Research Article

Selenium Increases the Efficiency of Bone Marrow Stem Cells in Repairing the Liver Damage Induced by CCL_4 in Male Rats

Faris A. M. Baawad[1], Saleha Y. M. Alakilli[2], Karima F. Mahrous[3]*
[1]Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Department of Biology, King Abdulaziz University, Jeddah, Saudi Arabia.
[3]Cell Biology Department, National Research Centre, 33 Bohouth St., Dokki, Giza, Egypt.
***Corresponding author's E-mail:** kfmahrous@hotmail.com

Accepted on: 29-03-2016; **Finalized on:** 30-04-2016.

ABSTRACT

Using of bone marrow stem cells (BMSCs) for transplantation to treat the damage in liver induced by either chemical compounds or by viruses is considered as in medical application. The availability, easy culture and surviving for a long time are optimistic advantages of using BMSCs in healing the degenerative of hepatic cells. Therefore, the aim of the present study was planned to use BMSCs in combination with selenium (Se) to treat the hepatic damage induced by carbon tetrachloride (CCL4) in male rats. To perform the aim of this study eighty adult albino male rats were divided in nine groups as follows: Group 1: animals were treated with solvent vehicle. Group 2: animals were injected with CCL4 twice weekly for 2 months. Groups 3 and 4: animals were treated with different doses of Se. Groups 5 and 6: animals were injected with CCL4 and low or high dose of Se, respectively. Group 7: animals were injected with CCL4 and single dose of Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs). Group 8: animals were injected with CCL4 and single dose of Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs) plus low dose of Se. Group 9: animals were injected with CCL4 and BM-MSCs plus high dose of Se. The results of this study revealed that treatment of male rats with CCL4 increased the levels of the following parameters including rate of the apoptosis, changes in the gene expression of three antioxidants related genes, DNA fragmentation as well as decreased the activity of the gutathione peroxidase (GPx) enzyme. However, treatment of CCL4-treated rats with BM-MSCs combined with low and high dose of Se decreased the apoptosis rates, alterations in the expression of antioxidant related genes, decreased the damage in the DNA induced by CCL4 as well as increased the activity of the antioxidant enzyme GPx in male rats. Therefore, our results suggest that BMSCs could be used in combination with Se in healing the liver damage induced by CCL4 treatment.

Keywords: Bone marrow stem cells, Selenium, Gene expression, DNA fragmentation, male rats.

INTRODUCTION

Liver is the important organ in the body for metabolism and may of physiological processes. Due to its biological properties it is usually exposed several harmful compounds and viruses as well as drugs for therapy strategies. However, until present medicine cannot find effective, safe and fast tools or agents for treat the damage and disorders occurs in liver tissues[1]. In most of the world especially the undeveloped countries liver diseases are considered as the most health problems which need a lot of money to use a good therapeutic strategy. Therefore, the world health organization (WHO) and many of universities worldwide are performing many of researches to find therapeutic agents and tools more effective and possible without any side effects than the current available one[2]. One of the promising agents are those extracted from natural products which exhibited promising results without side effects for treating liver toxicity.[3,4]

Indeed, the liver diseases occurs worldwide are due to increase the generation of the free radicals in the body. The free radicals cause several disorders and diseases including liver diseases and different kinds of tumors[5]. On the other hand, several studies for healing most of these diseases are carried out and suggesting that some of these diseases could be suppressed using good management such as antioxidants in the natural products.[5-7] Some works found that protective effects of the natural products extracted from medicinal plants are coincide with several compounds having many of the antioxidants[5]. These antioxidants having not only protection against liver diseases but also having protection against other several diseases such as cancer, heart disorders and diseases related to genetic toxicity[6,7]. However, the biological mechanism of the natural products relay on the protective action more than therapeutic action. Therefore, we are thinking in this study to use other compounds and agents against liver damage as therapeutic agents.

There are several tools to induce the liver damage in the laboratory animals in which induction of liver fibrosis using carbon tetrachloride (CCl4) is one of the famous methods. It has been reported that use of CCL4 to induce hepatic damage was found to be similar to the symptoms of the virus inducing hepatitis in human[8].

The biological way of CCL4 inducing damage in the liver of the laboratory animal is that it induce generation of the free radicals, and decline the activity of the antioxidant enzymes as well as induce lipid peroxidation[9].

In addition, CCL4 was found to be a suppressor for several drugs used for protection against liver toxicity[10].

Several studies revealed that CCL4 was able to suppress several antioxidants such as antioxidant enzymes (CAT, GPs and SOD), non-enzymatic antioxidants (GSH) and other antioxidants (α-tocopherol & selenium)[11]. These antioxidant when be affected due to CCL4 treatment the generation of ROS increases in the affected organs and tissues. One important antioxidant against CCL4 is Selenium (Se). It is considered as a component of the chemical structure of glutathione peroxidase enzyme and plays an important role against the free radicals in the body as good[12]. The results of research on Se indicated that it was able to prevent the transformation of the normal cells to malignant tumors due to its protective action against the oncogenes which they responsible for tumor progression[13,14]. Moreover, the results on the cancer research indicated that low level of selenium in the cells was coincide with increase the proliferation of tumor cells[15]. Additionally, the degree of cancer was related to the level of selenium deficiency[15]. Also, other studies revealed that In addition, some studies indicate that selenium deficiency was in deep correlation with liver damage (e.g. fibrosis)[16,17]. Therefore, supplementation of selenium was effective against suppression of cancer and protection of the live against fibrosis.[14]

From the modern tools to be taken in consideration in cancer and liver damage is stem cells application. Therefore, this new tool in therapy of cell degeneration is promising tool in the medical field. It has been found that use of bone marrow stem cells (BMSCs) for treatment of cell degeneration take increase attention due to its availability, easy and fast culture in very the cheaper laboratories as well as its long survivor time[18]. Furthermore, the BMSCs are able to be differentiating to several cell types depending on the media used in the culture program.[19]

Several studies revealed that BMSCs having different biological actions such as immune response[20], anti-inflammation characters[21], and trophical impacts[22]. In addition, they possess a variety of cytokines and regulators of which are able to enhance the growth, proliferation and differentiation as well as recovery processes.[23,24] It has been found that the first resources of stem cells were bone marrow and tissues of the adipose gland as well as blood cells[25,26].

From these very promising stem cells is bone marrow derived MSCs (BM-MSCs) which they easy to obtain from the bone marrow cells and having good biological properties.[27]

Although several works had been focused on the therapy of liver fibrosis, combination between BM-MSCs and Se has been not investigated yet.

Therefore, the present study aimed to use Se and BM-MSCs alone or in mixture to evaluate their protective effect on liver fibrosis induced by CCL4 treatment in male rats.

MATERIALS AND METHODS

Drugs and Reagents

Carbon tetrachloride (CCL4, 99.9% purity) was purchased from Sigma (St. Louis, MO, USA). Trizol was bought from Invitrogen (Carlsbad, CA, USA).

The reverse transcription and PCR kits were obtained from Fermentas (Glen Burnie, MD, USA). SYBR Green Mix was purchased from Stratagene (La Jolla, CA, USA).

Experimental Animals

Ninety adult albino male rats (100-120 g, purchased from the Animal House Colony, Giza, Egypt) were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum* at the Animal House Laboratory, National Research Center, Dokki, Giza, Egypt.

After an acclimation period of 1 week, animals were divided into groups (10 rats/ group) and housed individually in filter-top polycarbonate cages, housed in a temperature-controlled (23 ± 1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination.

All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of National Research Center, Egypt.

Experimental Design

The male rats were randomly divided in 9 groups (n=10 per group) and treated for 2 months as follows: Group 1, control group: animals were treated intragastrically with solvent vehicle control (olive oil). Group 2, animals were injected intraperitoneally with 0.5 ml/kg of CCL4 (suspended in olive oil, 1:9 v/v) twice weekly for 2 months to maintain hepatic damage. Groups 3 and 4, animals were treated orally with Selenium (Se, 1 and 2 mg/kg)[28], respectively. Groups 5 and 6, animals were injected intraperitoneally with 0.5 ml/kg of CCL4 and Se (1, and 2 mg/kg), respectively. Group 7, animals were injected intraperitoneally with 0.5 ml/kg of CCL4 and single dose of Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs, 3×10^6 cells/rat) intravenously[21]. Group 8, animals were injected intraperitoneally with 0.5 ml/kg of CCL4 and single dose of Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs, 3×10^6 cells/rat) intravenously[21] plus 1 mg/kg Se. Group 9, animals were injected intraperitoneally with 0.5 ml/kg of CCL4 and single dose of BM-MSCs (3×10^6 cells/rat) intravenously plus 2 mg/kg Se.

Afterwards, at 24 h after the last injection, rats in each group were sacrificed by decapitation after anesthetized. The liver tissues were collected on ice bath in order to investigate the apoptosis, ROS, total RNA (for the determination apoptotic related genes GLAST, GLT-1,

ASCT2 mRNA), total DNA (for determination DNA fragmentation) and for GPx activity.

Apoptosis assay

The tissue of cortex (100 mg per sample) was made into single-cell suspensions according to method of Villalba.[29] Cells apoptosis was determined by flow cytometry (FCM) assay using Annexin V/PI apoptosis detection kit. The single-cell suspension (1×10^6 cells/mL) was suspended in 200 µL ice-cold binding buffer and then 10 µL horseradish peroxidase FITC labeled Annexin V and 5 µL propidium iodide (PI) were added. The cell suspension was incubated in darkness at room temperature for 15 min. Apoptosis rate was determined by flow cytometer. In this study, both FITC and PI negative cells were considered as normal cells. FITC-single positive and PI negative cells were defined as early apoptotic cells, while both FITC and PI positive cells were considered as late apoptotic or necrosis cells.

Gene Expression Analysis

Extraction of total RNA and cDNA synthesis

Liver tissues of male rats were used to extract the total RNA using TRIzol® Reagent (Invitrogen, Germany) Kit. The isolation method was carried out according to the manufacturer's instructions of the above Kit. Approximately 50 mg of the liver tissues were mixed with some drops of liquid nitrogen and homogenized in 1 ml of TRIzol® Reagent in autoclaved mortar. Afterwards, total RNA was dissolved and preserved in diethylpyrocarbonate (DEPC)-treated water up to use.

To assess the RNA yield and purity of the total RNA, RNAse-free DNAse I (Invitrogen, Germany) was used to digest DNA contamination. A small drop of isolated RNA was examined photospectrometrically at 260 nm. The purity of total RNA was determined between 1.8 and 2.1 to be good purified when it examined by photospectrometer at the 260/280 nm ratio. To avoid RNA damaging, aliquots of RNA were prepared after isolation for either reverse transcription reaction or otherwise for storing at -80°C up to use.

To synthesize the complementary DNA (cDNA) isolated RNA from liver tissues was reverse transcribed into cDNA. The reaction volume was carried out in 20 µl. The reaction volume was prepared according to the instructions of the RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). The reverse transcription (RT) reaction was performed for 10 min at 25°C. Afterwards, the tubes of the reaction were put in thermo-cycler machine for 60 min at 42°C, and then the reaction was terminated for 5 min at 99°C. The PCR products containing the cDNA were kept at – 20°C up to use for DNA amplification.

Quantitative Real Time-PCR (qRT-PCR)

A StepOne Real-Time PCR System (Applied Biosystem, USA) was used to assess the copy of the cDNA of male rats to detect the expression values of the tested genes (Table 1). To perform the PCR reaction, a volume of 25 µL of reaction mixtures was prepared containing 12.5 µl of SYBR® green (TaKaRa, Biotech. Co. Ltd.), 0.5 µl of 0.2 µM forward and reverse primers, 6.5 µL DNA-RNA free water, and 2.5 µl of the synthesized cDNA. The cDNA was propagated using reaction program consisted of 3 steps. In the first step the PCR tubes was incubated at 95°C for 3 min. In the second step the reaction program consisted of 50 cycles. Each cycle of them consisted of 3 sub-steps: (a) 15 sec at 95°C; (b) 30 sec at 60°C; and (c) 30 sec at 72°C. In the third step the reaction program consisted of 71 cycles. The first cycle of them started at 60°C for 10 sec and then the followed cycles increased about 0.5°C every 10 sec up to 95.0°C. A melting curve of the reaction was performed for each qRT-PCR termination at 95.0°C to assess the quality of the primers. To verify that the reaction of the qRT-PCR does not have any contamination PCR tubes containing non template control were used. The sequences of specific primer of the genes used are listed in Table 1. The relative quantification of the target (GLAST, GLT-1 and ASCT2) to the reference (β-Actin) was determined by using the $2^{-\Delta\Delta CT}$ method.

Table 1: Primer sequences used for qPCR

Gene	Primer sequence (5′–3′)[a]	References
GLAST	GGGTTTTCATTGGAGGGTTGC	Deng[30]
	CCACGGGTTTCTCTGGTTCAT	
GLT-1	GGGTCATCCTGGATGGAGGT	Deng[30]
	CGTGTCGTCATAAACGGACTG	
ASCT2	gccagtccacggccaagatc	GenBank : BC080242
	gcctggtcgtgttcgctata	
β-actin	GGAGATTACTGCCCTGGCTCCTA	Deng[30]
	GACTCATCGTACTCCTGCTGCTG	

[a]F: forward primer; R: reverse primer. Tm: melting temperature.

DNA Fragmentation Analysis

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described according to Lu.[31] Briefly, liver tissues were homogenized, washed in PBS, and lysed in 0.5 ml of DNA extraction buffer (50 mM Tris–HCl, 10 mM EDTA. 0.5% Triton, and 100 µg/ml proteinase K, pH 8.0) for overnight at 37°C. The lysate was then incubated with 100 µg/ml DNase free RNase for 2h at 37°C, followed by three extractions of an equal volume of phenol/chloroform (1:1 v/v) and a subsequent re-extraction with chloroform by centrifuging at 15,000 rpm for 5 min at 4°C. The extracted DNA was precipitated in two volume of ice-cold 100% ethanol with 1/10 volume of 3 M sodium acetate, pH 5.2 at −20°C for 1 h, followed by centrifuging at 15,000 rpm for 15 min at 4°C. After washing with 70% ethanol, the DNA pellet was air-dried and dissolved in 10 mM Tris–HCl/1 mM EDTA, pH 8.0. The DNA was then electrophoresed on 1.5% agarose gel and stained with

ethidium bromide in Tris-acetate/EDTA buffer (TAE) (pH 8.5, 2 mM EDTA, and 40 mM Tris–acetate). A 100-bp DNA ladder (Invitrogen, USA) was included as a molecular size marker and DNA fragments were visualized and photographed by exposing the gels to ultraviolet transillumination.

Determination of Gutathione Peroxidase (GPx) Activity

Glutathione peroxidase activity measurements were carried out by a procedure according to Miranda.[32] The reaction mixture consisted of 8 mM H_2O_2, 40 mM guaiacol, 50 mM sodium acetate buffer, pH 5.5, and a suitable amount of the enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed at 30 s intervals. One unit of GPx activity was defined as the amount of enzyme which increases the O.D. 1.0/min under standard assay conditions.

Statistical Analysis

All results were expressed as mean ± SE of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when $P < 0.05$.

RESULTS

Effects of Se and BM-MSCs on apoptosis rate induced by CCL4

Assessment the effect of Se and BM-MSCs on the inhabitation of the apoptosis induced by CCL4 is summarized in Fig. 1. The results revealed that CCL4 increased apoptosis rates to 675.9% as compared to the normal control. In contrary, low apoptosis cells were observed in either low or high dose of Se (103.8 and 119.0%, respectively) compared with control rats (Fig. 1).

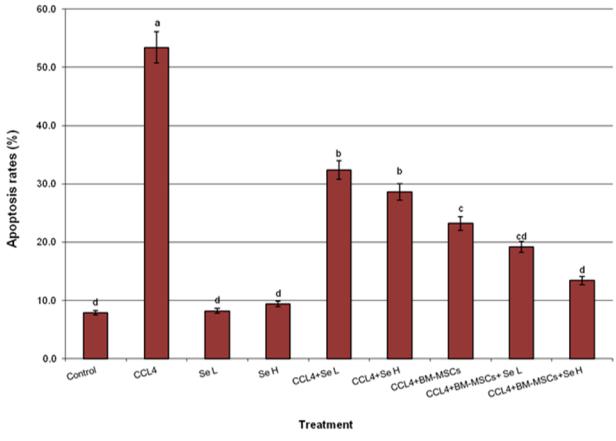

Figure 1: The changes of apoptosis in liver tissues of male rats exposed to CCL4 and/or BM-MSCs and selenium. Data are presented as mean ± SEM. a, b, c, d, followed by different superscripts are significantly different (P≤0.05).

Moreover, the results showed that Se supplementation reduced the apoptosis rates induced by the treatment of CCL4 exposure, where, the apoptosis rates declined to 265.8 and 313.9% in rats treated with CCL4+ low dose of Se and CCL4+high dose of Se, respectively (Fig. 1). Furthermore, the apoptosis rates were reduced further when CCL4 exposed-rats treated with BM-MSCs alone or plus low or high dose of Se (293.7, 243.0 and 169.6, respectively).

Effect of Se and BM-MSCs against CCL4 induced gene expression changes

The expression values of antioxidant related genes (GLAST, GLT-1 and SNAT) in liver tissues of male rats were quantified by real-time RT-PCR (Figures 2-4). Supplementation of male rats with Se and BM-MSCs exhibited high quantitative values of GLAST, GLT-1 and ASCT2 which were very close to those in control group animals (Figures 2-4).

However, exposure of male rats with CCL4 decreased the mRNA expression values of GLAST, GLT-1 and ASCT2 to 29.8, 28.7 and 27.1, respectively compared with control rats (Figures 2-4).

On the other hand, expression values of GLAST gene increased 2.3, 2.6, 2.7, 3.1 and 3.3-fold in CCL4+low dose of Se, CCL4+high dose of Se, CCL4+BM-MSCs, CCL4+BM-MSCs+ low dose of Se and CCL4+BM-MSCs+ high dose of Se, respectively (Fig. 2).

In the same line, expression values of GLT-1 gene increased 2.2, 2.7, 2.8, 3.2 and 3.4-fold in CCL4+low dose of Se, CCL4+high dose of Se, CCL4+BM-MSCs, CCL4+BM-MSCs+ low dose of Se and CCL4+BM-MSCs+ high dose of Se, respectively (Fig. 3).

In addition, expression values of ASCT2 mRNA increased 2.7, 3.0, 3.1, 3.4, and 3.5-fold in CCL4+low dose of Se, CCL4+high dose of Se, CCL4+BM-MSCs, CCL4+BM-MSCs+ low dose of Se and CCL4+BM-MSCs+ high dose of Se, respectively (Fig. 4).

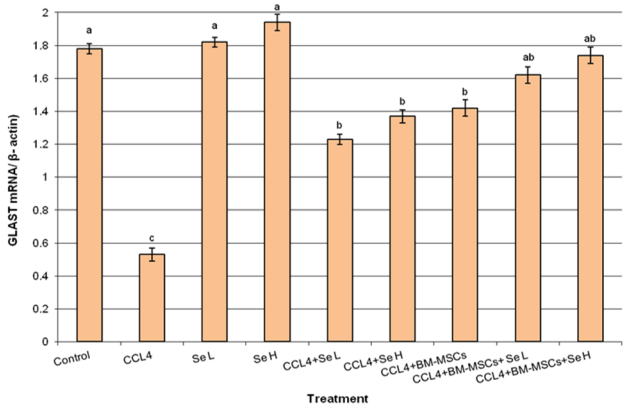

Figure 2: The alteration of GLAST mRNA in liver tissues of male rats exposed to CCL4 and/or BM-MSCs and selenium. Data are presented as mean ± SEM. a,b,c,d, followed by different superscripts are significantly different (P≤0.05).

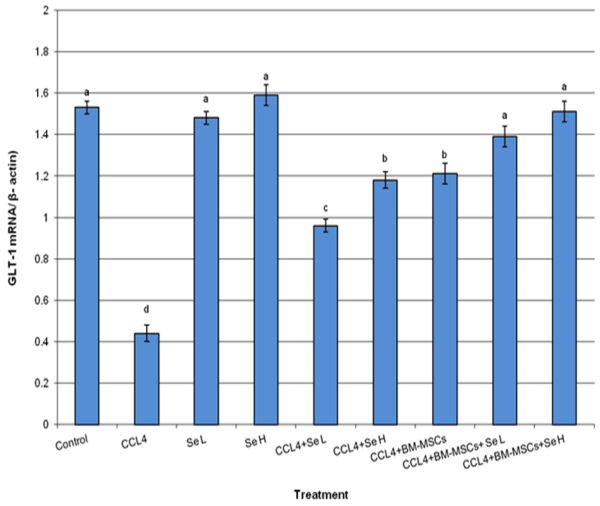

Figure 3: The alteration of GLT-1 mRNA in liver tissues of male rats exposed to CCL4 and/or BM-MSCs and selenium. Data are presented as mean ± SEM. a, b, c followed by different superscripts are significantly different (P≤0.05).

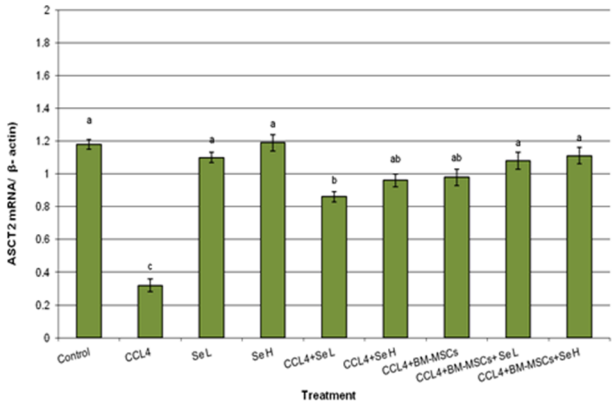

Figure 4: The alteration of ASCT2 mRNA in liver tissues of male rats exposed to CCL4 and/or BM-MSCs and selenium. Data are presented as mean ± SEM. a, b, c, d, followed by different superscripts are significantly different (P≤0.05).

Effect of Se and BM-MSCs against CCL4 induced DNA Fragmentation

Assessment of the rates of DNA fragmentation in liver tissues following CCL4 induced DNA damage and the protective role of Se and BM-MSCs in male rats are summarized in Figures 5.

The results observed that the rates of DNA fragmentation following low and high doses of Se administration were relatively near to that of the control animals. However, the DNA fragmentation increased significantly following CCL4 treatment in comparison to that of the control group (Fig. 5).

Meanwhile, the rate of DNA fragmentation decreased significantly following CCL4 plus low and high dose of Se and CCL4+BM-MSCs as well as CCL4+BM-MSCs+ low dose of Se and CCL4+BM-MSCs+ high dose of Se, respectively.

Whereas, the best treatments revealed low DNA fragmentation against CCL4 were CCL4+BM-MSCs+ low dose of Se and CCL4+BM-MSCs+ high dose of Se.

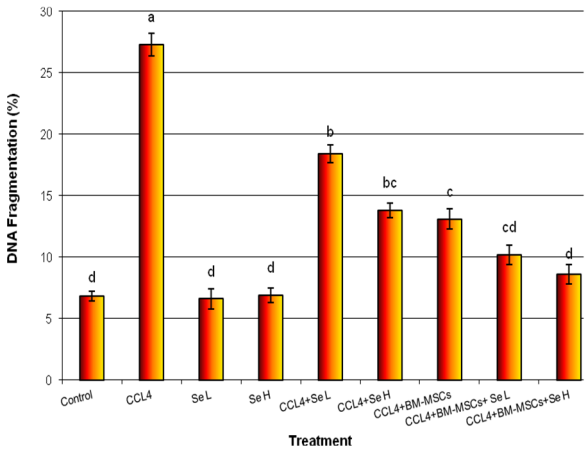

Figure 5: Rate of DNA fragmentation in liver tissues of male rats exposed to CCL4 and/or BM-MSCs and selenium. Data are presented as mean ± SEM. a, b, c followed by different superscripts are significantly different (P≤0.05).

Effect of Se and BM-MSCs on GPx activity levels

The suppression effect of BM-MSCs and Se on CCL4-induced alterations in the antioxidant enzyme GPx was presented in Table 2. In comparison with the control group, levels of GPx activity were relatively similar with those in low and high dose of Se treatments. However, the activity level of GPx decreased significantly in CCL4 treatment compared with control group. On the other hand, comparing with the CCL4 alone, GPx concentration increased significantly in CCL4+ low and high dose of Se and CCL4+BM-MSCs as well as CCL4+BM-MSCs+ low dose of Se and CCL4+BM-MSCs+ high dose of Se (Table 2).

Table 2: The amount of glutathione peroxidase activity in male rats exposed to CCL4 and/or Dobra extract.

Treatment	Glutathione peroxidase activity (U/mg tissues/min)
Control	5.7 ± 0.04^{a}
CCL4	1.7 ± 0.02^{b}
Se L	5.9 ± 0.03^{a}
Se H	6.4 ± 0.03^{a}
CCL4+Se L	4.3 ± 0.02^{a}
CCL4+Se H	4.7 ± 0.01^{a}
CCL4+BM-MSCs	4.9 ± 0.02^{a}
CCL4+BM-MSCs + Se L	5.1 ± 0.03^{a}
CCL4+BM-MSCs+Se H	5.4 ± 0.02^{a}

Data are presented as mean ± SEM. a, b, c, d, followed by different superscripts are significantly different (P≤0.05).

DISCUSSION

Liver is the important organ in the body for metabolism and may of physiological processes. Due to its biological properties it is usually exposed several harmful compounds and viruses as well as drugs for therapy strategies. However, until present medicine cannot find effective, safe and fast tools or agents for treat the damage and disorders occurs in liver tissues[1]. In most of the world especially the undeveloped countries liver diseases are considered as the most health problems which need a lot of money to use a good therapeutic strategy. Therefore, the world health organization (WHO) and many of universities and hospitals worldwide are performing many of researches to find therapeutic agents and tools more effective and possible without any side effects than the current available one.[2]

There are several tools to induce the liver damage in the laboratory animals in which induction of liver fibrosis using carbon tetrachloride (CCl4) is one of the famous methods. It has been reported that use of CCL4 to induce hepatic damage was found to be similar to the symptoms of the virus inducing hepatitis in human[8]. The biological way of CCL4 inducing damage in the liver of the laboratory animal is that it induce generation of the free radicals, and decline the activity of the antioxidant enzymes as well as induce lipid peroxidation[9]. In addition, CCL4 was found to be a suppressor for several drugs used for protection against liver toxicity[10].

The results of the present study showed that CCL4 was able to induce genetic toxicity (e.g. alterations in the expression of antioxidant related genes and DNA damage). These results are in same line with that reported by Abdou.[10,33]. They reported that rats exposed to CCL4 showed significant increase in the DNA fragmentation in comparison with untreated rats. They indicated that the toxicity action of the CCL4 on the DNA damage was attributed to several mechanisms such as direct modification on the DNA molecules, or during DNA repairing, or during DNA replication and or on generation of ROS inducing apoptosis[34].

The action mechanism of which CCL4 induced toxicity is that CCL4 administration activating the cytochrome P450 to produce the radical named trichloromethyl (CCL_3).

The trichloromethyl (CCL_3) plays its role through binding with the nucleic acids and proteins as well as with lipids structures. Several studies revealed that the binding of this radical with the lipids usually induce cell degeneration, while the binding with the DNA molecules induce cell cancer such as liver cancer[35]. On the other hand, this trichloromethyl could be also reacting with the oxygen molecules to form a strong reactive compound namely trichloromethylpheroxy (CCL_3OO) radical. The new compound enter the chain of the lipid peroxidation reaction making destruction and damage of the cells and therefore, increasing the cell apoptosis[35]. The postulation and results are in agreement with our findings that CCL4 increased the apoptosis rate (due to ROS generation) and declined the activity levels of GPx in male rats. In addition to these suggestion several studies indicated that the cell damage induced by CCL4 causes lose in the intracellular calcium. This action is resulted due to that CCL4 makes changes in the membrane of the mitochondria and the endoplasmic reticulum and subsequently the permeability of the calcium through the plasma membrane[36-38].

On the other hand, it has been found that the DNA damage induced by the oxidative stress of CCL4 treatment is attributed to the modification occurred in the DNA molecules in the form of lesions of the nitrogenous bases, sugar moleculaes, DNA strand breaks and DNA histones[10]. The lesions in the DNA and other binding molecules cause mutation, cell death and tumor formation[10]. Additionally, numerous studies indicated that the modification in the DNA molecules induced by CCL4 coincides with single mutation and number of complex mutations to rearrangement of several the chromosomes.[39,40]

The expression of antioxidant related genes is playing an important role in the immune system against any oxidative agents such as CCL4. From these antioxidants related genes are the GLAST, GLT-1 and ASCT2 genes which considered as glutamate transporters. Through their expression the glutamate will be up-taken into the mammalian central nervous system[41]. This glutamate transportation is maintaining support of the glutathione in the central nervous system. We found in the present study that the expression of these genes as glutamate transporters was decreased due to CCL4 treatment. Until present, there were very few data regarding the *in vivo* study focusing on the expression of GLAST, GLT-1 and ASCT2 genes in relation to CCL4 treatment.

Several studies revealed that BMSCs having different biological actions such as immune response[20], anti-inflammation characters[21], and trophical impacts[22]. In addition, they possess a variety of cytokines and regulators of which are able to enhance the growth, proliferation and differentiation as well as recovery processes[23,24]. It has been found that the first resources of stem cells were bone marrow and tissues of the adipose gland as well as blood cells[25,26]. From these very promising stem cells is bone marrow-derived MSCs (BM-MSCs) which they easy to obtain from the bone marrow cells and having good biological properties[27]. Therefore, the present study aimed to investigate the potential therapeutic effect of bone marrow mesenchymal stem cells (BM-MSCs) in recover damaging in liver tissues induced by CCL4 in male rate.

Moreover, supplementation of Se in combination with BM-MSCs was used in the current work to overcome the problem of using stem cells that tissue damage may attract migratory stem cell populations, particularly those from the BM.

The current study revealed that treatment of CCL4-treated rats with BM-MSCs combined with low and high dose of Se decreased the alterations in the expression of antioxidant related genes, decreased the damage in the DNA as well as decreased the rate of apoptosis induced by CCL4 better than those treated with CCL4+ BM-MSCs alone. Moreover, treatment of CCL4-treated rats with BM-MSCs combined with low and high dose of Se increased the activity of the antioxidant enzyme GPx.

Although the promising results of using BM-MSCs in therapy of degenerated hepatic cells some literature revealed that in fibroses case of the liver an excess of collagen precipitation take place[21]. This media of the fibroses cells makes the liver an undesirable site for the transplantation of the stem cells. Thus the transplantation of the BM-MSCs might be not achieved[21]. Therefore, an approach that takes into account is that the need for additional compounds to increase the efficiency of the stem cells to be more effective to recover the liver damage. Therefore, one of the most important aims of the present study is to use selenium with stem cells to be used as new therapeutic strategy to increase the therapeutic effect of the stem cells[21].

It has been found that selenium treatment can suppress the transformation of normal to tumor cells and for deactivation of oncogenes with anticarcinogenic impacts in dose depending manner[13,14]. Moreover, selenium is closely related to the inhibition of hepatic fibrosis[15,16].

To understand the mechanism of selenium in increasing the efficiency of stem cells in repairing the damage in the liver tissues is that selenium is able to inhibit the inflammatory related expression genes such as tumor necrosis factor- α (TNF-α). It has been found that TNF-α is considered as the most endogenous factor inducing inflammatory status[42-44]. Therefore, during the inflammatory status, TNF-α found to be highly expressed in the inflammatory cells of the liver diseases such as fibrosis. The role of TNF-α in increasing the inflammatory and liver damage is taking two forms in which one of them is indirect pathway. The role of TNF-α in this pathway is induce of liver damage through enhancing the generation of free radicals and several inflammatory mediators. The second pathway is direct way in which TNF-α induce liver damage due to promoting the process to produce nitric oxide from the diseased liver cells[45,46]. Our finding indicated that treatment of CCL4-treated rats with stem cells and Se increased the activity of anti-oxidant enzyme in which is an indicator to decrease the inflammatory compound (e.g. TNF-α).

Conclusion: Bone marrow stem cells could be used and interact with disease processes in a number of organs including the liver. The therapeutic action of BMSCs is increasing with the combination with Se supplementation. So, selenium is increasing the efficiency of stem cells in repairing the damage in the liver tissues due to inhibition the inflammatory related expression genes such as tumor necrosis factor-α (TNF-α). Therefore, our results suggest that BMSCs could be used in combination with Se in healing the liver damage induced by CCL4 treatment.

Acknowledgement: The skilful assistance of all staff members of the Biology Department and Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia is gratefully acknowledged.

REFERENCES

1. Huo HZ, Wang B, Liang Y K, Bao YY and Gu Y, Hepatoprotective and antioxidant effects of Licorice extract against CCl4-induced oxidative damage in rats. International Journal of Molecular Sciences, 12, 2011, 6529-6543.
2. Choi JH, Kim DW, Yun N, Choi IS, Islam MN, Kim YS and Lee SM, Protective effects of hyperoside against carbon tetrachloride-induced liver damage in mice. Journal of Natural Products, 74, 2011, 1055-1060.
3. Huang B, Ban X, He J, Tong J, Tian I and Wang Y, Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (Nelumho nucilero Gaertn) leaves. Food Chemistry, 120, 2010, 873-878.
4. Fakurazi S, Sharifudin SA and Arulselvan P, Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules, 17, 2012, 8334-8350.
5. Nayak SS, Jain R and Sahoo AK, Plepatoprotective activity of Glycosmis pentaphyllu against paracetamol-induced hepatotoxicity in Swiss albino mice. Pharmaceutical Biology, 49, 2011, 111-117.
6. Li-Weber M, New therapeutic aspects of havones: the anticancer properties of Sculellaria and its main active constituents. Cancer Treatment Reviews, 35, 2009, 57-68.
7. Kasolo JN, Bimenya CS, Ojok L and Ogwal-okeng JW, Phytochemicals and acute toxicity of Moringa oleifera roots in mice. Journal of pharmacognosy and Phytotherapy, 3, 2011, 38-42.
8. Kumar PV, Sivaraj A, Elumalai EK and Kumar BS, Carbon tetrachloride-induced hepatotoxicity in rats-protective role of aqueous leaf extracts of Coccinia grandis. International Journal of PharmTech Research, 1, 2009, 1612-1615.
9. Castro JA, De Ferreyra EC, De castro CR, Fenoes OM, Sasame H and Gillette JR. Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism-further studies on their mechanism of action. Biochemical Pharmacology, 23, 1974, 295-302.
10. Abdou HS, Salah SH, Booles HF and Abdel Rahim EA, Antioxidant effect of celery against carbon tetrachloride induced hepatic damage in rats. African Journal of Microbiology Research, 6, 2012a, 5657-5667.
11. Aboul-Soud MAM, Al-Othman AM, El-Desoky GE, Al-Othman ZA, Yusuf K, Ahmad J, Al-Khedhairy AA. Hepatoprotective Effects of Vitamin E/Selenium against Malathion-Induced Injuries on the antioxidant Status and Apoptosis-Related Gene Expression in Rats. J. Toxicol. Sci., 36, 2011, 285-296.

12. Atessahin A, Yilmaz S, Karahan I, Pirincci I, Tasdemir B. The effects of vitamin E and selenium on cypermethrin-induced oxidative stress in rats. Turk. J. Vet. Anim. Sci., 29, 2005, 385–391.
13. Schrauzer GN. Anticarcinogenic effects of selenium. Cell Mol Life Sci; 57, 2000, 1864-1873.
14. Schrauzer GN. Nutritional selenium supplements: product types, quality, and safety. J Am Coll Nutr; 20, 2001, 1-4.
15. Shamberger RJ, Frost DV. Possible protective effect of selenium against human cancer. Can Med Assoc J; 100, 1969, 682.
16. Zhang M, Song G, Minuk GY. Effects of hepatic stimulator substance, herbal medicine, Selenium/Vitamin E, and ciprofloxacin on cirrhosis in the rat. Gastroenterology; 110, 1996, 1150-1155.
17. Ianas O, Olinescu R, Badescu I, Simionescu L, Popovici D. The influence of "selenium organicum" upon the hepatic function of carbon tetrachloride poisoned rats. Rom J Intern Med; 33, 1995, 113-120.
18. Wyse R.D., Dunbar G.L., and Rossignol J. Use of genetically modified stem cells to treat neurodegenerative diseases. Int. J. Mol. Sci., 15, 2014, 1719–1745.
19. Taran R., Mamid M.K., Singh G., Dutta S., Parhar I.S., John J.P., Bhonde R., Pal R., and Das A.K. *In vitro* and *in vivo* neurogenic potential of mesenchymal stem cells isolated from different sources. J. Biosci, 39(1), 2014, 157–169.
20. Nauta A.J. and Fibbe W.E. "Immunomodulatory properties of mesenchymal stromal cells. Blood, 110, 2007, 3499–3506.
21. Ahmed H. H., Metwally F. M., Khalil W.K.B., Aglan H. A. Bone Marrow Derived Mesenchymal Stem Cells: A Unique Cytotherapy for Rescuing Degenerated Dopaminergic Neurons Neurochemical Journal, 9(4), 2015, 284–294.
22. Ankrum J. and Karp J.M. Mesenchymal stem cell therapy: Two steps forward, one step back. Trends Mol. Med, 16(5), 2010, 203–209.
23. Mahmood A1, Lu D, Qu C, Goussev A, Chopp M. Treatment of traumatic brain injury with a combination therapy of marrow stromal cells and atorvastatin in rats. Neurosurgery, 60(3), 2007, 546-53.
24. Lu D., Mahmood A., Wang L., Li Y., Lu M., and Chopp M. Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome Neuroreport, 12, 2001, 559–563.
25. Zuk P.A., Zhu M., Mizuno H., Huang J., Futrell J.W., Katz A.J., Benhaim P., Lorenz H.P., and Hedrick M.H. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng, 7, 2001, 211–228.
26. Bieback K., Kern S., Klüter H., and Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells Dayt Ohio, 22, 2004, 625–634.
27. Dharmasaroja P. Bone marrow-derived mesenchymal stem cells for the treatment of ischemic stroke J. Clin. Neurosci, 16, 2009, 12–20.
28. Włodarczyk B, Minta M, Biernacki B, Szkoda J, Żmudzki J. Selenium Protection against Cadmium Toxicity in Hamster Embryos. Polish Journal of Environmental Studies, 9(4), 2000, 323-327.
29. Villalba M., A. Martinez-Serrano, C. Borner, P. Blanco and J. Satrustegui,. NMDA-induced increase in [Ca2+](i) and 45Ca2+ uptake in acutely dissociated brain cells derived from adult rats. Brain Research, 570(1-2), 1992, 347–353.
30. Deng Y., Z.F. Xu, W. Liu, B. Xu, H.B. Yang and Y.G. Wei,. Riluzole-triggered GSH synthesis via activation of glutamate transporters to antagonize methylmercury-induced oxidative stress in rat cerebral cortex. Oxid Med Cell Longev, 2012, 534705.
31. Lu T., Y. Xu, M.T. Mericle and R.L. Mellgren, 2002. Participation of the conventional calpains in apoptosis. Biochimica et Biophysica Acta, 1590, 16-26.
32. Miranda M.V., H. M. Fernandez Lahore and O. Cascone. Horseradish peroxidase extraction and purification by aqueous two-phase partition. Appl Biochem Biotechnol, 53, 1995, 147–154.
33. Abdou HS, Salah SH, Booles HF. Abdel Rahim EA. Effect of pomegranate pretreatment on genotoxicity and hepatotoxicity induced by carbon tetrachloride (CCL4) in male rats. Journal of Medicinal Plants Research; 6(17), 2012b, 3370-3380.
34. Eastman A, Barry MA. The origins of DNA breaks: a consequence of DNA damage, DNA repair, or apoptosis? Cancer Invest; 10(3), 1992, 229-240.
35. Gruebele A, Zawaski K, Kaplan D, Novak R. Cytochrome P450 2E1- and cytochrome P4502B1/2B2-catalyzed carbon tetrachloride metabolism: effects on signal transduction as demonstrated by altered immediate-early (c-Fos and c-Jun) gene expression and nuclear AP-1 and NFKB transcription factor levels. Drug Metabol Disp; 24, 1996, 15-22.
36. Muriel P, Alba N, Perez-Alvarez VM, Shibayama M, Tsutsumi VK. Kupffer cell inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp. Biochem. Physiol. C Toxicol Pharmaco; 130, 2001, 219-226.
37. Weber LWD, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. Critical Reviews in Toxicology; 33(2), 2003, 105-136.
38. Saba AB, Oyagbemi AA, Azeez OI. Ameioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of Cnidoscolus conitifolius in rats. Nig. J. Physiol Sci; 25, 2010, 139-147.
39. Atienzar FA, Venier P, Jha AN, Depledge MH. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutat Res; 521, 2002, 151-163.
40. Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in Daphnia magna exposed to benzo[a]pyrene. Environ Toxicol Chem; 18, 1999, 2275-2282.

41. Yin Z., D. Milatovic, J. L. Aschner, T. Syversen, J. B. Rocha, D. O. Souza, M. Sidoryk, J. Albrecht and M. Aschner, Methylmercury induces oxidative injury, alterations in permeability and glutamine transport in cultured astrocytes. Brain Res, 1131(1), 2007, 1-10.

42. Chen L, Zhou J, Gao W, Jiang YZ. Action of NO and TNF alpha release of rats with cadmium loading in malfunction of multiple system organ. Shenli Xuebao; 55, 2003, 535-540.

43. Kimura K, Bowen C, Spiegel S, Gelmann EP. Tumor necrosis factor-alpha sensitizes prostate cancer cells to gamma-irradiation-induced apoptosis. Cancer Res; 59, 1999, 1606-1614.

44. Karck U, Peters T, Decker K. The release of tumor necrosis factor from endotoxin-stimulated rat Kupffer cells is regulated by prostaglandin E2 and dexamethasone. J Hepatol; 7, 1988, 352-361.

45. Al-Shabanah OA, Alam K, Nagi MN, Al-Rikabi AC, Al- Bekairi AM. Protective effect of aminoguanidine, a nitric oxide synthase inhibitor, against carbon tetrachloride induced hepatotoxicity in mice. Life Sci; 66, 2000, 265-270.

46. Hoebe KH, Witkamp RF, Fink-Gremmels J, Van Miert AS, Monshouwer M. Direct cell-to-cell contact between Kupffer cells and hepatocytes augments endotoxin-induced hepatic injury. Am J Physiol Gastrointest Liver Physiol; 280, 2001, G720-728.

Source of Support: Nil, **Conflict of Interest:** None.