

# The stem cell mobilizer StemEnhance reduces fibrosis and enhances proliferation in Thioacetamide induced-liver cirrhosis in the adult male albino rat

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## SUMMARY

StemEnhance (SE) is a haematopoietic stem cell mobilizer deduced from natural plant products. Preclinical studies showed that SE advances cardiovascular muscle recovery and relieves the symptoms and signs of diabetes. However, the efficacy of SE in improving liver cirrhosis has not been investigated. This study was done to assess the helpful effects of SE in a Thioacetamide (TAA)-induced rodent model of liver cirrhosis. Thirty adult male albino rats, 16~18 weeks old and 210~240 gm weight, were used in this study. They were divided equally into three groups: 1. normal control group: received saline intraperitoneal injection (i.p.) twice weekly for 12 weeks and served as a control; 2. cirrhosis group: liver cirrhosis was induced by (i.p.) injection of TAA (200 mg/kg body weight) twice a week for 8 weeks and left untreated; 3. cirrhosis/SE group: liver cirrhosis was induced the same way and then treated daily orally with SE at a dose of 300 mg/kg body weight dissolved in distilled water for 4 weeks. All the studied groups were sacrificed 12 weeks after beginning experiments. SE was found to alleviate hepatic fibrosis, improve histopathological changes and enhance hepatocyte proliferation. In addition, TNF- $\alpha$  mRNA expression was down-regulated in TAA-induced cirrhotic livers after SE administration. We

conclude that SE has a beneficial role in relieving liver fibrosis and improving liver function in the rat model of liver cirrhosis.

**Key words:** Liver cirrhosis – Thioacetamide – StemEnhance – TNF- $\alpha$

## INTRODUCTION

The liver is a vital organ that plays a key role in the detoxification of exogenous and endogenous substances. It also performs a wide range of metabolic activities required for the homeostasis, the nutrition and the immune defense (Lee et al., 2010; Raju et al., 2012). The liver fibrosis occurs as a result of chronic injury leading to excessive accumulation of the extracellular matrix and scar tissue formation. If not efficiently treated, the liver fibrosis may lead to cirrhosis, inducing permanent and irreversible damage to the liver structure and function with fatal consequences (Friedman et al., 2013).

The most common cause of the liver cirrhosis is the infection with either hepatitis B or C virus, which represents a major public health problem that affects millions of people worldwide. Studies on the epidemiology of hepatitis C virus (HCV) infections have suggested that Egypt has one of the highest prevalence rates of HCV in the world, with seroprevalence rate of 30-40% in the villagers over the age of 30 years old (Lehman and Wilson, 2009). Liver cirrhosis is the last phase of most the

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chronic hepatic diseases (Sporea et al., 2013). The new restorative methodologies to constrict liver scarring and to improve the liver recovery are liver transplantation (Dhingra et al., 2014), stem cell infusion (Zhang et al., 2012) and bone-marrow-derived haematopoietic stem cells (BM-HSCs) mobilization (Saito et al., 2013).

The bone-marrow-derived haematopoietic stem cells (BM-HSCs) at various stages of differentiation are localized normally in the bone marrow. But at a basal rate, low levels of stem/progenitor cells are released from their niche and circulate into the peripheral blood (Barnes et al., 1967). Many different soluble agents have the ability to mobilize the BM-HSCs from the bone marrow to the peripheral circulation and hence increase their total number (Weissman et al., 2001). A study found significant mobilization of cells expressing hematopoietic stem cell and endothelial progenitor cells (EPC) markers into the peripheral blood by stem cell mobilizers. This study also found that the stem cell mobilization may offer significant benefit in treatment of a wide variety of degenerative diseases (Mirkirova et al., 2010).

One of the important stem cell mobilizers, is the StemEnhance (SE), a natural water-soluble extract of the cyanophyta *Aphanizomenon flos-aquae* (AFA), which was shown to increase the number of circulating BM-HSCs by approximately 25% within 60 min after oral consumption (Jensen et al., 2007). Previous experimental studies reported that mobilization of BM-HSCs with SE promoted muscle regeneration in the cardiotoxin-induced muscle injury (Drapeau et al., 2010) and ameliorated manifestations of diabetes in rats (Ismail et al., 2013). SE was reported in humans to promote tissue repair. Significant improvements in the patient outcomes that associated with a wide variety of health problems linked to tissue damage or degeneration were improved by SE administration (Drapeau et al., 2012).

In the liver, the stem cell mobilizers increased the hepatocyte proliferation and the survival rate in the rodent model of acute liver failure induced by CCL4 (Mark et al., 2010). Clinical studies on humans revealed that the granulocyte-colony stimulating factor (G-CSF), is safe and effective in the mobilization of the hematopoietic stem cells. It improved the liver function and the survival rate in patients with hepatitis B virus-associated acute on chronic liver failure (Duan et al., 2013), and in patients with severe alcoholic hepatitis (Singh et al., 2014). However, the potential effectiveness of SE, as stem cell mobilizer, in ameliorating liver cirrhosis has not been clarified.

Given the possibility that BM-HSCs serve as endogenous "repair cells", and based on the fact that SE stimulates the mobilization of these endogenous BM-HSCs and that these mobilized stem cells have the ability to migrate to the sites of tissue damage and participate in the tissue regenera-

tion, SE may thus provide a promising non-invasive alternative to the exogenous stem cell transplantation. So the aim of the present study is to evaluate the effect of mobilization of BM-HSCs in an experimental model of TAA-induced liver cirrhosis by SE administration and its efficacy on the structure and function of the cirrhotic liver in adult male albino rat.

## MATERIALS AND METHODS

Thirty adult male albino rats (16~18 weeks' old) weighing 210~240 gm were used in this study. The animals were purchased and raised in the Medical Research Center in Minia University. They were housed in plastic cages with mesh wire covers and were given food and water ad libitum. The rats were handled according to the Ethics Committee recommendations of Minia University.

### Experimental groups

- Group I (normal control group): Ten male albino rats received saline intraperitoneal injection (i.p.) twice weekly for 12 weeks and served as a control.

- Group II (cirrhosis group): Ten male albino rats were used. Liver cirrhosis was induced by i.p. injection of Thio-acetamide (TAA) (200 mg/kg body weight) twice a week for 8 weeks (Hori et al. 1993). TAA powder was purchased from Sigma Chemical Company St. Louis, Mo., USA and dissolved in 0.9% saline.

- Group III (cirrhosis/SE group): Ten male albino rats were used. Liver cirrhosis was induced as scheduled above for 8 weeks and then the rats were treated daily orally with the StemEnhance 300 mg/kg body weight dissolved in distilled water by gastric gavage for one month (4 weeks). This dose was equivalent to the human dose of 6 capsules 3,000 mg/day as recommended by StemTech Health Sciences, Inc., UK.

- At the end of the experiment (after 12 weeks of the onset of this experiment), the rats were sacrificed using ether anaesthesia. Liver specimens were collected and processed for histological and immunohistochemical studies and for RNA extraction. Blood samples were also collected for biochemical analysis.

### Biochemical analysis

Blood samples were collected to measure the level of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and the level of Serum albumin. They were determined spectrophotometrically with an automatic analyzer using commercially available kits specific for each test.

### Statistical analysis

Biochemical measurements are expressed as mean  $\pm$  SE. Significant differences were determined by using SPSS 9.0 computer software. Results were considered significant at p value =

≤0.05.

### Histological study

For light microscopic examination and according to Bancroft and Cook (1994), the liver samples were collected and fixed in 10% buffered formalin for 24 hours. The serial 5- $\mu$ m paraffin sections were prepared and stained with hematoxylin and eosin (H& E), Masson Trichrome (MT) and immunohistochemistry. For the electron microscopic studies the liver tissues were collected and made in shapes of about 1 mm $\times$ 1 mm $\times$ 2 mm, fixed with glutaraldehyde and osmic acid, dehydrated with ethanol and embedded with ethoxyline resin then ultrathin sections were made for electron microscope examination.

### Immunohistochemistry

Anti-PCNA immunohistochemical staining was done according to Bancroft and Cook (1994). The paraffin sections were deparaffinized in xylene for 1~2 minutes and then rehydrated in descending grades of ethanol then brought to distilled water for 5 minutes. Sections were incubated in hydrogen peroxide for 30 minutes then rinsed in PBS (3 times, 2 minutes each). Each section was incubated for 60 minutes with 2 drops (=100  $\mu$ l) of the primary antibody anti-PCNA, Clone QBEnd/10 (Lab Vision Corporation Laboratories, CA 94539, USA). The slides were rinsed well in PBS (3 times, 2 min. each), incubated for 20 minutes with 2 drops of biotinylated secondary antibody for each section, then rinsed well with PBS. Each section was incubated with 2 drops (100  $\mu$ l) enzyme conjugate "streptavidin- horseradish peroxidase" for 10 minutes at room temperature, then washed in PBS. The substrate-chromogen (DAB) mixture, 2 drops was applied to each of the sections and incubated at room temperature for 5~10 min. then rinsed well with distilled water. The slides were counterstained with hematoxylin and dehydrated. The slides were mounted with aqueous mounting media (glycerine), 2 drops to each slide and covered with a coverslip. All the steps were performed in a humidity chamber to prevent drying of the tis-

sues. PCNA+ve cells showed brown deposits.

Semi-quantitative RT-PCR for *TNF- $\alpha$*  mRNA

RNA was extracted from five liver specimens in each of the study groups by the use of Gene JET™ RNA extraction kit (#K0731, Fermentas) according to the manufacturer's instructions. Purified RNA preparations were converted into first strand cDNA by the use of RevertAid™ First Strand cDNA Synthesis Kit (#K1631, Fermentas). The synthesized cDNA was diluted and amplified by Maxima Hot start Master mix (Fermentas) for 30 cycles using the designed *TNF- $\alpha$*  specific primers (forward: 5'-CTTCTCCTTCTGATCGTGG-3' and reverse:5'-CCCTGGGGAAGTCTTCCCTCT-3') and the GAPDH specific primers (Forward: 5'-CAAGGTCATCCATGACAACCTTTG-3' and reverse: 5'- GTCCACCACCCTGTTGCTGTAG-3'). The *TNF- $\alpha$*  and GAPDH amplification products of the same sample were loaded together in the same well of the gel. *TNF- $\alpha$*  amplicons were quantitated relative to GAPDH amplicons using Image J software.

## RESULTS

### SE improves liver function in the TAA-treated liver

This study showed that the serum ALT and AST levels were increased significantly ( $P < 0.05$ ) in the cirrhosis group compared to the normal control group, while these enzyme values were significantly decreased in the cirrhosis/SE group and returned to the normal levels comparable to that of the control (T-test: non-significant differences) (Table 1). The mean values of the serum albumin and the serum total protein were significantly decreased in the cirrhosis group compared to the normal control group, while these values were significantly increased by SE administration compared to the cirrhotic one (Table 1).

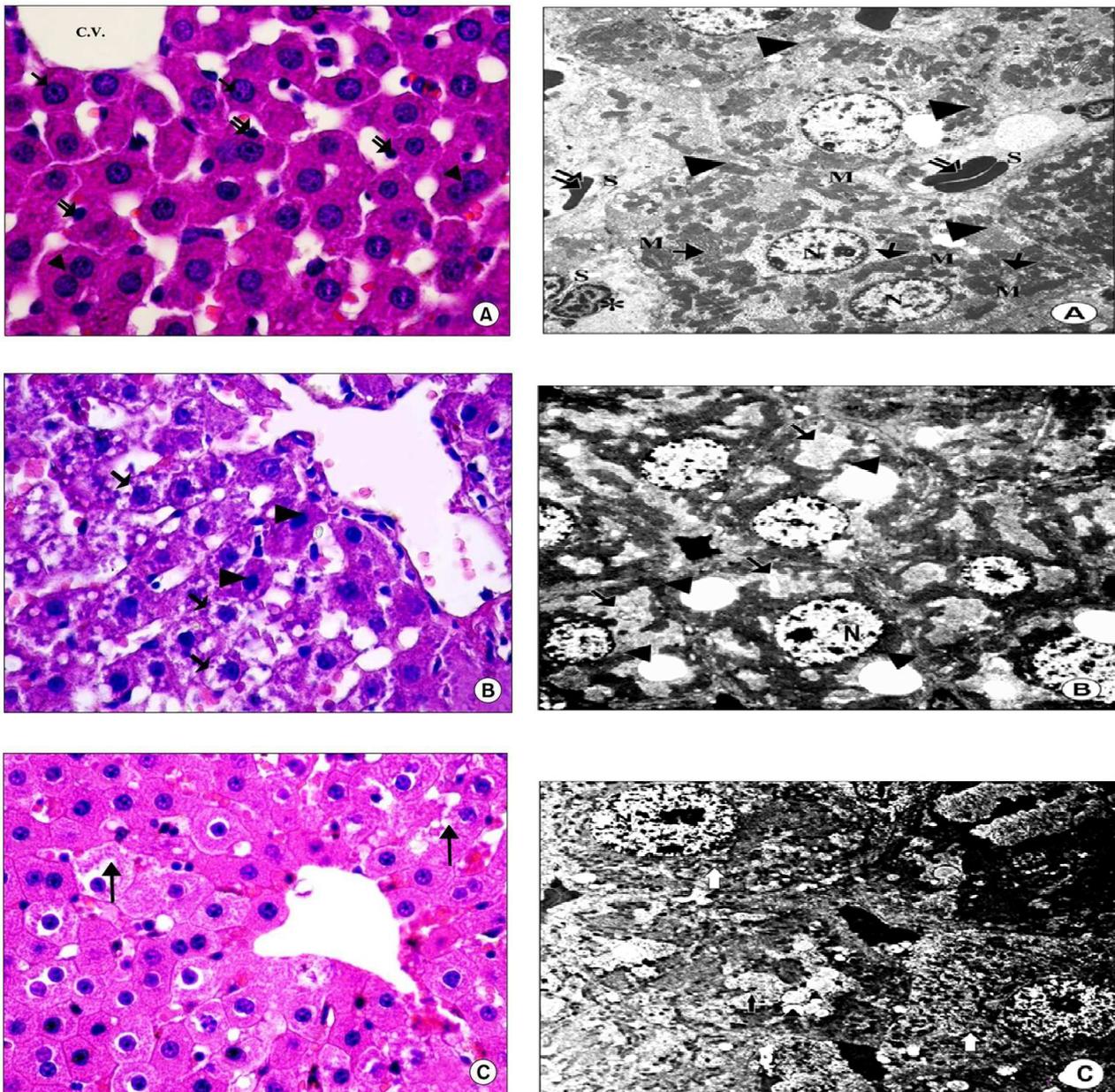
### SE ameliorates the histopathological changes in the TAA-treated liver

Light microscopic examination of the liver section stained by H&E of all rats of the normal control

**Table 1.** Liver functions in the studied groups

Groups	Serum ALT (U/l)	Serum AST (U/l)	Albumin (g/dl)	Total protein (g/dl)	Globulin (g/dl)	Albumin/globulin ratio
<b>Normal control</b>						
Mean $\pm$ SE	38 $\pm$ 0.33	84 $\pm$ 0.58	3.8 $\pm$ 0.04	7.6 $\pm$ 0.1	3.4 $\pm$ 0.1	1.0 $\pm$ 0.04
<b>Cirrhosis</b>						
Mean $\pm$ SE	90 $\pm$ 0.84	133 $\pm$ 1.15	2.5 $\pm$ 0.04	7.1 $\pm$ 0.12	2.9 $\pm$ 0.1	0.86 $\pm$ 0.03
<i>P</i> value (control Vs. cirrhosis)	0.001*	0.001*	0.002*	0.002*	0.005.	0.004*
<b>Cirrhosis/SE</b>						
Mean $\pm$ SE	35 $\pm$ 0.74	86 $\pm$ 1.21	3.9 $\pm$ 0.04	8.2 $\pm$ 0.13	4.2 $\pm$ 0.13	0.91 $\pm$ 0.04
<i>P</i> value (cirrhosis/SE Vs. Control)	NS	NS	NS	NS	NS	NS

\*= Significant i.e.  $P$  value  $<$  0.05 . NS= not significant.



**Fig. 1 (left column). Histological examination of the liver sections of the studied groups.** In the normal control (A), radiating regular cords of hepatocytes from the central vein (C.V.) are seen. The hepatocytes have central, rounded, vesicular nuclei (↑) and acidophilic cytoplasm. Some of the cells appear bi-nucleated (▲). Note the lining endothelial cells (arrows) of the blood sinusoids. (B) The cirrhosis group: most of the hepatocytes are vacuolated (arrows). Few hepatocytes appear with acidophilic cytoplasm and deeply stained nuclei (▲). In the cirrhosis/SE group (C), most of the hepatocytes have granular acidophilic cytoplasm and vesicular nuclei. Only few cells show cytoplasmic vacuolation (↑) in this group. (H&E stain; magnification x720 in A, x560 in B&C).

**Fig. 2 (right column). Electron microscopic examination of ultrathin sections of the liver of the studied groups.** In (A) normal control group shows adjacent hepatocytes with sinusoidal spaces (S) in-between. Hepatic satellite cells (HSCs) (\*) are seen in the peri-sinusoidal space. The hepatocytes appear with large rounded, central vesicular nuclei (N). The nuclei show usual characteristic chromatin distribution. The cytoplasm contains numerous mitochondria (M), rER (arrows) and fat droplets (▲). In (B) the cirrhosis group is showing adjacent hepatocytes. The nuclei of the hepatocytes, in this group, show abnormal chromatin distribution (N). The cytoplasm contains multiple vacuoles (arrows) and fat droplets (▲). In the cirrhosis/SE group (C) the hepatocytes (white) are comparable in morphology to that of the normal control group. They contain many mitochondria and show usual distribution of chromatin in their nuclei (N). Small vacuoles (arrows) and few fat droplets (▲) are seen in some hepatocytes (x4050 in A, B, C).

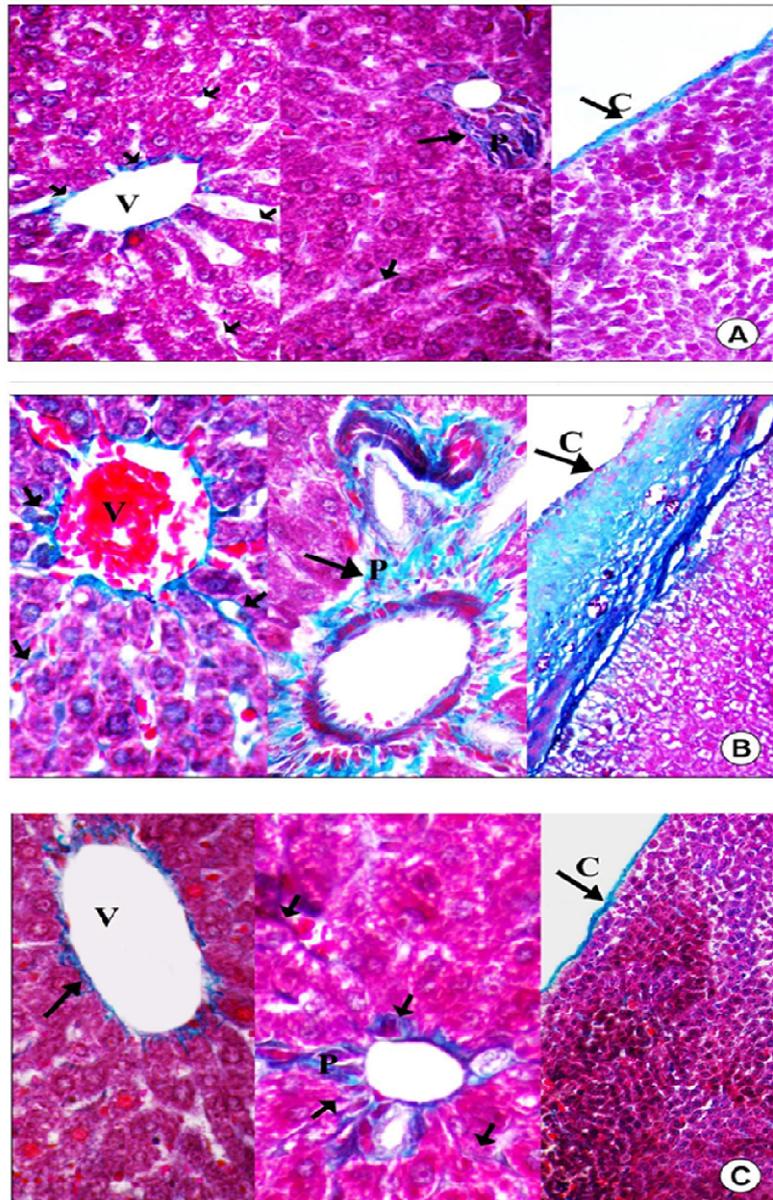
showed nearly the same histological picture. In the normal control liver (Fig. 1A), the hepatocytes were arranged in the form of branching and anastomosing cords that radiate from the central veins and were separated by blood sinusoids, which were lined by flat endothelial cells. The hepatocytes showed acidophilic cytoplasm with single central rounded vesicular nuclei and some of the cells were binucleated (Fig. 1A). In the cirrhosis group, most of the hepatocytes contained multiple, large cytoplasmic vacuoles. Some hepatocytes had deeply acidophilic cytoplasm and deeply stained nuclei (Fig. 1B). In the cirrhosis/SE group, a remarkable improvement in the hepatocytes morphology was noticed. Also, in this group the hepatocytes appeared nearly similar to that of the normal control rats. Only few hepatocytes showed slight vacuolated cytoplasm in this group (Fig. 1C).

Electron microscopic examination of the ultrathin sections of the liver showed in the normal control group (Fig. 2A) adjacent hepatocytes with sinusoidal spaces in-between. The hepatic stellate cells (HSCs) were seen in the peri-sinusoidal space. The hepatocytes appear with large rounded, central vesicular nuclei (N). The nuclei showed the usual characteristic chromatin distribution. The cytoplasm contained numerous mitochondria and prominent rough endoplasmic reticulum (rER), as well as few fat droplets (Fig. 2A). In the cirrhosis group (Fig. 2B), the hepatocytes showed large vacuoles and many fat droplets in the cytoplasm. An apparent reduction of mitochondria and rER were also observed. The nuclei showed abnormal distribution of the chromatin in this group (Fig. 2B), while the liver of the cirrhosis/SE group (Fig. 2C), showed that the ultrastructure of most of the hepatocytes was nearly comparable to that of the normal control

group. The hepatocytes showed numerous mitochondria, rER, and glycogen granules. Only some hepatocytes contained small vacuoles and few fat droplets (Fig. 2C).

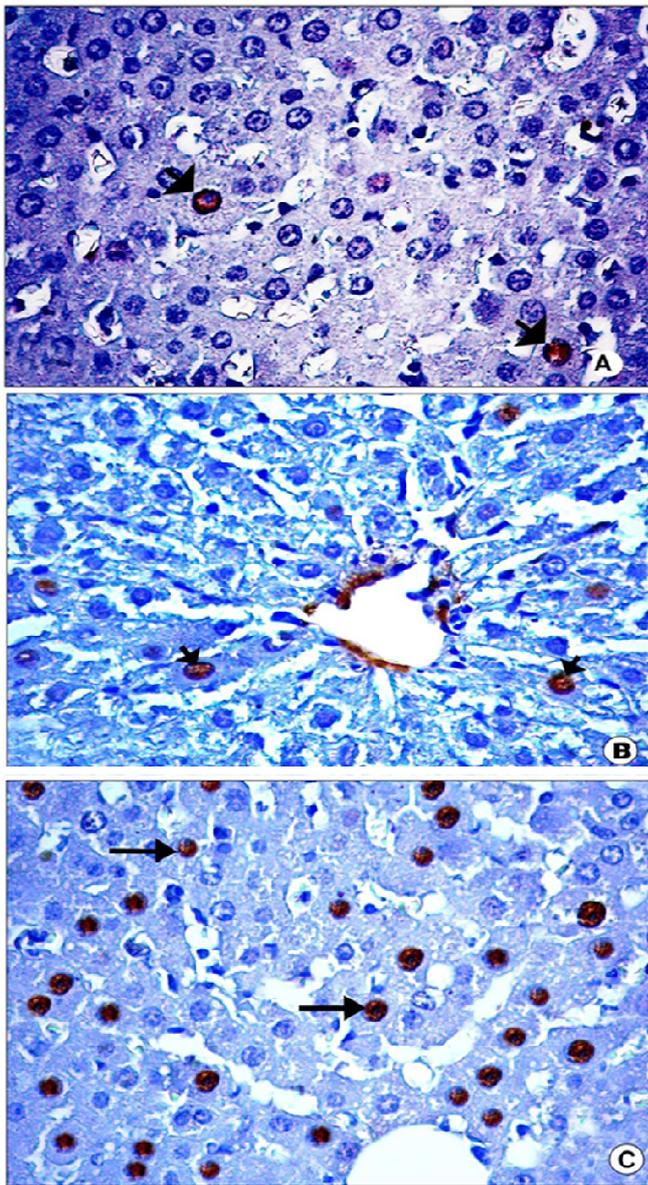
#### **SE has an antifibrogenic effect in the TAA-treated liver**

Light microscopic examination of Masson's trichrome stained sections of the liver was used to compare the amount of collagen fibers in all the studied groups. The parenchyma of the liver in the normal control appeared to be supported with



**Fig. 3. Examination of fibrosis in the liver sections of the studied groups.** In the normal control group (A) few collagen fibers (arrows) surrounding the central vein (V), in portal area (P) and in the capsule (C) were observed. In the cirrhosis group (B) there are numerous collagen fibers (arrows) surrounding the central veins (V), portal area (P) and the capsule (C). In (C) the collagen fibers of the cirrhosis/SE group are more or less comparable to that of the normal control group. (magnification: A,  $\times 400$ ; B,  $\times 400$ ; C,  $\times 160$ ).

a stroma of very delicate meshwork of collagenous fibers. Also few collagenous fibers surrounding the central veins, the portal area and the capsule were seen (Fig. 3A). In the cirrhosis group, the stroma was well defined. There was thick connective tissue capsule. There was an increase in the collagen fibers around the central veins, in between the hepatocyte cords and in the portal areas in the cirrhosis group (Fig. 3B). However, in the cirrhosis/SE group, few collagen fibers were detected. The amount of the collagen fibers were more or less comparable to that of the normal control group

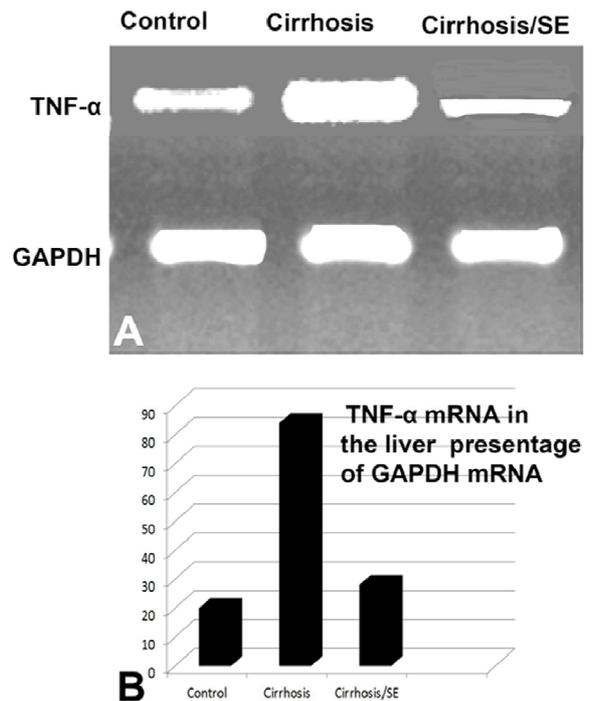


**Fig. 4. Immunohistochemistry for the hepatocyte proliferation in the studied groups.** In (A) few hepatocytes are positive for PCNA immunoreaction (brown in color; arrows) in the normal control group. (B) A few hepatocytes show positive immune reaction for PCNA (arrow) in the cirrhosis group. (C) Many PCNA positive immuno-reactive hepatocytes (arrows) are apparent in the cirrhosis/SE group. (Anti-PCNA immunostaining; magnification,  $\times 560$  in (A,B,C).

(Fig. 3C).

#### **SE promotes proliferation in the hepatocytes of the TAA- treated liver**

The cell proliferation marker Anti-PCNA immunostaining of the liver sections was done to compare the rate of hepatocyte proliferation in the studied groups. Few hepatocytes had a positive nuclear reaction in both the normal control group (Fig. 4A), and the cirrhosis group (Fig. 4B), while the positive PCNA hepatocytes were many in the cirrhosis/SE group (Fig. 4C), indicating increased hepatocyte proliferation by SE treatment in the cirrhotic liver.



**Fig. 5. TNF- $\alpha$  mRNA expression in the liver of the studied groups.** In (A), RT-PCR of the liver shows that the relative expression of TNF- $\alpha$  mRNA in the cirrhosis group is much higher than that of the normal control. While the relative expression of TNF- $\alpha$  mRNA in cirrhosis/SE group is more or less comparable to that of the normal control (A). In (B) the histogram shows that the mean value of the relative TNF- $\alpha$  mRNA expression in the cirrhosis group was 84% compared to 20% in the normal control liver and the difference between the two groups was statistically significant by T-test ( $p=0.0001$ ). In the cirrhosis/SE group the TNF- $\alpha$  is down-regulated (A) and becomes 28% (B) (T-test between the cirrhosis group and cirrhosis/SE shows P value  $<0.003$  and t-test between the normal control and the cirrhosis/SE shows non-significant differences).

#### **SE down-regulates TNF- $\alpha$ mRNA expression in the TAA-treated liver**

To compare the expression of TNF- $\alpha$  mRNA in the studied groups, semi-quantitative RT-PCR was done. A primer pair was designed specifically to amplify the TNF- $\alpha$  cDNA and was used to compare TNF- $\alpha$  mRNA expression relative to GAPDH mRNA expression. In the majority of samples, the relative expression of TNF- $\alpha$  mRNA is upregulated in the cirrhosis group and it was much higher than that of the normal control group (Fig. 5). The mean value of relative TNF- $\alpha$  mRNA expression in the cirrhosis group was 84% compared to 20% in the normal control liver. The difference was statistically significant by T-test ( $p$  value = 0.0001). In the cirrhosis/SE group, the TNF- $\alpha$  is down-regulated and becomes 28% (T-test with the cirrhosis group shows P value  $<0.003$  and T-test with the normal control shows non-signifi-

cant difference).

## DISCUSSION

Bone marrow mesenchymal stem cells (BM-MSCs) were described as multipotent cells because of their ability for differentiation into a variety of cells and tissue lineages (Zhang et al., 2012). They could also differentiate into functional hepatocyte-like cells (Dong et al., 2013). Previous studies detected that acute injury such as myocardial infarction (Wojakowski and Tendera 2005; Xin et al. 2008) and stroke (Sobrinho et al. 2007) were associated with up-regulated levels of these cells.

In this study on the liver and four weeks after finishing the TAA administration, light microscopic examination of the liver revealed loss of the usual hepatic architecture. Many vacuoles with dark stained nuclei in most of the liver cells were detected. Moreover, few mitochondria, few rER, many fat droplets and large irregular vacuoles were also observed by electron microscopic examination. This structural damage was due to edema of the organelles (Tasci et al. 2008), and it explained the significant increase in the serum AST and ALT and significant decrease in the serum albumin, which were observed in the present work in the cirrhosis group, as compared to the normal control one. The ALT and AST are useful serum markers for inflammation and necrosis of the liver (Cheung et al., 2006). Also, in the present work, the liver fibrosis was clearly evidenced by a significant increase in the areas of the collagen fibers by Masson trichrome staining of the TAA-induced liver cirrhosis sections.

In this study, the light and the electron microscopic examination of the cirrhosis/SE group revealed that the administration of SE resulted in an improvement of the liver structure. Most of the hepatocytes appeared nearly as those of the normal control group. Non-significant change was noticed in the level of the liver enzymes (ALT, AST) and albumin in the cirrhosis/SE group. Thus, the liver function in cirrhosis/SE group was nearly normal when compared to that of the normal control group. These results indicated that SE improved the histopathological changes and regained the normal function in the TAA-induced liver cirrhosis. Similarly, a previous study showed that the transplanted BM-MSCs could restore the serum albumin level and significantly suppressed the transaminase activity and the liver fibrosis in the injured liver of the rats. BM-MSCs differentiated into hepatic oval cells and then to hepatocyte-like cells. The damaged hepatocytes were repaired and the fibrosis was resolved, resulting in an overall improvement in the liver functions (Oyagi et al., 2006).

In the present study, the liver fibrosis was significantly relieved after SE administration in cirrhosis/SE group as compared to the cirrhosis group. Pre-

vious study showed that BM-MSCs ameliorated liver fibrosis by down-regulating the profibrotic genes and upregulating anti-fibrotic hepatic genes (Ali et al. 2012).

Anti-PCNA immune-staining technique was used in the present study to detect the presence of proliferating liver cells. The PCNA positively stained hepatocytes were significantly higher in number in the cirrhosis/SE group when compared to that of normal control and the cirrhosis groups. This result could be explained by the previous findings that reported that the normal hepatocytes were generally quiescent and replicate in a limited and regulated manner. The replicative activity of hepatocytes diminishes in advanced cirrhosis in humans and in chronic liver injury in mouse, reaching finally to a state of replicative senescence (Cheung et al., 2006). Bone marrow stem cell mobilization may enhance the intrinsic capability of hepatocytes to proliferate by releasing of proliferative cytokines and/or reducing fibrosis, thereby removing the block in the way of the hepatocyte proliferation (Wang et al., 2010). A previous study also revealed that the infusion of BM-MSCs facilitated the proliferation of hepatocytes after massive hepatectomy in rats. The increased proliferation of hepatocytes are reflected by the elevated PCNA-positive cells in the liver (Yu et al., 2013).

Tumor necrosis factor (TNF)- $\alpha$  is a pleiotropic cytokine with diverse biological effects on all mammalian cells. TNF- $\alpha$  is considered the central mediator in the liver damage and its expression is up-regulated in the liver injury induced by a variety of hepatotoxic agents (Le et al., 1987). In the present study the TNF- $\alpha$  was up-regulated in cirrhotic liver of the rat, however it was down-regulated in the liver of cirrhosis/SE group. In the previous studies down-regulation of pro-inflammatory cytokines, such as TNF- $\alpha$ , has been described in kidney, lung injury and fulminant hepatic failure models after bone marrow stem cells transplantation (Togel et al., 2005 and Ortiz et al., 2007). Furthermore, TNF- $\alpha$  signal is important for regulating the improvement of the liver fibrosis after bone marrow cell infusion (Hisanaga et al., 2011). These results, along with the present findings, suggest that mobilized bone marrow stem cells down-regulate pro-inflammatory cytokines, such as TNF- $\alpha$  in the TAA-induced liver cirrhosis.

Previous experimental studies reported that mobilization of BM-HSCs with SE promoted muscle regeneration in cardiotoxin-induced muscle injury (Drapeau et al., 2010) and ameliorated manifestations of diabetes in rats (Ismail et al., 2013). Recently, a study done by using SE on a TAA-induced mouse model of liver cirrhosis revealed that SE mobilized the CD34-positive cells in the peripheral blood. SE improved the histopathological changes and had a protective effect on liver function. SE also enhanced endogenous hepatic

proliferation in the cirrhotic mouse liver. SE upregulated vascular endothelial growth factor (VEGF) and downregulated the TNF- $\alpha$  expression in the TAA-induced liver cirrhosis in the mouse (El-Akabawy, and El-Mehi . 2015).

In the present study, SE administration improved liver function, reduced fibrosis, and ameliorated histological alterations in TAA-injured livers. In accordance with these findings, the granulocyte-colony stimulating factor (G-CSF) treatment, a common BM-HSC mobilizer, significantly improved survival and liver histology in chemically injured animals (Yannaki et al., 2005, Quintana-Bustamante et al., 2006, Mark et al., 2010 and Tsolaki et al., 2014). A clinical trial on the humans showed that the patients with advanced decompensated liver cirrhosis who were treated with a course of BM-HSC mobilizer, G-CSF, had either an improved or a stable liver function than those no treated with it (Gaia et al., 2013). Another clinical trial revealed that G-CSF therapy promoted CD34(+) cell mobilization in patients with hepatitis B virus-associated acute on chronic liver failure, and improved the liver function and the survival rate of those patients (Duan et al., 2013).

### Conclusion

Bone marrow stem cell mobilizer StemEnhance (SE) administration in a Thioacetamide-induced rat model of liver cirrhosis revealed that SE had a protective effect on liver function and improved histopathological changes. SE enhanced endogenous hepatic proliferation and down-regulated TNF- $\alpha$  expression.

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