The hepato- protective Effect of Stem Cells and Levamisole against Carbon-tetrachloride induced Liver Fibrosis.

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ABSTRACT

This study designed to evaluate the hepatoprotective effect of stem cells only or stem cells and levamisole on experimentally Carbon tetrachloride (CCl_4) induced liver damage by evaluation of haematological parameters, biochemical parameters, immunological parameters and histopathological findings. Sixty (60) Wister rats were divided into four groups with 15 animals in each group. Group (1): normal group. Group (2): rats injected with CCL_4 (0.2ml/100gm) s/c twice a week for 9 weeks. Group (3): rats injected with CCL_4 then given MSCs ($3x10^6$cell) I/v once a week for 4 weeks. Group (4): rats injected with CCL_4 then given levamisole (2.5mg/kg) three successive days for 4 weeks and stem cells (MSCs) ($3x10^6$cell) I/v once a week for 4 weeks. Results showed that injection of CCL_4 lead to significant increase in activity of hepatic enzymes (AST, ALT, ALP) and Bilirubin level while it decreased serum total protein, albumin, IL- 6, Phagocytic index, Phagocytic %, IgG and IgM. While after treatment with stem cells only or stem cells and levamisole lead to significant decrease in activity of hepatic enzymes (AST, ALT, ALP) and Bilirubin level while it increased serum total protein, albumin, IL- 6, Phagocytic index, Phagocytic %, IgG and IgM.

Keywords: liver fibrosis, Carbon tetrachloride, Levamisole and Mesenchymal Stem Cells.

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1. INTRODUCTION

The liver is the largest organ in the body and serves many vital functions such as remove damaged red blood cells from the blood in coordination with spleen, produces bile, clotting factors, stores vitamins, minerals, protein, fats and glucose from diet (Dyce et al., 1987; Waugh and Grant, 2001). CCL_4 commonly used as a hepatotoxin in experimental hepatopathy (Hsu et al., 2008) and (Geetha et al., 2008) because it induced a cirrhotic response in animals which is similar to human cirrhosis of the liver (Lee et al., 2007; Taira et al., 2004). In liver, CCL4 is biotransformed by cytochrome P450 to produce its active metabolite trichloromethyl free radical (CCl3) which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (Kaplowitz et al., 1986). CCL4 significantly increases ALT, AST, ALP activities and total bilirubin level while decreasing total protein, albumin and total cholesterol. Also, liver tissue of rats treated with CCL_4 showed hepatocellular necrosis, fatty vacuole and microvesicular fatty changes (Bigoniya et al., 2009). Levamisole is a synthetic imidazothiazole derivative which is a highly acceptable anti nematodal drug because of its broad range of activity in a large number of hosts. The drug appears to restore depressed immune function rather than to stimulate response to above normal levels. It also, stimulate formation of antibodies to various antigens, by stimulating T-cell activation and proliferation, potentiate monocyte and macrophage functions including phagocytosis and chemotaxis and increase neutrophil adherence (Mariam et al., 2015). The bone marrow (BM) is a convenient and rich source of stem cells. Mesenchymal stem cells (MSCs) are a type of multi potent adult stem cells that can be readily obtained from BM aspirates and expanded into large quantities *in vitro* (Le Blanc et al., 2004). Mesenchymal stem cells can be a rescue for liver diseases as they differentiate to hepatocytes, stimulate the regeneration of endogenous

parenchymal cells, and enhance fibrous matrix degradation (Christ and Dollinger, 2011).

2. MATERIALS AND METHODS

2.1. Chemicals and agents used in the experimental protocol:

Carbon tetrachloride CCL4 (hepatotoxic agent): CCL4 is a volatile colorless, clear and heavy liquid with a characteristic sweet nonirritant odor (EL-Nasr Pharmaceutical Comp. Cairo. Egypt), used as (40%) dissolved in paraffin oil. Levamisole: Levamisole hydrochloride: levamisole® 10%. The active substance of levamisole is L. isomer of dl-tetramisole, manufactured by Memphis. Stem Cells: Mesenchymal stem cells obtained from bone marrow of rats. Animals: 60 adult male Albino rats were divided into four groups (15 rat /cage) in room temperature, for a week before starting the experiment, under natural day and night periods and supplied with a balanced stable commercial diet and water.

2.2. Experimental design:

In this experiment, 60 adult Wister rats were divided into four groups used as following: Group 1: consists of 15 rats served as a control negative. Group 2: consists of 15 rats were injected subcutaneously with CCL4 40% dissolved in paraffin oil (0.2 ml/100gm) s/c twice a week for nine weeks for induction of liver fibrosis served as control positive (Zhao et al., 2005). Group 3: consists of 15 rats were injected s/c with CCL4 40% dissolved in paraffin oil (0.2 ml/100gm) twice a week for nine weeks then injection of MSCs (3x106/ml) I/V once a week for four weeks (Zhao et al., 2005). Group 4: consists of 15 rats were injected subcutaneously (s/c) with CCL4 40% dissolved in paraffin oil (0.2 ml/100gm) twice a week for nine weeks then were given Levamisole HCL (2.5 mg/kg) orally three successive days for four weeks. in addition to injection of MSCs (3x106 /ml) I/V once for four weeks. At the end 9th, 13th and 15th weeks, all animals were sacrificed and the blood from every animal was taken into clean tubes. The blood, serum and liver tissue were collected from animals for biochemical, immunological and histopathological examinations.

2.3. Assessment of biochemical parameters:

Alanine aminotransferase and aspartate aminotransferase (AST) activities were determined according to Reitman and Frankel (1957) and bilirubin according to Tietz (1986). While alkaline phosphate activity according (Tietz et al., 1983). Total proteins according to Weichselbum (1964). Albumin and globulins according to Keyser and Watkins (1972). IL-6 level according to Dalrymple et al. (1995).

2.4. Assessment of Immunological parameters:

Phagocytic activity of peripheral blood neutrophils was performed according to Wilkinson (1981). Determination of Immunoglobulins (IgG and IgM) was measured according to Ovary (1966).

2.5. Histopathological examination:

Specimens from liver were obtained from all groups stained by H&E.

2.6. Statistical analysis:

All quantitative measurements were expressed as means ± SD of control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) on SPSS (statistical package for social sciences). Statistical significance was set up $P < (0.05)$.

3. RESULTS

3.1. Serum ALT, AST, ALP and bilirubin level.

After 9 weeks, CCL4 injected rats showed significant increase in serum ALT, AST, ALP and bilirubin levels when compared with the control (-ve) group (Table 1). After 13 weeks, CCL4 injected rats showed significant increase in serum ALT, AST, ALP and bilirubin levels when compared with the control (-ve) group**.** On the other hand, rats treated with stem cells only or stem cells and levamisole revealed that there were significant decrease in ALT, AST, ALP activities and bilirubin level when compared with their CCL_4 control group. In addition, there were significant decrease in ALT, AST, ALP activities and bilirubin level between rats treated with stem cells and levamisole than those treated with stem cells only. After 15 weeks**,** CCL_4 injected rats showed significant increase in serum ALT, AST, ALP activities and bilirubin level when compared with the control (-ve) group**.** On the other hand, rats treated with stem cells only or stem cells and levamisole revealed that there was significant decrease in ALT, AST, ALP activities and bilirubin level when compared with their CCL_4 control group. In addition, there were significant decrease in ALT, AST, ALP activities and bilirubin level between rats treated with stem cells and levamisole than those treated with stem cells only.

3.2. Serum total protein albumin and globulin levels:

Regarding to the results in table (2), after 9 weeks, there were significant decrease in serum total proteins, albumin and globulin levels when compared with their control (-ve) group. After 13 week, Rats injected with CCL4 showed that there was significant decrease in serum total proteins, albumin and globulin levels when compared with their control (-ve) group, while after treatment with stem cells or stem cells and levamisole, there were significant increase in serum total proteins, albumin and globulin levels when compared with their control CCL4 group. After 15 weeks, our results showed that there were significant decrease in serum total proteins, albumin and globulin levels when compared with their control (-ve) group, while after treatment with stem cells or stem cells and stem cells, there were significant increase in serum total proteins, albumin and globulin levels when compared with their control CCL4 group.

3.3. Serum interleukin –6 level:

After 15 weeks, results showed that carbon tetrachloride injected groups showed a significant increase in IL-6 when compared with control (-ve) group. While after injection of stem cells or stem cells and levamisole, there were a significant decrease in IL-6 when compared with their CCL4 control group. On the other hand, there were non-significant changes between group treated with stem cells only when compared with group treated with stem cells and levamisole (Table 3).

3.4. Humoral immune response:

After 15 weeks, our data in table showed that carbon tetrachloride injected groups showed a significant decrease in IgG and IgM levels when compared with control (-ve) group. While after injection of stem cells or stem cells and levamisole, there were a significant increase in IgG and IgM when compared with their CCL4 control group. On the other hand, there were significant increase in groups treated with stem cells and levamisole in IgG with non-significant changes in IgM level than treated with stem cells only.

3.5. Cell mediated immune response:

CCL4 injected groups showed significant decrease in Phagocytic percentage (%) and Phagocytic index at 15th weeks when compared with control (-ve) group. While after injection of stem cells, stem cells and levamisole there were significant increases in Phagocytic % and phagocytic index when compared with their corresponding CCL4 control groups. In addition to there were increase in group treated with stem cells and levamisole in Phagocytic percentage and Phagocytic index than rats treated with stem cells (table 3).

3.6. Pathological findings:

Group of rats kept as control: There was normal hepatic architecture with radial arrangement of hepatocytes that contain pronounced nuclei and obvious nucleoli were recorded in fig (1). Group of rats experimentally induced rats by administration of carbon tetrachloride showing that hepatic lobules are demarcated and separated by collagen bundles with formation of pseudo lobules. The portal area showing proliferation of bile ductules with high grades, marked dilatation of sinusoids, congestion of portal veins and also, hydropic degeneration were detected in diffuse manner all over the hepatocytes in the parenchyma by different grades from the central to peripheral zones of the lobules and necrosis in hepatocytes and dilatation of central vein fig (2).

Liver of rats injected with CCL_4 then treated by stem cells after 13 week showing that normal structure of liver was destroyed but the hepatic lobules were encysted and separated by fine collagen, moderate proliferation of bile ductules and moderate sinusoidal dilatation, mild vacuolar degeneration of hepatocytes, dilatation of central vein and mild activation of kuppfer cells. After 15 weeks, liver showing almost normal hepatic architecture with mild congestion of portal vasculature, mild dilatation of portal veins and hepatic regeneration (normal central vein, almost normal hepatocytes) fig (4).

Liver of rats injected with CCL_4 then treated by stem cells and levamisole at 13 week showing congestion of portal veins and absence of inter-lobular septae, almost normal structure of portal area with activation of kuppfer cells and marked activation of kuppfer cells and moderate dilatation of sinusoids fig (5). After 15 week showing mild congestion of portal veins with mild dilatation of inter lobular sinusoids, normal structure of portal area and advanced hepatic regeneration with marked activation of kuppfer cells fig (6).

4. DISCUSSION:

According to the biochemical result, CCl_4 treated rats showed increase in activities of serum ALT, AST, ALP and Bilirubin level. These results agree with Kamel et al. (2010); Sahar (1998) and Elshater et al. (2013).

Table (1): ALT, AST, ALP and total bilirubin at 9th, 13th and 15th week after treatment with stem cells, stem cells and levamisole in CCL_4 injected rats.

Weeks	Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (mg/dl)
9weeks	Control(-ve)	15±0.58[a]	29.33±0.88[a]	191.67±6.89[a]	0.63±0.04[a]
	CCL_4 (control+ve)	33.67±4.10[b]	54.33±5.36[b]	209.67±3.41[b]	0.84±0.06[b]
	CCL_4 before stem cells	50±1.54[c]	50.33±2.96[b]	220±5.29[b]	0.77±.02[b]
	CCL_4 before stem cells and levamisole	44±4.01[c]	44±4.5[b]	212±1.54[b]	0.72±0.03[b]
13weeks	Control(-ve)	21.33±2.19[a]	54±0.58[a]	146.33±4.41[a]	0.72±0.02[a]
	CCL_4 (control +ve)	61.33±1.13[b]	87±2.08[b]	194.67±6.39[b]	0.91±0.04[b]
	Stem cells	52±0.88[c]	70±2.52[c]	171.7±3.8[c]	0.80±0.01[c]
	Stem cells and levamisole	37.67±1.18[d]	61.67±3.71[d]	154.33±6.49[a]	0.75±0.01[a]
15weeks	Control (-ve)	23.00±2.31[a]	56.33±2.01[a]	177.33±6.57[a]	0.69±0.02[a]
	CCL_4 (Control +ve)	52.67±1.11[b]	83.67±3.28[b]	210.00±8.72[b]	0.86±0.01[b]
	Stem cells	40±1.6[c]	70.33±2.4[c]	193.7±2.61[c]	0.80±0.02[c]
	Stem cells and levamisole	31.33±3.71[d]	61±3.79[a]	180.33±1.72[a]	0.72±0.03[a]

Mean ± SE. Different superscript represents significant changes within the same column in each check point at $p \leq 0.05$.

Table (2): Total protein, Albumin and globulin after treatment stem cells and levamisole in CCL_4 injected rats.

Weeks	Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
9 week	Control (-ve)	6.88±0.22[a]	4.38±0.55[a]	3.33±0.13[a]
	CCL_4 (control+ ve)	4.06±0.32[b]	2.19±0.63[b]	2.88±0.08[b]
	CCL_4 before stem cells	5.19±0.19[c]	3.61±0.29[c]	2.83±0.03[b]
	CCL_4 before stem cells and levamisole	4.22±0.19[b]	2.40±0.61[b]	2.98±0.09[b]
13 week	Control (-ve)	7.14±0.29[a]	5.04±0.33[a]	3.74±0.11[a]
	CCL_4 (control +ve)	5.19±0.07[b]	1.84±0.02[b]	3.25±0.01[b]
	Stem cells	6.77±0.09[a]	4±0.15[c]	3.53±0.08[c]
	Stem cells and levamisole	6.02±0.10[c]	3.98±0.11[c]	3.45±0.03[c]
15 week	Control (-ve)	6.22±0.19[a]	5.41±0.26[a]	3.49±0.08[a]
	CCL_4 (control +ve)	5.04±0.03[b]	2.70±0.30[b]	3±0.11[b]
	Stem cells	5.90±0.14[a]	4.81±0.16[a]	3.35±0.11[a]
	Stem cells and levamisole	5.48±0.09[c]	3.36±0.05[c]	3.22±0.09[a]

Table: IL- 6, IgG, IgM, Phagocytic % and Phagocytic index after treatment stem cells and levamisole in CCL_4 injected rats.

Groups	IL- 6	IgG (mg/dl)	IgM (mg/dl)	Phagocytic %	Phagocytic index
Control (-ve)	120.63±1.1[a]	1180.07±20.82[a]	42.00±1.19[a]	62.4±4.02[a]	1.15±0.08[a]
CCL_4 (Control +ve)	144.2±4.23[b]	898.67±10.72[b]	34.67±1[b]	37.8±2.69[b]	0.66±0.03[b]
Stem cells	130.13±5.98[a]	1160.33±14.74[a]	39.33±1.14[c]	55.6±1.5[a]	1.1±0.012[a]
Stem cells and levamisole	126.87± 3.11[a]	1110±12.56[c]	38.33±1.06[c]	47.4±1.71[c]	0.96±0.022[c]

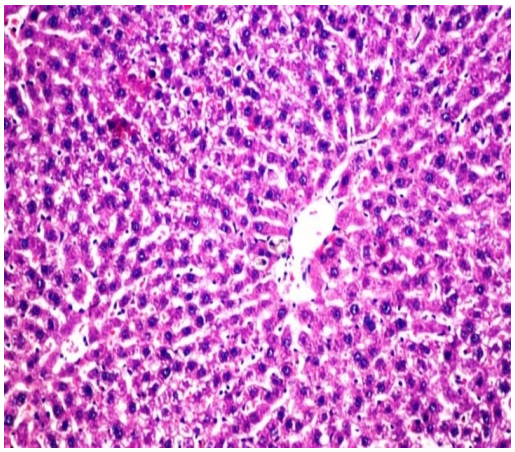

Figure (1): Liver of rats of control group showed normal hepatic architecture with radial arrangement of hepatocytes that contain pronounced nuclei and obvious nucleoli (H&E x 400).

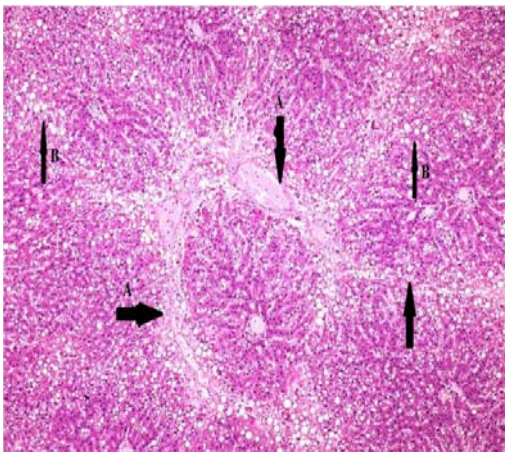

Figure (2): Liver of rats injected with CCL_4 (after 9 weeks) showing hepatic lobules demarcation, separated by thick collagen bundles with formation of pseudo lobules (A arrow), marked vacuolar degeneration of hepatocytes with congestion of hepatic vasculature (B arrow) (H&E x 100).

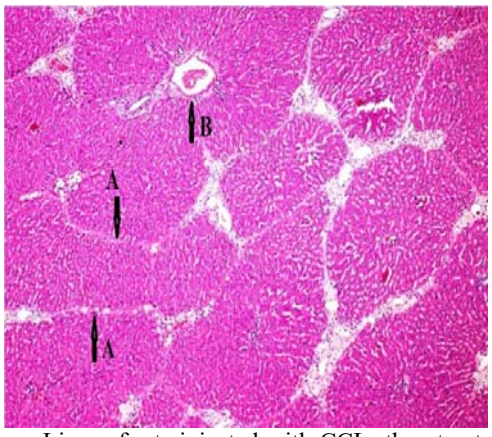

Figure : Liver of rats injected with CCL_4 then treated by stem cells after 13 week showing that the hepatic lobules were encysted and separated by fine collagen (A), mild congestion of hepatic vasculature (B) (H&E x 100).

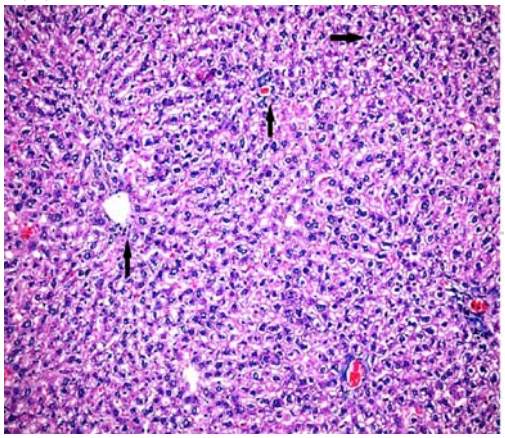

Figure (4): Liver of rats injected with CCL_4 then treated by stem cells after 15 week showing absence of interlobular septae, mild congestion of hepatic vasculature (H&E x 400).

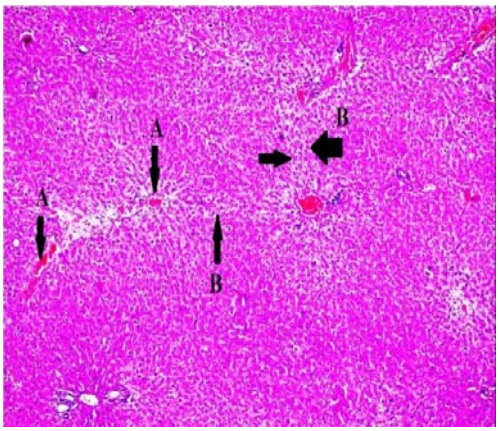

Figure (5): Liver of rats injected with CCL_4 and treated with stem cells and levamisole after13 week showing congestion of portal veins (A arrow) and absence of inter-lobular septae (B arrow) (H&E x 100).

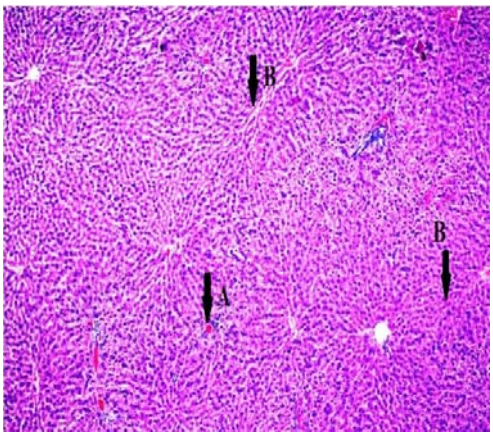

Figure (6): Liver of rats injected with CCL_4 and treated with stem cells and levamisole after 15 week showing mild congestion of portal veins (A arrow) with absence of inter lobular sinusoids (B arrow) (H&E x 100).

This increase in enzyme activities, reflecting the damage of the liver cells or changes in the cell membrane permeability which led to leakage of enzymes from cells to the circulation. These biochemical results confirmed to the histopathological changes in liver of rats injected with CCL_4 showing that hepatic lobules are demarcated and separated by collagen bundles with formation of pseudo lobules. The portal area showing proliferation of bile ductules with high grades, marked dilatation of sinusoids, congestion of portal veins and also, hydropic degeneration were detected in diffuse manner all over the hepatocytes in the parenchyma by different grades from the central to peripheral zones of the lobules and necrosis in hepatocytes and dilatation of central vein with thrombus formation. These results agree with Eidi et al. (2012); Jawad et al. (2013) and Mariam et al. (2015).

On the other hand, data showed improvement (decrease) in liver enzyme activities and bilirubin level after treatment with stem cells only. This supported by Oyagi et al. (2006) and Abdel Aziz et al. (2007)), they reported that bone marrow (BM) cells were able to transdifferentiate into hepatocytes. In addition, the trans differentiation ability, soluble factors produced by MSCs may play an important role in regeneration and protection from hepatocellular death. Moreover, they reported that treatment with the isolated mesenchymal stem cells from bone marrow (MSCs) decreases serum AST and ALT activities These biochemical results confirmed to the histopathological changes in liver of rat administered stem cells. After 13 weeks, which that normal structure of liver was destroyed but the hepatic lobules were encysted and separated by fine collagen, moderate proliferation of bile ductules and moderate sinusoidal dilatation, mild vacuolar degeneration of hepatocytes, dilatation of central vein and mild activation of kuppfer cells. After 15 weeks, liver showing almost normal hepatic architecture with mild congestion of portal vasculature, mild dilatation of portal veins and hepatic regeneration (normal central vein, almost normal hepatocytes).

Our data showed improvement (decrease) in liver enzyme activities and bilirubin level after treatment with stem cells and levamisole after 13 and 15 week. These data agreed with Shereen (2007) and El Boshy and Nasr-EL Deean (2013). Who reported the hepatoprotective effect, antioxidant and immunomodulatory effect of levamisole. These biochemical results confirmed to the histopathological changes in liver of rat administered stem cells and levamisole. After 13-week liver showed congestion of portal veins with absence of inter-lobular septae, normal structure of portal area with activation of Kupffer cells. After 15 week showing mild congestion of portal veins with mild dilatation of inter lobular sinusoids, normal structure of portal area, marked activation of Kupffer cells.

Regarding to total protein, albumin, globulin, our data showed that there was significant decrease in total protein, albumin and globulin in rats injected with CCL_4. This data is in agreement with Elshater et al. (2013); Navarro and Senior (2006); Rao et al. (2006) and Mona et al. (2014). This may due to liver damage as liver consider the main organ responsible for synthesis of most proteins. Hypoalbuminemia occurs commonly in association with chronic hepatic disease and liver cirrhosis. Also, may be due to low feed intake, decrease absorption of proteins. In addition, CCL_4 affects kidney which led to albuminuria.

In addition, our result showed significant increase in total protein, albumin and globulin levels after administration of stem cells and levamisole due to the marked improvement of the liver lesions. This result agrees with Hussein et al. (2003) and Shereen (2007). They found that serum total protein and globulin significantly increased in rabbits treated with levamisole due to the ability the levamisole to enhance both cellular and humoral immune responses.

While Rats treated with the isolated mesenchymal stem cells from bone marrow cause significant increase in serum total protein, albumin, globulin levels. This finding is supported by Zhao et al., (2005), Abdel Aziz et al., (2007) and Qiao et al. (2011). They reported that treatment with BM-MSCs in CCl_4 induced liver fibrosis in rats leads to improvement in serum protein activity and an increase in serum albumin levels. This may be due to MSCs was able to promote partial recovery of liver function, suppression of liver inflammation, and had the best therapeutic effect for hepatic fibrosis**.**

Concerning to serum IL-6, After 15 week, our results showed that carbon tetrachloride injected groups showed a significant increase in IL-6. These data agree with Davis et al. (2010) who indicated that CCl_4-induced acute liver injury could activate hepatic non-parenchymal cells (including Kupffer cells and stellate cells) and increase the production of TNF-α and IL-6. The release of TNF-α, as a pro-inflammatory mediator in liver apoptosis, is also linked to cytotoxicity induced by CCl_4. Kupffer cells (macrophages in liver) produce TNF-α in rapid response to tissue injury, which then up-

regulates the expression of IL-6. TNF-α and IL-6 together activate the neighboring hepatocytes (Wu et al., 2010). While after injection of stem cells, data showed that there was significant decrease in IL-6. This result agrees with Nemeth et al. (2009). They showed that an intravenous injection of MSCs can beneficially modulate the response of the host immune system and improve survival. And their results suggested that the injected MSCs interact with monocytes and macrophages in circulating and tissue and reprogram them. Treated monocytes and macrophages produce large amounts of IL-10, and the treatment decreases the amounts of circulating TNF-α and IL-6. In addition, treatment with stem cells and levamisole showed significant decrease in IL-6. These data agree with Sun et al. (2009). They reported that treatment with levamisole and colchicine result in significant reduction of IL-6and TNF-α level in mucocotaneous type of Behcet's disease patients. This attributed to the modulating effect of levamisole on both cell- mediated and humoral immunity.

Concerning to immunological parameters, Carbon tetrachloride injected groups showed significant decrease in Phagocytic percentage (%) and phagocytic index also showed significant decrease in IgG and IgM at 15th weeks when compared with control (-ve) group. This result supported by Bishayi et al. (2002) and Biswajit and Mahuya (2010) who reported that carbon tetra chloride (CCl_4) not only causes liver damage but also confers an immunocompromised state particularly concerning macrophage function. Biswajit and Mahuya, (2012) mentioned that rats injected with CCl_4 at a dose of 0.5 ml/kg b.w (i/p) showed significant decrease in the phagocytic capacity of CCl_4 intoxicated splenic macrophages when allowed to ingest heat killed *S. aureus*. This can be explained in the light of morphologic alteration, reduction in membrane integrity and reduced adhering capacity of macrophages. While after injection of stem cells, data showed significant increase in Phagocytic % and phagocytic index and also showed there were significant increase in IgG and IgM. This data agrees with Ramasamy et al. (2007). Who stated that MSCs have the ability to modify and influence almost all the cells of the innate and adaptive immune systems to interfere with and affect cellular proliferation, differentiation, maturation, and function to induce an anti-inflammatory phase.

After treatment with stem cells and levamisole, there were a significant increase in Phagocytic % and phagocytic index and also there were significant increase in IgG and IgM. These data are in agreement with Findlay and Munday (2000); Ispir and Yonar (2007); Li et al. (2006) and Nevien et al. (2008). This increase was attributed to the immunostimulant effects of levamisole due to its ability to enhance both the innate and specific humoral and cellular immune responses. In addition, these data are in agreement with Bilandzic et al. (2010) who reported that levamisole treatment of boars for three consecutive days in a dose of 2.5 mg/kg body weight led to a significant increase in serum IgG concentration after treatment whereas serum IgM levels were unaffected by levamisole treatment.

5. CONCLUSION

We could conclude from our study that there were improvements in erythrogram (RBCs, Hb, PCV, red blood indices), leukogram (WBCs, lymphocytes, granulocytes), liver functions (ALT, AST, ALP, Bilirubin), IL-6, immunological responses (phagocytic activity, IgG, IgM) and also liver tissue after treatment with stem cells or stem cells and levamisole compared with CCL_4 with superiority of stem cells and levamisole together.

6. REFERENCES

Abdel Aziz, M.T., Atta, H.M., Mahfouz, S., Fouad, H.H., Roshdy, N.K., Ahmed, H.H., Rashed, L.A., Sabry, D., Hassouna, A.A., Hasan, N.M., 2007. Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis. Clin Biochem 40, 893-899.

Bigoniya, P., Singh C. S., Shukla, A., 2009. A Comprehensive Review of Different Liver Toxicants Used in Experimental Pharmacology. International Journal of Pharmaceutical Sciences and Drug Research 1, 124-135.

Bilandzic, N., Mitak, M., Simic, B., 2010. Levamisole Increases Leukocyte Count and Immunoglobulin Levels in Young Boars. Slov Vet Res 47, 13-19.

Bishayi, B., Roychowdhury, S., Ghosh, S., Sengupta, M., 2002. Hepatoprotective and immunomodulatory properties of Tinospora cordifolia in CCl4 intoxicated mature albino rats. The Journal of toxicological sciences 27, 139-146.

Biswajit, C., Mahuya, S., 2010. Supporting the immune system through functional modulation of carbon tetrachloride intoxicated splenic macrophages by administering Tinospora cordifolia. Journal

of Applied Pharmaceutical Science 2, 117-124.

Christ, B., Dollinger, M.M., 2011. The generation of hepatocytes from mesenchymal stem cells and engraftment into the liver. Current opinion in organ transplantation 16, 69-75.

Dalrymple, S.A., Lucian, L.A., Slattery, R., McNeil, T., Aud, D.M., Fuchino, S., Lee, F., Murray, R., 1995. Interleukin-6 deficient mice are highly susceptible to Listeria monocytogenes infection: correlation with insufficient neutrophilia. Inf. Immun, 63, 2262-2268.

Davis, J.M., 3rd, Knutson, K.L., Strausbauch, M.A., Crowson, C.S., Therneau, T.M., Wettstein, P.J., Matteson, E.L., Gabriel, S.E., 2010. Analysis of complex biomarkers for human immune-mediated disorders based on cytokine responsiveness of peripheral blood cells. Journal of immunology (Baltimore, Md. : 1950) 184, 7297-7304.

Dyce, K.M., Sack, W.O., Wensing, C.J., 1987. Text Book of Veterinary Anatomy. W B Saunders Co, Philadelphia.

Eidi, A., Mortazavi, P., Tehrani, M.E., Rohani, A.H., Safi, S., 2012. Hepatoprotective effects of pantothenic acid on carbon tetrachloride-induced toxicity in rats. EXCLI journal 11, 748-759.

El Boshy, M.E., Nasr-EL Deean, N., 2013. Comparative study on the effect of fucoiden and levamisole on some selective Biochemical and Hematological parameters in heat stress rabbits. International Journal of Pharmaceutical and Medical Research 1, 1-5.

Elshater, A.A., Salman, M.M.A., Moahmed, S., 2013. The hepato-ameliorating effect of Solanum nigrum against CCL4 induced liver toxicity in Albino rats. Egypt Acad J Biolog Sci 5, 59-66.

Findlay, V., Munday, B., 2000. The immunomodulatory effects of levamisole on the nonspecific immune system of Atlantic salmon, Salmosalar L. J. Fish Dis. 23, 369-378.

Geetha, S., Jayamurthy, P., Pal, K., Pandey, S., Kumar, R., Sawhney, R.C., 2008. Hepatoprotective effects of sea buckthorn (Hippophae rhamnoides L.) against carbon tetrachloride induced liver injury in rats. J Sci Food Agr 88, 1592-1597.

Hsu, Y.W., Tsai, C.F., Chang, W.H., Ho, Y.C., Chen, W.K., Lu, F.J., 2008. Protective effects of Dunaliella salina--a carotenoids-rich alga, against carbon tetrachloride-induced hepatotoxicity in mice. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 46, 3311-3317.

Hussein, S.H., Abd-El Hamid, S., Sahlaby, S., 2003. Evaluation of levamisole as antihelmintic in sheep naturaly infested with gastrointestinal nematod. Egy. J. Ag. Res 87, 415-431.

Ispir, U., Yonar, M.E., 2007. Effects of levamisole on phagocytic activity of rainbow trout (Oncorhynchus mykiss W.). Acta Vet Brno 76, 493-497.

Jawad, K.A., Bassim, M.J., Suad, A.M., 2013. Toxicopathological and biochemical effects of Carbon Tetrachloride CCl4 with residual accumulation in Liver of mice. Kufa Journal for Veterinary Medical Sciences 4, 57-68.

Kamel, H.H., Azza, H.A., Walaa, M.S.A., Amira, H.M., 2010. Protective effect of some antioxidants against CCl4 induced toxicity in liver cells from BRL3A cell line. Clinical Pathology Dept., Fac. of Vet. Med. Beni-Suef University, Beni-Suef, Egypt.

Kaplowitz, N.T.Y., Simon, F.R., Stolz, A., 1986. Drug induced hepatotoxicity. Annals of Internal Medicine 104, 826-839.

Keyser, J.W., Watkins, G.L., 1972. Estimation of serum proteins by electrophoresis on cellulose acetate Clin. Chem. 18, 1541-1542.

Le Blanc, K., Rasmusson, I., Sundberg, B., Gotherstrom, C., Hassan, M., Uzunel, M., Ringden, O., 2004. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363, 1439-1441.

Lee, T.Y., Chang, H.H., Chen, J.H., Hsueh, M.L., Kuo, J.J., 2007. Herb medicine Yin-Chen-Hao-Tang ameliorates hepatic fibrosis in bile duct ligation rats. J Ethnopharmacol 109, 318-324.

Li, G., Guo, Y., Zhao, D., Qian, P., Sun, J., Xiao, C., Liang, L., Wang, H., 2006. Effects of levamisole on the immune response and disease resistance of Clariasfuscus Aquaculture 253, 212-217.

Mariam, G.E., Hassanenem M.M., Ibrahim M.F., Nermeen, M.S., M.A., A.e., 2015. Evaluation of protective and therapeutic role of moringa oleifera leaf extract on CCL4 induced genotoxicity, hemotoxicity and hepatotoxicity in rats Int. J. Pharm Tech Res 7, 392-415.

Mona, N.M., Olfat A.H., Fatma, H.S., M.E., M., S., S.A., 2014. Transplantation of Modified and

Fresh Hepatocyte Reduces Hepatotoxicity Induced by Carbon Tetrachloride. Life Science Journal 11.

Navarro, V.J., Senior, J.R., 2006. Drug-Related Hepatotoxicity N. Engl. J. Med. 354, 731-739.

Nemeth, K., Leelahavanichkul, A., Yuen, P.S., Mayer, B., Parmelee, A., Doi, K., 2009. Bone marrow stromal cells attenuate sepsis via prostaglandin E2- dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 15.

Nevien, K.M.A., Viola, H.Z., Yousef, M.A.A., 2008. effect of some immunostimulants on health status and disease resistance of niletilapia (oreochromis niloticus) In: 8th international symposium on tilapia in aquaculture.

Ovary, Z., 1966. The structure of various immunoglobulins and their biological activities. A nal. NY Acad. Sci. 242 2606.

Oyagi, S., Hirose, M., Kojima, M., Okuyama, M., Kawase, M., Nakamura, T., Ohgushi, H., Yagi, K., 2006. Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCl4-injured rats. J. Hepatol 44, 742–748.

Qiao, H., Tong, Y., Han, H., 2011. A novel therapeutic regimen for hepatic fibrosis using the combination of mesenchymal stem cells and baicalin. Pharmazie 66, 37–43.

Ramasamy, R., Fazekasova, H., Lam, E.W.F., Soeiro, I., Lombardi, G., Dazzi, F., 2007. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. Transplantation 83, 71–76.

Rao, G.M., Rao, V.C., Pushpangadan, P., Shirwaikar, A., 2006. Hepatoprotective effects of rubiadin, a major constituent of Rubiacordifolia Linn Journal of Ethnopharmacology 103, 484- 490.

Reitman, S., Frankel, F., 1957. Colorimetric method of determination of serum glutamic oxaloacetic and glutamicpyruvic transminases. Am. J. Clim. Pathol 28, 26-34.

Sahar, N.E. 1998. Clinicopathological studies in experimental animal suffering from hepatic fibrosis before and after treatment by colchicines. M.V.Sc, Zagazig University.

Shereen, M. 2007. Clinicopathological studies on the effect of levamisole in rabbits. Ph.D., Fac of Vet. Med., Zagazig Univ.

Sun, A., Wang, Y.P., Chia, J.S., Liu, B.Y., Chiang, C.P., 2009. Treatment with levamisole and colchicine can result in a significant reduction of IL-6, IL-8 or TNF-alpha level in patients with mucocutaneous type of Behcet's disease. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology 38, 401-405.

Taira, Z., Yabe, K., Hamaguchi, Y., Hirayama, K., Kishimoto, M., Ishida, S., Ueda, Y., 2004. Effects of Sho-saiko-to extract and its components, Baicalin, baicalein, glycyrrhizin and glycyrrhetic acid, on pharmacokinetic behavior of salicylamide in carbon tetrachloride intoxicated rats. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 42, 803-807.

Tietz, N.W., 1986. Determination of bilirubin and glucose. Text book of Clinical Chemistry. W.B. Saunders Co., London Philadelphia.

Tietz, N.W., Burtis, C.A., Ducan, P., Ervin, K., 1983. Refrence method for measurement of alkaline phosphatase activity in human serum. Journal of Clinical Chemistry 29, 751-761.

Waugh, A., Grant, A., 2001. Ross and Wilson Anatomy and Physiology in Health and Illness, 9 ed. Churchill Livingstone, Spain.

Weichselbum, T.E., 1964. An accurate rapid method for determination of protein in small amounts of food, serum and plasma. Am. J. Clin. Pthol. 7, 40.

Wilkinson, P.C., 1981. Techniques in Clinical Immunology, 2nd ed. Blackwell Scientific Publications, London.

Wu, Y.L., Lian, L.H., Wan, Y., J.X., N., 2010. Baicalein inhibits nuclear factor-κB and apoptosis via c-FLIP and MAPK in D-GalN/LPS induced acute liver failure in murine models. Chem. Biol. Interact. 188, 526–534.

Zhao, D.C., Lei, J.X., Chen, R., Yu, W.H., Zhang, X.M., Li, S.N., Xiang, P., 2005. Bone marrow-derived mesenchymal stem cells protect against experimental liver fibrosis in rats. World J Gastroenterol 11, 3431-3440.