Experimental pathological studies on the ability of Stem cells for regeneration of hepatocytes in rats

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A B S T R A C T

Stem cell therapy is an important catigory of regenerative medicine has the potential to provide a valuable adjunct and alternative to liver transplantation with treatment of liver fibrosis and cirrhosis. In this approch, 70 male albino rats were randomly divided into 4 groups: Group 1: Control group (20 rats), Group 2: TAA-treated group (20 rats), Group 3: Mesenchymal stem cells treated group (15 rats) and Group 4: TAA- Mesenchymal stem cell –treated group (15 rats). Our results showed that TAA is significantly increase the activities of ALT, AST, and ALP and reduced the levels of total protein, albumin and globulins in the serum. Moreover, TAA successfully induced liver cirrhosis characterized by coarse nodular liver surface with severe fibrous connective tissue proliferation led to pseudolobulation of hepatic parenchyma. Meanwhile, after treatment with Mesenchymal stem cells (MSCs) the liver of the rats was grossly nearly normal and microscopically thin strands of fibrous connective tissue in between the hepatic lobules with mild degenerative and necrotic changes and significant improvement in the liver blood parameters. These findings concluded that MSCs have ameliorative effect on chemically induced liver cirrhosis through their regenerative capacity and antifibrotic effect.

Keywords: Liver cirrhosis, TAA, Antifibrotic, MSCs

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1. INTRODUCTION:

Hepatotoxicity is a chemical-driven liver damage. There are many chemical agents that cause hepatotoxicity e.g. carbon tetrachloride, thioacetamide, galactosamine and alcohol. TAA was first reported as a hepatotoxic agent by Fitzhugh and Nelson, (1948) and it has some advantages as a model hepatotoxin consist in its high specifity for the liver, regiono-specifity for the perivenous area and long time between its necrogenic effects and liver failure (Chilakapati et al., 2005 and Mehendale, 2005). TAA chronic intoxication was established as a reliable and reproducible experimental model of fibrosis and cirrhosis in rodents by either the oral or intraperitoneal (IP) routes (Zimmermann *et al.*, 1986 and Muñoz *et al.*, 1991). In addition, TAA is a well-accepted animal model induced hepatic diseases which perfectly mimic human chronic hepatic diseases, including fibrosis and cirrhosis (Huang *et al.*, 2012). Recent treatments for cirrhosis are limited to remove the underlying injurious stimulus and liver transplantation. Transplantation is a highly successful treatment for end stage cirrhosis, with a 75% five-year survival rate. But limited availability of organs, growing lists of patients needing a transplant, issues of compatibility, high risks associated with surgery and comorbid factors mean that not everyone is eligible for transplantation. As a result, effective antifibrotic treatments are needed urgently (Iredale, 2003a). Nowadays, mesenchymal stem cells (MSCs) found to serve as a potentially relevant therapeutic agents for the treatment of liver diseases because of their potential to differentiate into hepatocytes, suppress the pathophysiological process that is mediated by chronic inflammation, and this immunosuppressive mechanism contributes to a modification of the microenvironment, and ability to secrete trophic factors diminished tissue fibrosis, increased resident stem cell proliferation and eventually tissue regeneration (Sun et al., 2011 and Eom et al., 2015). So herein our aim were to investigate the role of TAA as hepatotoxic agent and its ability for induction of liver cirrhosis, besides, to evaluation the role of BM-MSCs in treatment of liver cirrhosis caused by TAA.

2. MATERIALS AND METHODS:

2.1. Experimental design:

This study was done on 70 male albino rats randomly divided into 4 groups as following: Group 1: Control group (20 rats) received physiological saline 1ml I/P for 8 weeks. Group 2: TAA-treated group (20 rats) received TAA 200 mg/kg body weight I/P three times a week for 8 weeks (Kasahara, 1977). Group 3: mesenchymal stem cells-treated group (15 rats) treated with single dose of $3x10^6$ BM- MSCs per rat by intravenous infusion at tail vein Group 4: TAA-mesenchymal stem cell –treated group (15 rats) received TAA in dose of 200 mg/kg body weight three times a week for 8 weeks then treated with single dose of $3x10^6$ BM- MSCs per rat by intravenous infusion at tail vein (Lee et al., 2009). Five rats from group 1 and group 2 were sacrificed after 8 weeks post administration to ensure induction of liver cirrhosis and five rats from each of four groups were sacrificed after 10, 12 and 14 weeks post treatment. Serum sample and liver sample were collected for biochemical and histopathological examination.

2.2. Determination of liver functions in serum:

Serum ALT and AST were determined according to Bergmeyer et al., (1978), also ALP according to Bowers and McComb, (1966). Meanwhile, total protein and serum Albumin levels were estimated according to Weichselbaum, (1946) and Doumas *et al.*, (1997) respectively. Subsequently, globulin and A/G ratio were calculated.

2.3. Histopathological Examination:

Specimens from the liver tissue were taken, fixed in neutral buffered formalin solution (10%). After proper fixation, the specimens were trimmed, washed in running tap water, dehydrated in different ascending grades of ethyl alcohol, cleared in xylene, embedded in paraffin. Thin sections at 5 µm were done then stained hematoxylin and eosin stain according to Bancroft et al., (1996) and Crossman trichrome stain according to Gomori, (1950).

2.4. Stem cell isolation:

6 weeks-old male rats were anesthetized then sacrificed by head dislocation then hind limbs were harvested. Limbs were removed above the hip and below the ankle and the collected bones were washed by 70% alcohol for 30 seconds, then by PBS (1) 1 minute and finally by PBS (2) for 2-5minutes. A 22-gauge needle was inserted into tibia's modularly canal then bone marrow samples were taken then added to 5 ml (DMEM) supplemented by 15% FBS, 100U/ml penicillin and 100U/ml streptomycin (complete media). Then washed by centrifugation at 1200 rpm for 5minute. The cell pellet was collected and cultured in a 75-cm3 flask in a DMEM medium supplemented by 15% FBS and 100U/ml penicillin and 100U/ml streptomycin then the cultures were incubated at 37 C° in a 5% CO2 environment. After three days, flasks were examined under the microscope and media was changed then the media was changed every 3 days. Upon confluence (70%-80%), the cells were lifted by Trips in EDTA using tissue scraper. The viability of the cells tested by using Geimsa stain, as only viable cells could be stained. The cells were counted by using a flow cytometer as described by Aziz et al., (2007). Cells were suspended in phosphate buffer solution (PBS) at a concentration of $3x10^6$ cells/mL.

2.5. Detection of MSCs homing in liver tissue:

Liver tissue was stained with PKH26 and examined with a fluorescence microscope to detect the homing of the injected MSCs.

2.6. Statistical Analysis:

Statistical analysis was carried out by one way ANOVA followed by Tukey test using SPSS V. 19 software (SPSS, Chicago, IL, USA) (Howitt and Cramer, 2011).

3. RESULTS:

3.1. Results of biochemical analysis:

TAA–treated rats after 8 weeks showed significant increase in ALT, AST and ALP activities when compared with control group. On the other hand, total protein, albumin, globulin and A/G ratio revealed significant reduction in their levels (table. 1) . TAA- MSCs treated group after ten weeks from experimental start showed non-significant changes in ALT, AST and ALP activities when compare with TAA-treated group but there was significant elevation in their activities when compared with control group. It also showed non-significant changes in the levels of total protein, albumin, globulins and A/G ratio when compared with TAA-treated group. Meanwhile, there were significant decreases in their levels when compared with control group (table. 2). In TAA- MSCs treated group after twelve weeks from experimental start revealed significant reductions in the activities of ALT, AST and ALP when compared with TAA-treated

group and showed significant increases in the level of total protein, albumin and A/G ratio when compared with TAA- treated rats, but globulins showed non- significant alteration in TAA-MSCs treated group when compared with TAA-treated group (table. 3). TAA-MSCs treated group after`14 weeks from experimental start revealed significant reduction in the activities of ALT, AST and ALP when compared with TAA-treated group. On the other hand, total protein, albumin, and globulin and A/G ratio showed significant increases near to normal levels (table. 4)

3.2. Results of Macroscopic examination:

The liver of TAA -treated rats showed severe enlargement with congestion and mottling of liver surface. Frequently, uneven surface of the liver with coarse nodular appearance. Multiple grayish white firm nodules of variable sizes (few mm up to 1cm in diameters) were seen in all hepatic lobules (Fig. 1). Meanwhile, after treatments with MSCs the surfaces of liver become finely nodulated with presence of minute focal grayish white areas on the hepatic surface particularlly after 12 weeks. Nearly normal liver observed after 14 weeks of MSCs treatment (Fig. 2).

3.3. Results of microscopic examination

The liver of TAA treated rats f 8 and 10 weeks post administration showed severe congestion of the central veins, portal blood vessels and hepatic sinusoids. The hepatocytes exhibited severe vacuolar and hydropic degeneration and fatty changes (Fig. 3). The hepatocytes showed also megalocytosis with severe enlargement of nucleus and nucleolus and the presence of two or more nuclei in the hepatic cell. Moreover, focal areas of necrosis and apoptic changes of hepatocytes were also observed in the hepatic parenchyma. The prominent microscopical findings were represented by fibrosis with formation of fibrous bridges connecting the portal areas. Moreover, distinct nodular feature with formation of pseudolobulation of the liver which separate the hepatic lobe from other lobules (Fig. 4). Liver cirrhosis with severe fibrosis of hepatic tissue which takes positive reaction by Crossman trichrome stain was observed (Fig. 5). The lining epithelium of bile ducts exhibited marked proliferation characteristic of cholangiocarcinoma. The tumor composed of polygonal or columnar cells arranged in irregular tubules and small acini, supported by a moderate to dense fibro vascular stroma (Fig. 6). The liver of TAA treated rats 12 and 14 weeks post TAA administration showed large areas of distortion of hepatic architecture than that observed after eight and ten weeks. The degenerative changes characteristic for hepatocytes evidenced by vacuolar and hydropic degeneration with occasionally fatty change appeared more pronounced and affected large areas of hepatocytes. The amount of fibrous connective tissue proliferation in between the hepatic lobules were markedly observed separating the lobules from each other with occasional formation of pseudolobulation of the hepatic tissue (Fig. 7). The hepatocytes exhibited also neoplastic changes characteristic of hepatocellular carcinoma. Neoplastic cells were polygonal, had variably distinct cell borders, abundant eosinophilic granular to vacuolated cytoplasm and around vesiculate nucleus with one or two prominent magenta nucleoli (Fig. 8). The liver of rats treated with TAA-MSCs and sacrificed after 10 weeks showed congestion of central vein and hepatic sinusoids. Moreover, the hepatocytes showed moderate degree of degenerative changes in the form of vacuolar and hydropic degeneration. Fibrous connective tissue proliferation in between the hepatic lobules leading to psedudolobulation of the hepatic parenchyma were also noticed (Fig. 9).The portal area showed congestion of the portal blood vessels with hyperplasia of bile duct. Moreover, adenomatous proliferation of the epithelial cell lining the bile duct with formation of neoplastic tubules were also detected. The liver of rats treated with TAA-MSCs and sacrificed after 12 weeks showed mild congestion of hepatic blood vessels. The hepatocytes exhibited mild degree of hydropic degeneration and minute fatty vacuoles were seen. There was reduction of the degree of fibrosis and the central vein still occupied the centrolobular space in the hepatic parenchyma (Fig. 10). The bile duct showed mild degree of hyperplasia with formation of less number of newly formed bile ductules with mild congestion of portal blood vessels. Some examined sections showed reminant of adenomatous proliferation of the bile ductules with mildest preductular fibrosis. The liver of rats treated withTAA- MSCs and sacrificed after 14 weeks showed mild congestion of hepaic blood vessels. The hepatocytes exhibited mild vacuolar and hydropic degeneration. Thin strands of the fibrous connective tissue in between hepatic lobules were seen in most examined sections (Fig. 11), which were positive by Crossman trichrome stain (Fig. 12).

The portal area showed focal mononuclear leucocytic infiltration practically around the bile duct. The bile duct in some examined cases showed mild degree of hyperplasia with few mononuclear preductular infiltration. Esinophilic

debris were also seen in the lumen of newly formed bile ductules. Focal areas of regenerated hepatocytes were seen in all examined sections. These areas appear in the form of vesiculated nucleus and more eosinophilic cytoplasm (Fig. 13). Meanwhile, some examined areas showed normal histological structure of hepatic tissue.

3.4. Results of Fluorescent microscope examination:

Liver of rats treated with labeled PKH26 MSCs and sacrificed after 10 weeks showed red autoflouresence confirming that MSCs homed into the liver tissue. PKH26 labelled cells showed moderate red autoflurosence indicate distribution of MSCs in the hepatic tissue mainly around blood sinusoids after 12 weeks from MSCs treatment**.** The liver of rats treated with PKH26 labelled cells after 14 weeks showed diffuse pattern of strong red autoflouresence (Fig.14).

Table (1): Serum biochemical changes in different treated groups compared to their corresponding controls (mean ±SE) after 8 weeks of treatment with thioacetamide.

Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	27.19±1.01	46.91±3.63	84.59±0.46	6.11±0.01	3.69±0.01	2.41±0.01	1.53±0.01
TAA	136.46±2.70**	208.30±1.51**	184.70±1.74**	4.50±0.02**	2.44±0.02**	2.05±0.01**	1.18±0.01**

Data represent mean values ± SEM (n = 5). * ($p \leq 0.05$) with the corresponding control groups. ** Indicate significance

Table (2): Serum biochemical changes in different treated groups compared to their corresponding controls (mean ±SE) 10 weeks after treatment with MSCs.

Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	28.26±1.91 ^{b}	49.45±0.61 ^{b}	77.71±10.45 ^{b}	6.36±0.01 ^{a}	3.78±0.11 ^{a}	2.57±0.06 ^{a}	1.46±0.01 ^{a}
TAA	136.90±1.40 ^{a}	207.60±1.40 ^{a}	183.90±5.20 ^{a}	4.48±0.01 ^{b}	2.44±0.03 ^{b}	2.03±0.01 ^{b}	1.18±0.01 ^{b}
CMSCs	28.93±0.83 ^{b}	48.42±1.16 ^{b}	78.68±4.75 ^{b}	6.38±0.04 ^{a}	3.81±0.03 ^{a}	2.57±0.01 ^{a}	1.48±0.01 ^{a}
TAA/MSCs	135.26±1.01 ^{a}	206.90±0.56 ^{a}	173.23±6.16 ^{a}	4.49±0.01 ^{b}	2.46±0.01 ^{b}	2.03±0.02 ^{b}	1.20±0.01 ^{b}

Data represent mean values ± SEM (n = 5). * ($p \leq 0.05$) with the corresponding control groups. The same characters in the same column means no significance

Table (3): Serum biochemical changes in different treated groups compared to their corresponding controls (mean ±SE) 12 weeks after treatment with MSCs.

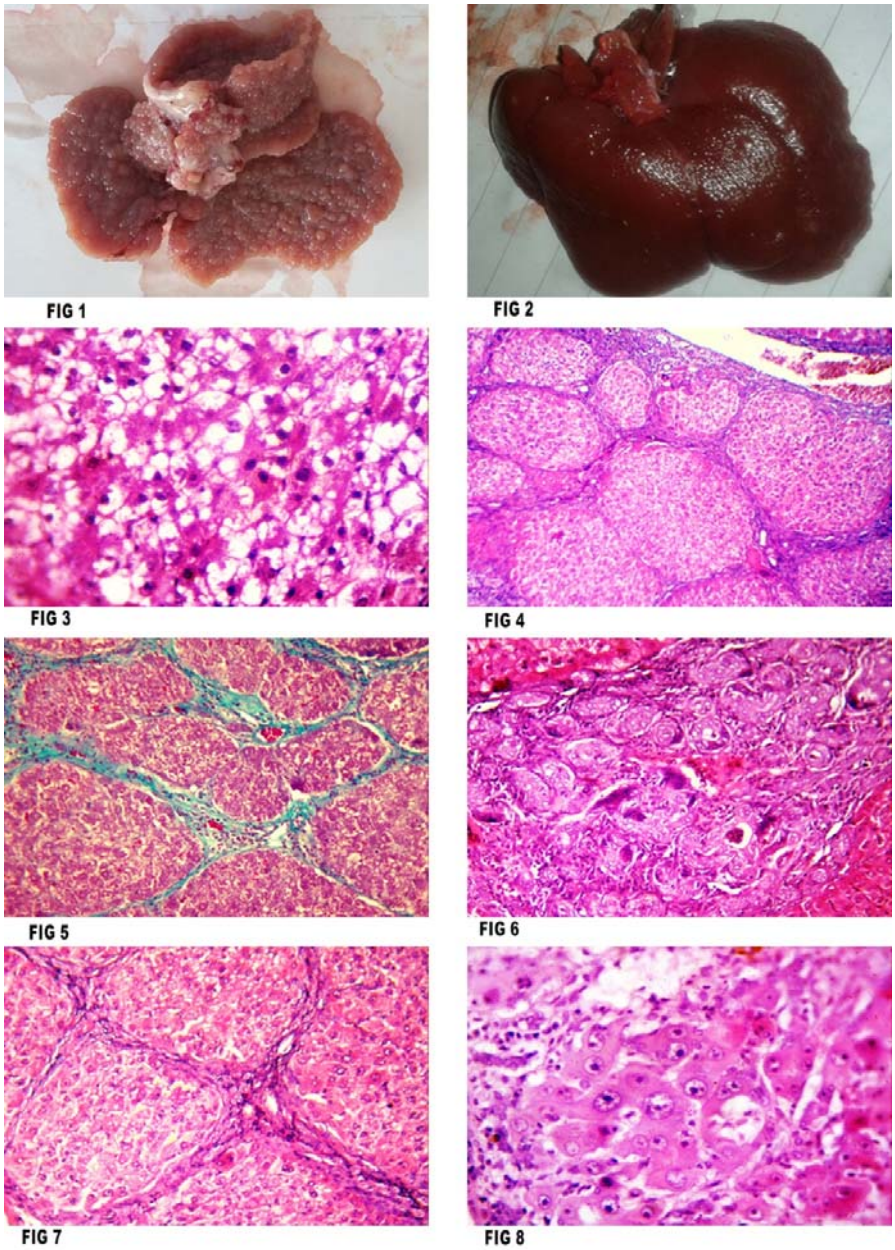
Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	27.42±0.98 ^{c}	49.42±0.58 ^{c}	74.97±9.19 ^{c}	6.30±0.14 ^{a}	3.76±0.07 ^{a}	2.54±0.07 ^{a}	1.48±0.01 ^{b}
TAA	136.95±0.65 ^{a}	212.15±1.05 ^{a}	168.05±1.75 ^{a}	4.52±0.02 ^{c}	2.51±0.01 ^{c}	2.03±0.01 ^{b}	1.23±0.01 ^{c}
CMSCs	28.40±0.94 ^{c}	49.58±1.07 ^{c}	85.00±2.48 ^{c}	6.25±0.08 ^{a}	3.72±0.05 ^{a}	2.52±0.03 ^{a}	1.47±0.01 ^{b}
TAA/MSCs	112.12±2.02 ^{b}	182.02±2.80 ^{b}	155.88±1.44 ^{b}	5.24±0.01 ^{b}	3.17±0.01 ^{b}	2.07±0.01 ^{b}	1.52±0.01 ^{a}

Data represent mean values ± SEM (n = 5). * ($p \leq 0.05$) with the corresponding control groups. The same characters in the same column means no significance

Table (4): Serum biochemical changes in different treated groups compared to their corresponding controls (mean ±SE) 14 weeks after treatment with MSCs.

Parameters Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	26.60±0.43 [c]	46.46±0.13 [c]	89.07±7.66 [c]	6.31±0.12 [a]	3.77±0.08 [a]	2.53±0.03 [a]	1.48±0.01 [b]
TAA	141.46±2.16 [a]	212.66±1.27 [a]	170.63±4.22 [a]	4.53±0.01 [c]	2.50±0.01 [c]	2.02±0.01 [c]	1.23±0.01 [c]
CMSCs	28.67±1.02 [c]	49.50±0.81 [c]	82.94±9.24 [c]	6.21±0.11 [a]	3.70±0.05 [a]	2.50±0.05 [a]	1.47±0.01 [b]
TAA/MSCs	87.80±3.90 [b]	145.92±1.29 [b]	134.88±1.66 [b]	5.39±0.01 [b]	3.26±0.02 [b]	2.13±0.01 [b]	1.53±0.01 [a]

Data represent mean values ± SEM (n = 5). * ($p \leq 0.05$) with the corresponding control groups. The same characters in the same column means no significance



(Fig.1) The liver of rats received TAA (200 mg/kg b.wt) three times a week and sacrificed eight weeks post TAA administration showing severe nodular cirrhosis in the hepatic tissue which mostly taken grayish white coloration. (Fig.2.). The liver of rats received TAA (200mg/k.gb.wt I.P) three times a week for eight weeks then treated with MSCs

dose (3x106 cells/ mL I.V) and sacrificed after 14 weeks showing nearly normal liver similar to control one. (Fig.3) The liver of rats received TAA (200 mg/kg b.wt) three times a week and sacrificed eight weeks post TAA administration showing fatty change of hepatocytes characterized by clear encircled areas in the cytoplasm which push the nucleus to one side giving signet ring appearance. H&E (X 400). Fig. 4) The liver of rats received (200 mg/kg b. wt) TAA three times a week and sacrificed after eight weeks showing pseudolobulation with formation of unusual hepatic lobules giving classic microscopical picture of cirrhosis. H&E (X100). (Fig. 5) The liver of rats received (200 mg/kg b. wt) TAA three times a week and sacrificed after eight weeks showing pseduolobulation of liver parenchyma. Crossman trichrome stain (X200). (Fig.6) The liver of rats received (200 mg/kg b.wt) TAA three times a week and sacrificed after ten weeks showing adenomatous proliferation of the bile duct giving picture of cholangiocarcinoma. H&E (X200). Fig.7) The liver of rats after received TAA (200mg/k.gb.wt I.P) three times / week for eight weeks and sacrificed after 12 weeks showing monolobular cirrhosis in which the connective tissue encircling individual hepatic lobule. H&E (X 400). (Fig. 8) The liver of rats received TAA (200mg/k.gb.wt I.P) three times / week for eight weeks and sacrificed after14weeks showing moderate anisocytosis and anisokaryosis and multifocal cytomegalic cells that occasionally contain multiple nucleiwith mono nuclear leucocytic infiltration around the affected area. H&E (×400).

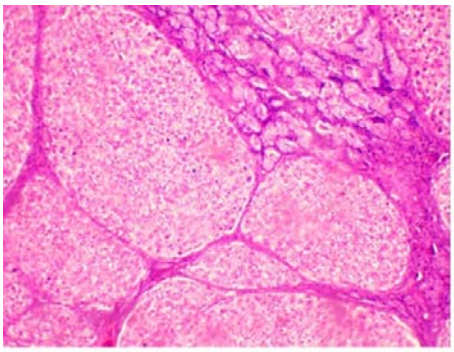


FIG 9

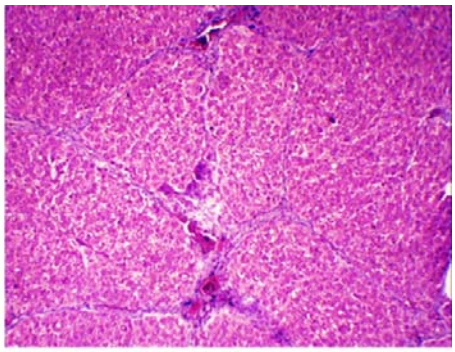


FIG 10

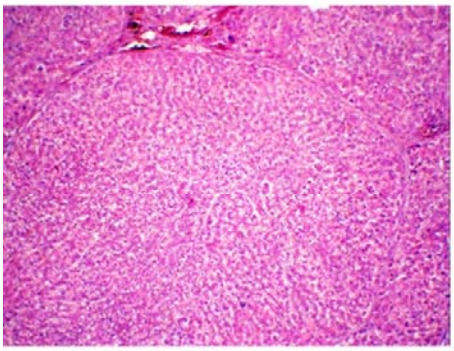


FIG 11

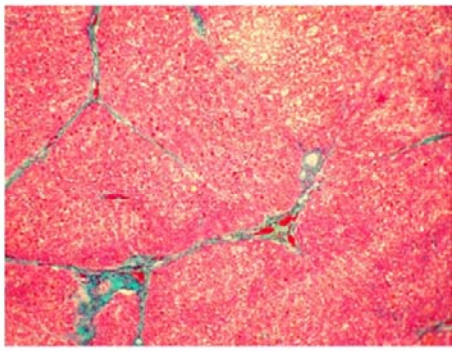


FIG 12

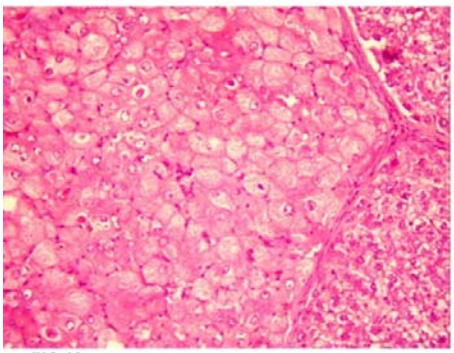


FIG 13

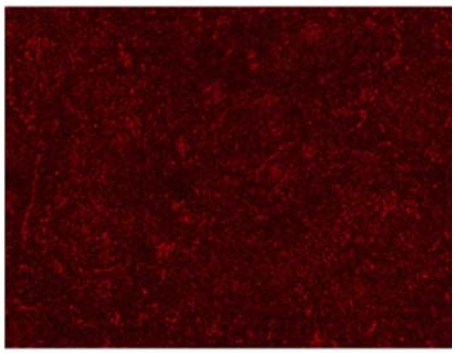


FIG 14

Fig.9). The liver of rats that received TAA (200mg/k.gb.wt I.P) three times a week for eight weeks then treated with MSCs dose ($3x10^6$ cells/ mL I.V) and sacrificed after 10 weeks showing adenomatous proliferation of neoplastic bile ductules. H&E (X 100). Fig.10). The liver of rats that received TAA (200mg/k.gb.wt I.P) three times a week for eight weeks then treated with MSCs dose ($3x10^6$ cells/ mL I.V) and sacrificed after 12 weeks showing fibrous strands in between hepatic lobules. H&E (X 100). (Fig.11). The liver of rats that received TAA (200mg/k.gb.wt I.P) three times a week for eight weeks then treated with MSCs dose ($3x10^6$ cells/ mL I.V) and sacrificed after 14 weeks showing reminant of fibrous connective tissue proliferation in between the hepatic lobules. H&E (X 100). (Fig.12). The liver of rats that

received TAA (200mg/k.gb.wt I.P) three times a week for eight weeks then treated with MSCs dose ($3x10^6$ cells/ mL I.V) and sacrificed after 14 weeks showing thin fibrous strands in between the hepatic lobules. Crossman trichrome stain(X 100). (Fig.13) The liver of rats that received TAA (200mg/k.gb.wt I.P) three times a week for eight weeks then treated with MSCs dose ($3x10^6$ cells/ mL I.V) and sacrificed after 14 weeks showing focal regenerated area in the hepatic parenchyma. H&E (X 100). (Fig.14). The liver of rats that received TAA (200mg/k.gb.wt I.P) three times a week for eight weeks then treated with labeled PKH26 MSCs dose (3x106 cells/ mL I.V) and sacrificed after 14 weeks showing diffuse pattern of strong red autoflouresence of MSCs.

4. DISCUSSION:

Chronic administration of TAA produces liver cirrhosis via increase ROS formation and disruption of calcium homeostasis are factors that could induce an increase in the permeability of the mitochondrial inner membrane, disrupt mitochondrial membrane potential and inhibit mitochondrial respiration and finally hepatic toxicity resulted (Bernardi *et al.*, 2001).

In the present study, rats were injected with TAA and sacrificed after 8 weeks when compared with control group showed high significant increase in the activities of ALT, AST and ALP. Also, it showed high significant decrease in the levels of albumin, globulin, total protein and A/G ratio (table 1). These findings were in complete agreement with the result of Ahmad *et al.*, (2002), Alshawsh et al., (2011),Wang *et al.*, (2011),Amin *et al.*, (2012) and Zaky, (2015). The elevated serum liver enzymes such as ALT, AST, and ALP in intoxicated rats could be attributed to necrosis of hepatocytes that results in the leakage of transaminase (Kaplowitz, 2001). The total protein and albumin levels are depressed in hepatotoxic conditions due to disturbances in the carbohydrate, protein and lipid metabolisms (Low et al., 2004). In Group of rats which injected with TAA for 8 weeks and sacrificed after 10 weeks when compared with control group and MSCs-control group showed significant difference in the liver biochemical parameter. But no significant difference was detected when compared with MSCs-treated group. Additionally, in group of rats treated with MSCs and sacrificed after 12 and 14 weeks we detected significant reduction in the activities of ALT, AST and ALP. Meanwhile significant elevation in the levels of total protein, albumin, globulin and A/G ratio when compared with TAA group (table 3, 4). Our results were in complete agreement with Aziz et al., (2007), El-khayat et al., (2013), Ahmed et al., (2014), Shao et al., (2014) and Quintanilha et al., (2014) who reported that the rats that received BM-MSCs infusions by tail vein showed better results for the biochemical parameters. The serum injury markers (ALT and AST) were reduced with successive cell infusions, suggesting protection of hepatocytes from necrosis and apoptosis and MSCs can protect hepatocytes by reducing ROS damage induced by TAA.

In the present study, the prolonged administration of TAA in rats led to micronodular cirrhosis which grossly give the nodular appearance of liver.These results agreed with the result of Amin et al., (2012), Salama *et al.*, (2013) and Ling et al., (2013). Meanwhile, after treatment with MSCs the liver grossly showed nearly normal apperance

The microscopical finding in cirrhotic group releved severe degree of hydropic degeneration and fatty changes. Moreover, focal areas of necrosis and apoptic changes with mononuclear leucocytic infiltration were also observed in the hepatic parenchyma. These results were in compelete agreement with the results of Geng et al., (2010), Madani et al., (2008), Buko et al., (2014) and Rui et al., (2014).These findings may be attributed to metabolism of TAA which metabolized by cytochrome P450 enzymes of liver microsomes and converted to a toxic intermediate called thioacetamide S- oxide due to oxidation process which induced oxidative stress in the hepatic cells responsible for the changes in cell permeability, increase intracellular concentration of Ca++, increase in nuclear volume, enlargement of nucleoli and also inhibits mitochondrial activity leads to cell death and severely affecting hepatic cells which are located in the perivenous acinus region as previesly mentioned by Zaragoza et al., (2000) and Bigoniya et al., (2009). Moderate degree of degenerative changes in hepatocytes were observed in treated rats with MSCs the form of vacuolar and hydropic degeneration. These results completely agreed with the results of Choi et al., (2013) who demonstrated that migration and engraftment of MSCs to the injured liver area, and differentiation into functional hepatocytes from 1 to 3 weeks.

Severe fibrous connective tissue proliferation forming fibrous bridges connecting the portal areas led toformation of pseudolobulation which separate the hepatic lobe from other lobules. These finding were agreed with the results of Yeh *et al.*, (2004) and Hessin et al., (2015). Meanwhile, after MSCs treatment there was thin strands of fibrous connective tissue in between the hepatic lobules.

The hepatic tissue preserved it is nearly normal hepatic lobular architecture with central veins and radiating hepatic cords. This results came in agreement with previous studies (Ahmed *et al.*, 2014, Volarevic et al., 2014 and Mansour et al., 2015) who found that MSCs could reduce the proliferation of stellate cells and collagen type I synthesis through the secretion of IL-10, and to promote hepatic stellate cell apoptosis through the secretion of HGF and NGF lead to a significant decrease in collagen deposition and proliferation. MSCs can exert antifibrotic effect in liver cirrhosis through the expression of matrix metalloproteinase-9 (MMP-9) that degrades the

Moreover, the portal areas showed severe congestion of the portal blood vessels with mild vasculitis, multiple thrombosis and perivascular edema as well as perivascular mononuclear leucocytic infiltration. The bile duct in the portal areas showed hyperplasia of their epithelial cell lining with formation of newly formed bile ductules, besides, inter aciner mononuclear leukocytic infiltration. Additionally, severe proliferation of the bile ductual epithelium with multiple formation of consulted cell mass giving the picture of cholangiocarcinoma. These results agreed with the results of Al-Bader et al., (2000), David et al., (2002), Yeh *et al.*, (2004) and Ling et al.,(2013). Meanwhile, after MSCs treatment the bile duct showed mild degree of hyperplasia with fewer numbers of newly formed bile ductules and mildest preductular fibrosis. Multiple focal regeneration of some areas of hepatic parenchyma was seen scattered in the hepatic tissue. These results agreed with Hwang et al., (2012) who indicated that MSCs administrated, fristlly underwent transdifferentiation into hepatic oval cells and later to hepatocyte-like cells. During this process, inflammation was reduced, damaged hepatocytes were repaired, and fibrosis was resolved, resulting in an overall improvement in liver function.

The hepatocytes exhibited also neoplastic changes characteristic of hepatocellular carcinoma. Neoplastic cells were polygonal, had variably distinct cell borders, abundant eosinophilic granular to vacuolated cytoplasm and around vesiculate nucleus with one or two prominent magenta nucleoli. These results were in agreement with Heindryckx et al., (2009) and Newell et al., (2008) who reported that HCC universally arises secondary to inflammation and fibrosis. TAA in drinking water (0.03%) or by intraperitoneal injection induces fibrosis and hepatocellular carcinoma in rats and mice over a period of 2–3 months, which may be secondary to the oxidant properties of TAA and the induction of hepatic oxidative stress. Meanwhile, these results disagreed with the result of Hessin et al., (2015) who found that TAA administration (200 mg/kg/biweekly, ip) for four successive weeks cause fibrosis without the presence of hepatocellular carcinoma. These neoplastic changes decreased after MSCs treatment.

In all examined sections after 14 weeks of MSCs treatment showed focal areas of regenerated hepatocytes which appeared in the form of vesiculated nucleus and more eosinophilic cytoplasm. Similar results were detected by Ahmed *et al.*, (2014) and Tarek et al., (2014) who showed that MSCs could secrete many cytokines and growth factors, such as VEGF that has antifibrotic effect, HGF which shows anti-apoptotic activity in hepatocytes and could promote the MSCs to undergo the process of transdifferantiation into parenchymal hepatocytes, and NGF which can induce apoptosis of HSCs and also agreed with results of Berardis et al., (2015) and Eom *et al.*, (2015) who reported that the cell therapy using MSCs may represent an attractive therapeutic option, based on their potential to differentiate into hepatocytes, allowing the replacement of damaged hepatocytes, their potential to promote residual hepatocytes regeneration and their capacity to inhibit hepatic stellate cell activation or induce their apoptosis.

Homing of the injected MSCs into the liver tissue was confirmed by fluorescent technique (labeled MSCs with the PKH26 dye). These finding agreed with the results of Liu et al., (2009), El-khayat *et al.*, (2013b) and Mansour et al., (2015) who found that homing of MSCs into liver tissue was confirmed by labeled MSCs with the PKH26 dye, these cells showed strong red auto fluorescence after transplantation into rats, confirming that these cells were actually seeded into the liver tissue.

5. CONCLUSIONS:

From the results observed in the present study, we can conclude that TAA is a perfect model for induction of most types of liver injury. Short term administration of TAA led to elevation the activities of serum enzymes and decrease the levels of serum proteins with degenerative and necrotic changes of hepatocytes. Meanwhile, long-term administration of TAA characterized by hepatic fibrosis and cirrhosis ending with hepatic carcinogenesis as cholangiocarcinoma and CHC.

BM-MSCs injected intravenous in the tail vein of rat were successfully homed to the injured

liver. BM-MSCs have an ameliorative effect on hepatic enzymes (ALT, AST and ALP), serum proteins (TP, albumin and globulin) and on the degenerative and necrotic changes of hepatocytes. BM-MSCs could be successfully used in treatment of liver fibrosis and cirrhosis. BM-MSCs don't have therapeutic effect on hepatic carcinogenesis.

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