCs could contribute to scavenging activity against free radicals by stimulating the activities of CAT and SOD, which are the major biological defense mechanisms against ROS in different tissues **(Burra *et al*., 2012)**. **Kuo *et al*. (2008)** previously concluded that MSCs could resist oxidative damage through the following mechanisms; *1)* the up-regulation of the anti-apoptotic genes Bcl-2 and Bcl-xL, *2)* the up-regulation of the cell-survival signals and *3)* the over-expression of cytosolic SOD **(Kuo *et al*., 2008)**. There was another important previously illustrated mechanism for antioxidant activities of MSCs, concerned with the overexpression of a transcription factor, nuclear factor 2 (Nrf2). The Nrf2 antioxidant response pathway is "the primary cellular defense against the cytotoxic effects of oxidative stress" **(Gold *et al*., 2012)**. Among other effects, Nrf2 increases the expression of several antioxidant enzymes. Several drugs that stimulate the Nrf2 pathway are being studied for treatment of diseases that are caused by oxidative stress **(Gold *et al*., 2012)**.

In the present study, INH and RIF induced a significant elevation of serum levels of cholesterol, triglycerides and total lipids. Our results coincide with different studies **(Jyothi *et al*., 2013; Kumar *et al*., 2010; Saad *et al*., 2010)**. One of the major disorders encountered in anti-TB drugs-induced hepatotoxicity is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition mechanisms **(Saad *et al*., 2010; Santhosh *et al*., 2007)**. A strong association between hypercholesterolemia and increased free radical production was previously documented. ROS produced during oxidative stress react with lipoproteins to produce oxidation states, diminishing the cellular uptake of lipids from the blood.

Schwartz *et al*. (2002) postulated that MSCs from the BM of human, mice as well as rats, when cultured with HGF and FGF, to enhance hepatocyte differentiation, they had the ability to take up LDL from the medium, hence decreasing cholesterol, triglycerides and total lipid levels. We suggest that, the uptake of LDL may be the explanation for the decreased levels of serum

lipid profile in BM-MSCs-treated rats **(Sato *et al*., 2005; Schwartz *et al*., 2002)**.

DNA fragmentation has been recognized as the onset of many diseases including cancer and could be a useful indicator for the oxidative status and antioxidant defense system of organism **(Thirunavukkarasu and Sakthisekaran, 2003)**. The data obtained from the present study showed that INH and RIF induced a highly significant DNA damage, indicated by the increased tail moment in comet assay, in liver and kidney tissues (547% and 1191%, respectively). These results are in accordance with previous studies **(Chen *et al*., 2011; Zhang *et al*., 2011; Yue *et al*., 2009)**. A previous study showed that hydrazine and therapeutic agents with hydrazine functionality, including phenelzine and hydralazine, cause time- and concentration-dependent strand scission of DNA in human hemolysate **(Runge-Morris *et al*., 1994)**. INH caused significant elevation of the liver tissue content of 8-OH deoxyguanosine, an early biomarker of DNA oxidative damage, suggesting that free radicals generated after INH treatment caused oxidative DNA damage **(Yue *et al*., 2009)**. DNA fragmentation into oligonucleosomal fragments forming ladder pattern following gel electrophoresis, is a feature of apoptosis that was induced by INH and RIF **(Chen *et al*., 2011).**

Table (1): Changes in serum levels of albumin, total protein and albumin/globulin (A/G) ratio in 30 days INH and RIF-induced hepatotoxic rats (HT), treated with rat bone marrow mesenchymal stem cells (HT + MSC).

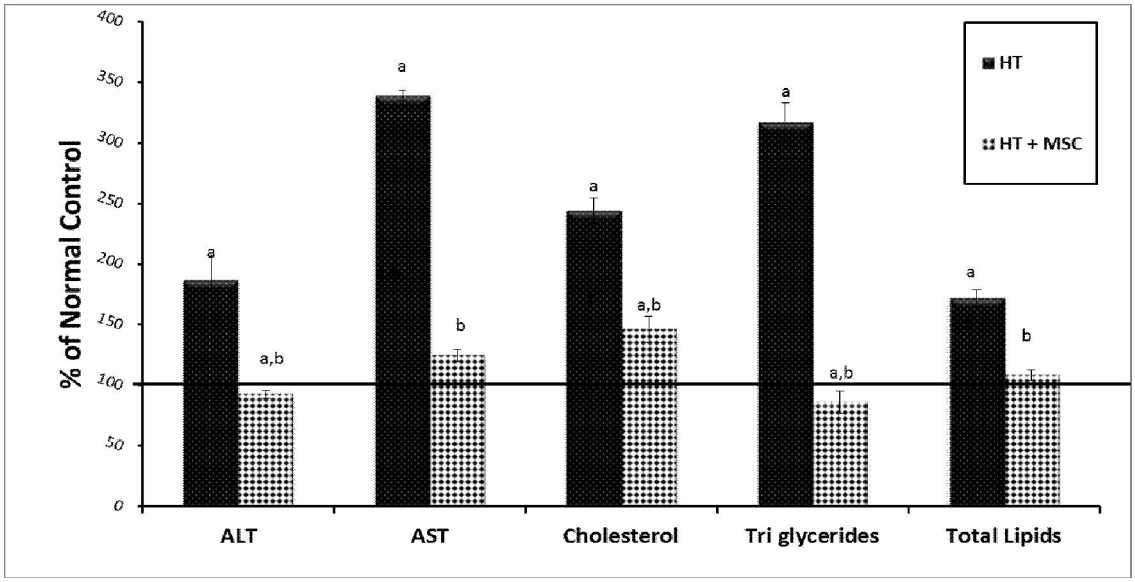
	Albumin(mg/dl)		**T. Protein(mg/dl)**		**(A/G) ratio**	
	Mean ± SEM *(Range)*	*% of NC*	**Mean ± SEM** *(Range)*	*% of NC*	**Mean ± SEM** *(Range)*	*% of NC*
Control	**3.89±0.13**[(b)] *(3.45-4.34)*	*100%*	**7.34±0.17**[(b)] *(6.86-8.43)*	*100%*	**1.28±0.09**[(b)] *(0.89-1.73)*	*100%*
HT	**1.65±0.14**[(a)] *(0.99-2.2)*	*42%*	**4.84±0.24**[(a)] *(3.69-5.65)*	*66%*	**0.43±0.01**[(a)] *(0.21-1.04)*	*34%*
HT + MSC	**3.63±0.90**[(b)] *(3.28-3.92)*	*93%*	**6.77±0.15**[(b)] *(6.13-7.62)*	*92%*	**1.36±0.22**[(b)] *(0.79-2.8)*	*106%*

Data are presented in mean ± SEM (n=8).
(a) Significantly different from normal control group ($p<0.05$).
(b) Significantly different from hepatotoxicity group ($p<0.05$).

Table (2): Changes in serum levels of urea and creatinine in 30 days INH and RIF-induced hepatotoxic rats (HT), treated with rat bone marrow mesenchymal stem cells (HT + MSC).

	UREA(mg/dl)		**CREATININE(mg/dl)**	
	Mean ± SEM *(Range)*	*% of NC*	**Mean ± SEM** *(Range)*	*% of NC*
Control	**39.4±1.8** [(b)] *(33.2-46.8)*	*100%*	**0.65±0.05** [(b)] *(0.43-0.91)*	*100%*
HT	**14.7±1.0** [(a)] *(11.4-18.7)*	*37%*	**1.12±0.04** [(a)] *(1.00-1.30)*	*172%*
HT + MSC	**31.1±1.5** [(a,b)] *(25.7-37.1)*	*79%*	**0.66±0.03** [(b)] *(0.43-1.30)*	*101%*

Data are presented in mean ± SEM (n=8).
(a) Significantly different from normal control group ($p<0.05$).
(b) Significantly different from hepatotoxicity group ($p<0.05$).



Figuure 1: Changes in activities of serum transaminases (ALT and AST) and serum levels of cholesterol, triglycerides and total lipids in 30 days INH and RIF-induced hepatotoxicity in rats (HT), treated with rat bone marrow mesenchymal stem cells (HT + MSC).
Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group ($p<0.05$).
(b) Significantly different from hepatotoxicity group ($p<0.05$).

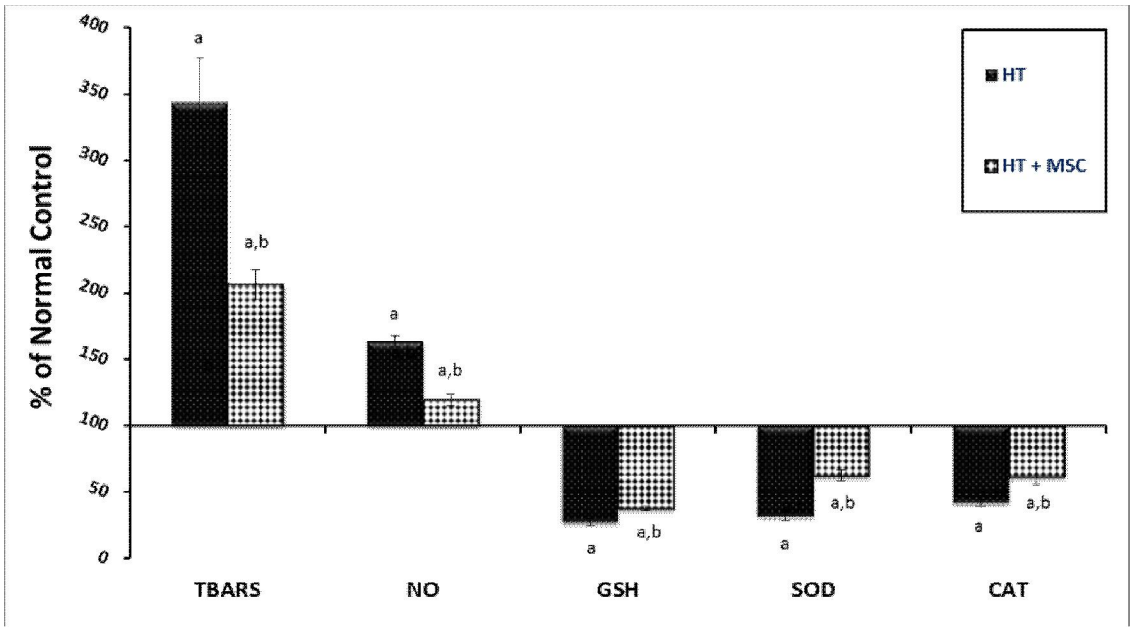


Figure 2: Changes of liver oxidative stress parameters (TBARS and NO) and the non-enzymatic antioxidant (GSH) contents and the enzymatic activities of (SOD, CAT) in 30 days INH and RIF-induced hepatotoxic rats (HT), treated with rat bone marrow mesenchymal stem cells (HT + MSC).
Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group ($p<0.05$).
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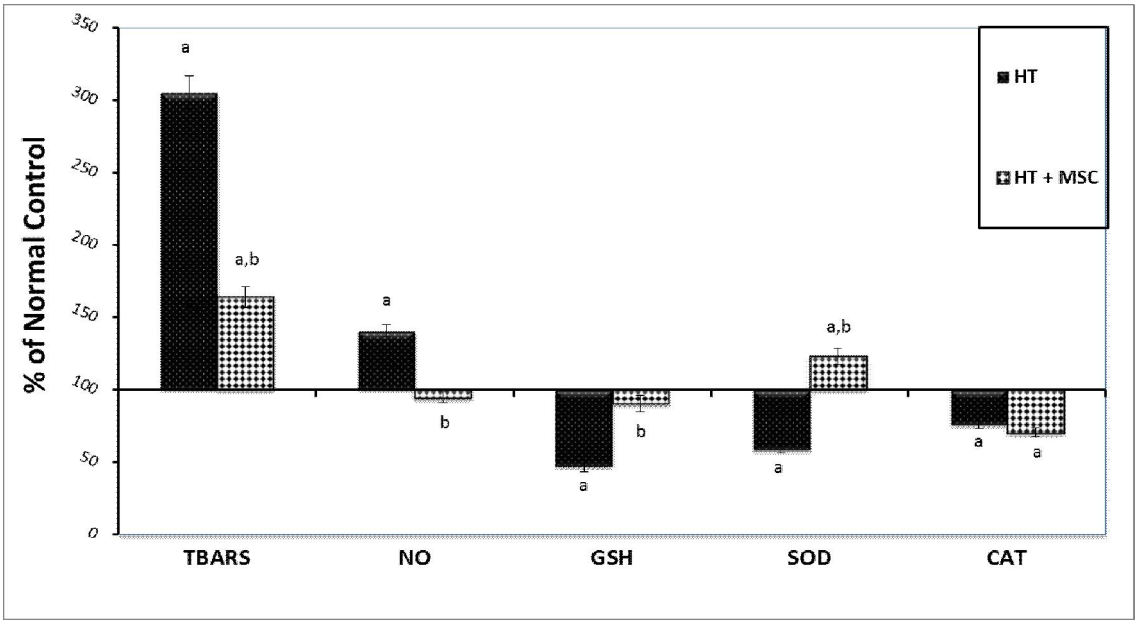

Figure 3: Changes of kidney oxidative stress parameters (TBARS and NO) and the non-enzymatic antioxidant (GSH) content and the enzymatic activities of (SOD, CAT) in 30 days INH and RIF-induced hepatotoxic rats (HT), treated with rat bone marrow mesenchymal stem cells (HT + MSC).
Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group ($p<0.05$).
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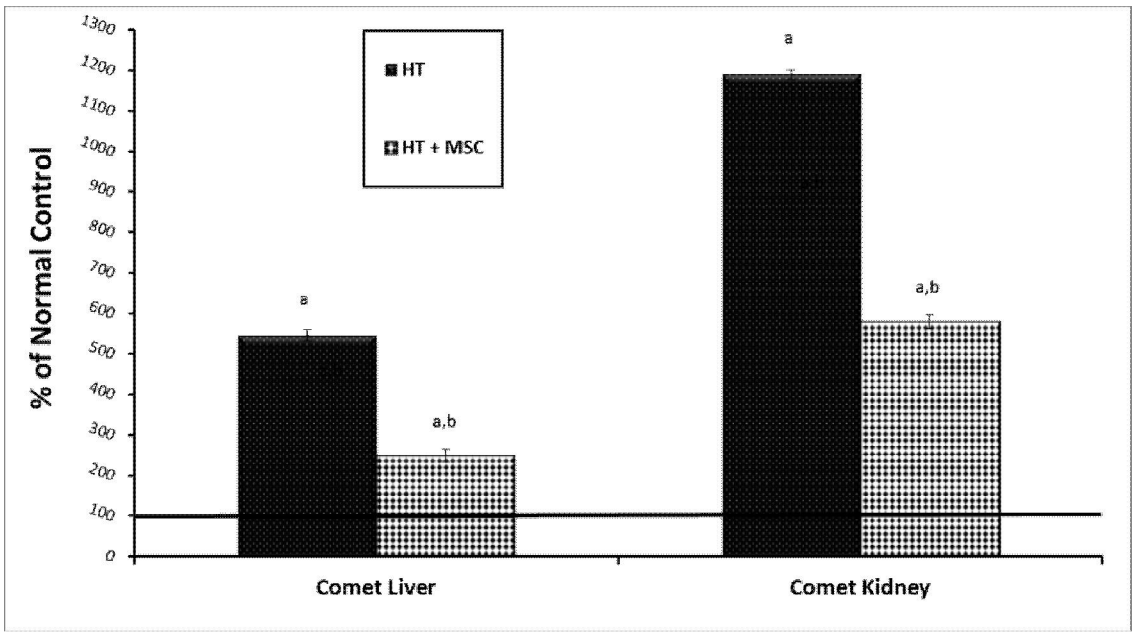

Figure 4: Changes of liver and kidney DNA fragmentation indicated by comet assay in 30 days INH and RIF-induced hepatotoxic rats (HT), treated with rat bone marrow mesenchymal stem cells (HT + MSC).
Data are presented in percentage of normal control ± SEM (n=8).
(a) Significantly different from normal control group ($p<0.05$).
(b) Significantly different from hepatotoxicity group ($p<0.05$).

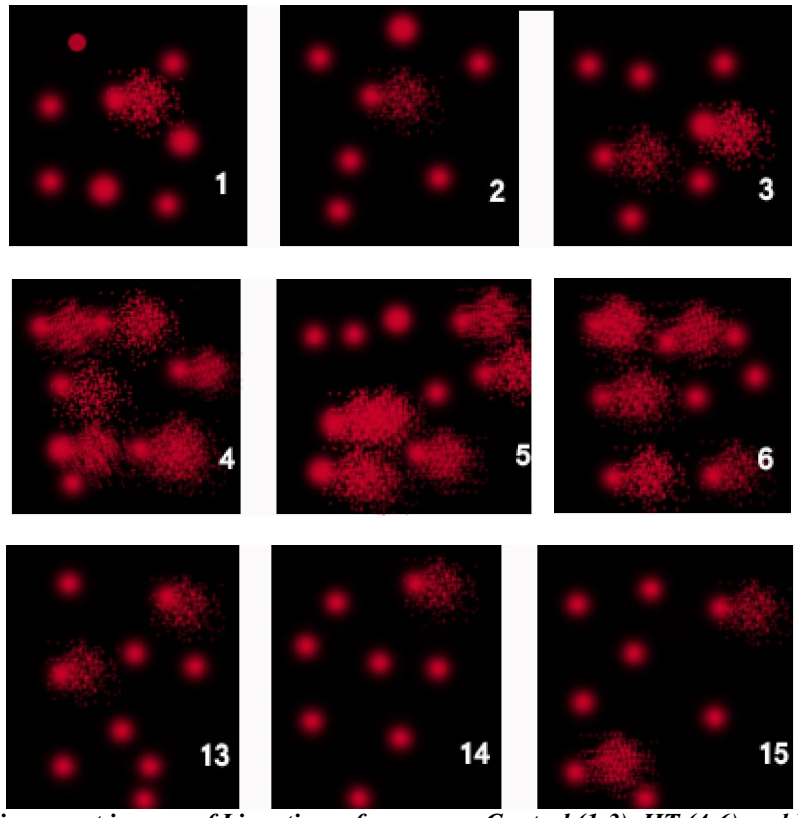

Figure 5a: ***Representative comet images of Liver tissue for groups: Control (1-3), HT (4-6) and HT+MSC (13-15)***

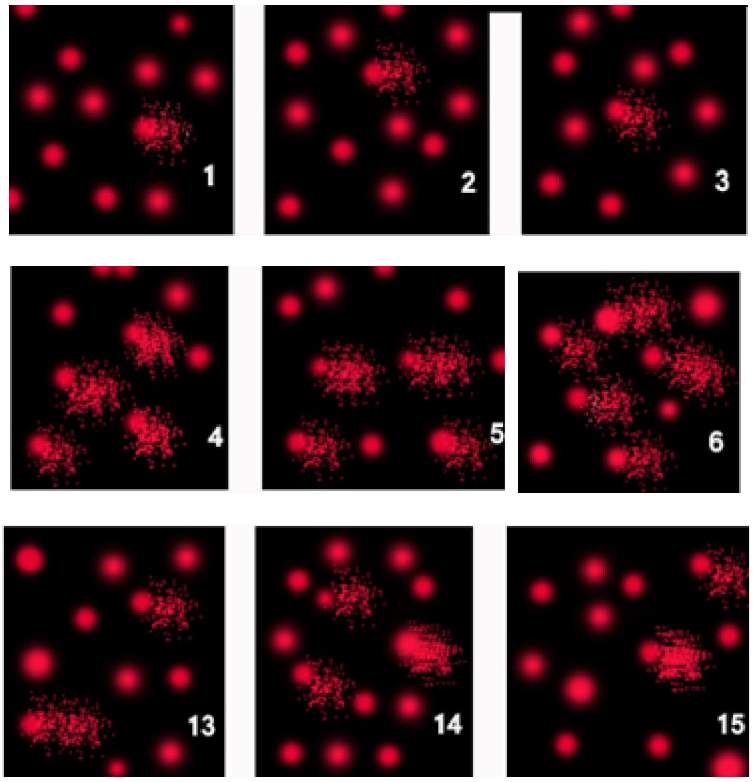

Figure 5b: ***Representative comet images of Kidney tissue for groups: Control (1-3), HT (4-6) and HT+MSC (13-15)***

Conclusion

The present study indicates that simultaneous administration of BM-MSCs along with INH-RIF protected the experimental animals against hepatotoxic and renal toxicity reactions. Moreover, it can be concluded that this therapeutic treatment may be taken as a valuable tool for protection against hepatotoxicity induced by INH-RIF in an animal model by increasing the antioxidant defense systems, minimizing the free radical induced tissue injury and overall maintenance of the endogenous scavengers of free radicals.

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