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 (CCl4) 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 CCL4 (0.2ml/100gm) s/c twice a week for 9 weeks. Group (3): rats injected with CCL4 then given MSCs (3x10⁶cell) I/v once a week for 4 weeks. Group (4): rats injected with CCL4 then given levamisole (2.5mg/kg) three successive days for 4 weeks and stem cells (MSCs) (3x10⁶cell) I/v once a week for 4 weeks. Results showed that injection of CCL4 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.

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). CCL4 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 CCL4 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, potentiates cytotoxic 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 a 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 CCL4 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, 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 was significant decrease in ALT, AST, ALP activities and bilirubin level when compared with their CCL4 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 CCL4 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 kupffer 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 CCL4 then treated by stem cells and levamisole at 13 week showing congestion of portal veins and absence of interlobular septae, almost normal structure of portal area with activation of kupffer cells and marked activation of kupffer 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 kupffer cells fig (6).

4. DISCUSSION:

According to the biochemical result, CCl4 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 CCL4 injected rats.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control(-ve)</td>
<td>15±0.58a</td>
<td>29.33±0.88a</td>
<td>191.67±6.89a</td>
<td>0.63±0.04a</td>
</tr>
<tr>
<td></td>
<td>CCL4(control+ve)</td>
<td>33.67±4.10b</td>
<td>54.33±5.36b</td>
<td>209.67±3.41b</td>
<td>0.84±0.06b</td>
</tr>
<tr>
<td></td>
<td>CCL4 before stem cells</td>
<td>50±1.54c</td>
<td>50.33±2.96b</td>
<td>220±5.29b</td>
<td>0.77±0.02b</td>
</tr>
<tr>
<td></td>
<td>CCL4 before stem cells and levamisole</td>
<td>44±4.01c</td>
<td>44±4.5b</td>
<td>212±1.54b</td>
<td>0.72±0.03b</td>
</tr>
<tr>
<td>9weeks</td>
<td>Control(-ve)</td>
<td>21.33±2.19a</td>
<td>54±0.58a</td>
<td>146.33±4.41a</td>
<td>0.72±0.02a</td>
</tr>
<tr>
<td></td>
<td>CCL4(control +ve)</td>
<td>61.33±1.13b</td>
<td>87±2.08b</td>
<td>194.67±6.39b</td>
<td>0.91±0.04b</td>
</tr>
<tr>
<td></td>
<td>Stem cells before stem cells</td>
<td>52±0.88c</td>
<td>70±2.52c</td>
<td>171.7±3.8c</td>
<td>0.80±0.01c</td>
</tr>
<tr>
<td></td>
<td>Stem cells and levamisole</td>
<td>37.67±1.18d</td>
<td>61.67±3.71d</td>
<td>154.33±6.49a</td>
<td>0.75±0.01a</td>
</tr>
<tr>
<td>13 weeks</td>
<td>Control(-ve)</td>
<td>23.00±2.31a</td>
<td>61.67±3.28b</td>
<td>190.00±8.72b</td>
<td>0.86±0.01b</td>
</tr>
<tr>
<td></td>
<td>CCL4(control +ve)</td>
<td>44±0.88c</td>
<td>70.33±2.4c</td>
<td>193.7±2.61c</td>
<td>0.80±0.02c</td>
</tr>
<tr>
<td></td>
<td>Stem cells and levamisole</td>
<td>44±0.88c</td>
<td>70.33±2.4c</td>
<td>193.7±2.61c</td>
<td>0.80±0.02c</td>
</tr>
<tr>
<td>15 weeks</td>
<td>Control(-ve)</td>
<td>23.00±2.31a</td>
<td>61.67±3.28b</td>
<td>190.00±8.72b</td>
<td>0.86±0.01b</td>
</tr>
<tr>
<td></td>
<td>CCL4(control +ve)</td>
<td>44±0.88c</td>
<td>70.33±2.4c</td>
<td>193.7±2.61c</td>
<td>0.80±0.02c</td>
</tr>
<tr>
<td></td>
<td>Stem cells and levamisole</td>
<td>44±0.88c</td>
<td>70.33±2.4c</td>
<td>193.7±2.61c</td>
<td>0.80±0.02c</td>
</tr>
</tbody>
</table>

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

Table (2): Total protein, Albumin and globulin after treatment stem cells and levamisole in CCL4 injected rats.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Groups</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control(-ve)</td>
<td>6.88±0.22a</td>
<td>4.38±0.55a</td>
<td>3.33±0.13a</td>
</tr>
<tr>
<td></td>
<td>CCL4 (control+ve)</td>
<td>4.06±0.32b</td>
<td>2.19±0.63b</td>
<td>2.88±0.08b</td>
</tr>
<tr>
<td>9 weeks</td>
<td>CCL4 before stem cells</td>
<td>5.19±0.19c</td>
<td>3.61±0.29c</td>
<td>2.83±0.03b</td>
</tr>
<tr>
<td></td>
<td>CCL4 before stem cells and levamisole</td>
<td>4.22±0.19b</td>
<td>2.40±0.61b</td>
<td>2.98±0.09b</td>
</tr>
<tr>
<td></td>
<td>Control(-ve)</td>
<td>7.14±0.29a</td>
<td>5.04±0.33a</td>
<td>3.74±0.11a</td>
</tr>
<tr>
<td></td>
<td>CCL4 (control+ve)</td>
<td>5.19±0.07b</td>
<td>1.84±0.02b</td>
<td>3.25±0.01b</td>
</tr>
<tr>
<td>13 week</td>
<td>Stem cells</td>
<td>6.77±0.09a</td>
<td>4±0.15a</td>
<td>3.53±0.08a</td>
</tr>
<tr>
<td></td>
<td>Stem cells and levamisole</td>
<td>6.02±0.10c</td>
<td>3.98±0.11c</td>
<td>3.45±0.03c</td>
</tr>
<tr>
<td></td>
<td>Control(-ve)</td>
<td>6.22±0.19c</td>
<td>5.41±0.26a</td>
<td>3.49±0.08a</td>
</tr>
<tr>
<td></td>
<td>CCL4 (control+ve)</td>
<td>5.04±0.03b</td>
<td>2.70±0.30b</td>
<td>3±0.11b</td>
</tr>
<tr>
<td></td>
<td>Stem cells</td>
<td>5.90±0.14a</td>
<td>4.81±0.16a</td>
<td>3.35±0.11a</td>
</tr>
<tr>
<td>15 week</td>
<td>Stem cells and levamisole</td>
<td>5.48±0.09a</td>
<td>3.36±0.05c</td>
<td>3.22±0.09a</td>
</tr>
</tbody>
</table>

Table: IL-6, IgG, IgM, Phagocytic % and Phagocytic index after treatment stem cells and levamisole in CCL4 injected rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-6 (mg/dl)</th>
<th>IgG (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>Phagocytic %</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(-ve)</td>
<td>120.63±1.1a</td>
<td>1180.07±20.82a</td>
<td>42.00±1.19a</td>
<td>62.4±4.02a</td>
<td>1.15±0.08a</td>
</tr>
<tr>
<td>CCL4 (Control+ve)</td>
<td>144.2±4.23b</td>
<td>898.67±10.72b</td>
<td>34.67±1b</td>
<td>37.8±2.69b</td>
<td>0.66±0.03b</td>
</tr>
<tr>
<td>Stem cells</td>
<td>130.13±5.98a</td>
<td>1160.33±14.74a</td>
<td>39.33±1.14c</td>
<td>55.6±1.5a</td>
<td>1.1±0.012a</td>
</tr>
<tr>
<td>Stem cells and levamisole</td>
<td>126.87±3.11a</td>
<td>1110±12.56c</td>
<td>38.33±1.06a</td>
<td>47.4±1.71c</td>
<td>0.96±0.022c</td>
</tr>
</tbody>
</table>
The hepato-protective Effect of Stem Cells and Levamisole against Carbon-tetrachloride induced Liver Fibrosis

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 CCL₄ (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 CCL₄ 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 CCL₄ 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 CCL₄ and treated with stem cells and levamisole after13 week showing congestion of portal veins (A arrow) and absence of interlobular septae (B arrow) (H&E x 100).

Figure (6): Liver of rats injected with CCL₄ 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 CCL4 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 kupffer 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 CCL4. 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, CCL4 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 CCL4 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 CCL4-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 CCL4. 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-6 and 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 (CCl₄) not only causes liver damage but also confers an immunocompromised state particularly concerning macrophage function. Biswajit and Mahuya, (2012) mentioned that rats injected with CCl₄ at a dose of 0.5 ml/kg b.w (i/p) showed significant decrease in the phagocytic capacity of CCl₄ 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 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 CCL₄ with superiority of stem cells and levamisole together.

6. REFERENCES


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