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The therapeutic effect of using the stem cells for treating liver toxicity in rats

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ABSTRACT

Objective: The objective of our study is to evaluate the therapeutic effect of Bone marrow mesenchymal stem cells (BMMSCs) on the hepatotoxicity induced by carbon tetrachloride(CCL_4) relative to Curcumin(Cur) as a treatment derived from herbal plants. ***Material & Methods:*** Thirty-five adult male Western Albino Rats were used, were divided into four groups. group(I) : Control gp ,group (II): CCL_4 gp ,group (III) : Cur treated gp , and group (IV): BMMSCs treated gp . Isolation and preparation of BMMSCs, detection of cell-surface markers by flow cytometry, blood samples were collected which in turn used to analyse liver function tests: glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP), total bilirubin, total protein (TP), total antioxidant (TAC) , malondialdehyde (MDA) ,Hemoglobin concentration (HB), White blood cells(WBCs) count and Platelet (PLT) count . Histopathology and immunohistochemistry were also performed. ***Results:*** The liver damage that resulted from CCl_4 showed a significant rise in (ALT, AST , ALP, MDA, T. bilirubin, WBCs count, as well as a decrease in T.P , TAC, HB concentration, and PLT count. treatment with BM- MSCs enhanced liver state more than curcumin ,. It elevated the levels of T.P, TAC, HB, and PLT while considerably lowering the levels of AST, ALT, ALP, MDA, T.bilirubin, and WBCs. Histopathological was marked BM-MSCs improved liver construction. ***Conclusions***:Curcumin and BM-MSCs improve liver function and lessen the toxicity by restoring liver structure and function in a rat model of CCL_4-induced hepatotoxicity ,but treatment with BM-MSCs improved liver condition more successfully.

Introduction:

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The liver is essential organ for continuing homeostasis and metabolic control. It has a basic role in removing of chemicals, toxins, & metabolites, as well as in the synthesis of carbohydrate proteins, hormones, lipid, proteins, and nitrogen metabolism [1] . Hepatotoxicity can result from exposure of pathological causes as viral hepatitis (specially hepatitis B & C), chemicals, drugs, alcohol, and ecological pollutant [2].

Carbon tetrachloride is a public ecological pollutant. $CCl_4$is the organic composite. $CCL_4$was manufactured by the reaction of both chloroform and chlorine. nowadays $CCL_4$is made from methane. fire extinguishers ,refrigerants and a cleaning agent broadly used CCL_4 [3] . CCL_4 is a clear liquid and has sweet odour and does not occur naturally [4] . CCL_4 usages nowadays barred due to its harmful effects,so CCL_4 is just used in rare manufacturing applications. possible ways of person exposure to CCL_4 are breathing, dermal interaction and ingestion [5] . CCL_4 has free radicals which are composed of trichloromethyl (CCL_3) and peroxy trichloromethyl ($OOCCL_3$) radicals. CCL_4 hepatotoxicity are due to the release of These free radicals which produce lipid peroxide which may lead to cell membrane harm, variation in enzyme activity and lastly generation of hepatotoxicity [6].

Curcumin, a bioactive composite, is a phytochemical constituent of turmeric curcuma longa which known as a natural herb used in old-style Chinese medicine. Curcumin has an positive function in pharmacological assets, working as an anti-oxidative, anti-inflammatory, anti-proliferative, anti-fibrotic, and immune-modulatory agent [7].Curcumin destroys inflammatory mediators and related pathways involved in cellular proliferation, migration, and invasion in liver cell lines [8].

cell treatment has developed as a new alternate treatment to replace or mend injured cells in a certain organ. Stem cells have self-renewal and differentiation abilities and are a potential way for cell-based treatment [9]. It is said that stem cells are self-renewing cells which have ability to differentiated to a variety of specialized cell kinds. Embryonic stem cells (ESCs) and adult stem cells are the two essential kinds of stem cells. Tissue-specific stem cells were taken from numerous organs of adult body, as the liver, blood, skin, pancreas, skeletal muscle, and brain [10]. Adult mesenchymal stem cells are not hematopoietic. which can distinguish into adipose tissue, cartilage, or bone. They can be insulated from the adipose tissue, bone marrow, and umbilical cord blood.

Mesenchymal stem cells from bone marrow (BM-MSCs) can be used in hepatotoxicity therapy and renewal of hepatic cells [11]. The effects of BM-MSCs include anti-fibrotic and encouraged renewal of damaged hepatocytes when using animal models of hepatotoxicity [12]. BM_MCs have the ability to release numerous bioactive components which are have capability to Encourage the reclamation liver cells that have been destroyed and stopping reactions of inflammatory responses[13]The BM-MSCs cause apoptosis and destroy collagen fibres creation in hepatocytes in vitro study has shown that. The BM-MSCs were discovered in vitro testes to capable of distinguishing to stellate cells of liver, the new distinguished hepatocyte cells similar morphologies and exhibit particular liver markers [14].

Materials and Methods:

Animals

In our investigation, 35 adult male Western Albino rats were employed .The selected rats weights (190-200 gm) and all of the ages ranging from (6-8 weeks) .All rats were examined thoroughly afore the choice and reserved under observation for one week before being used as experimental animals to exclude the infectiously diseased animals. the rats

obtained and housed in Faculty of Medicine Ain shams Medical Research Institute Animal Facility (MASRI-animal) . Rats were kept in metal cages with wire coverings were supplied food and water ad libitum. All study processes were achieved in agreement with and authorized by the"Institutional Animal Care and Use Committee for Fayoum University (FU-IACUC) ", with approval number (AEC2353 -2024)

Chemicals

(CCL_4): Carbone tetrachloride with 100% concentration & Olive oil were bought from (Algomhoria company) ,Curcumin purchased from (sigma company), Dulbecco's Modified Eagle's Medium (DMEM),Sterile (PBS) ،Fetal Bovin Serum (FBS) ،Penicillin-Streptomycin Mixture ،Fungizone media ،0.25%Trypsin and 0.02%EDTA solution were bought from (Capricorn Scientific Company).anti-CD105, anti-CD19 obtained from (Becman Coulter), and kits to assess the level of AST,ALT ،Total Protein(T.P) ،Alkaline Phosphatase(ALP), T.Bilirubin , Total antioxidant (TAC) ,Malondialdehyde (MDA) were obtained by (Bio Diagnostic company, Egypt).

Isolation, Purification and Characterization of bone marrow-derived mesenchymal stem cells (BM-MSC):

Bone marrow was harvested from the femurs and tibia bones of ten (10) male rats of middling weight 100gm then completely involved in 70% ethanol for 1min. then femur and tibia were washed by (PBS) and fungizone. Removing bone marrow was beneath a Cabinets with laminar air flow (Nuair), The bone marrow was drained from the diaphysis by inserting a syringe needle loaded with 3ml of complete culture media (CM) which consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented,13% Fetal Bovine Serum (FBS) , 1.5% penicillin/streptomycin mixture , and 0.05% fungizone into one of the bone ends. The marrow plugs were then dissociated by pipetting and the dispersed cells were centrifuged at 1800 rpm for 15 minutes at 4 ℃.The cellular pellets were washed twice with a CM and centrifuged (by Hettich Centrifuge) . adding 20 ml CM to cells to be sure we have single cells. Isolated cells with CM were incubated in 5% humidified CO2 incubator for 14days with changing medium each 3 days and daily examination by Inverted microscope. Identification of BM- MSCs via adhesion and morphology features, as elongated, spindle, or fibroblastic shapes, The BM-MSC were digested and stained with anti-CD105 as a positive marker – and anti-CD19 as a negative marker. after three days The non-adherent cell was removed. When cultures become related to 90 percent confluence, cells were rinsed two times by PBS and treated by 5ml of 0.25 percent trypsin 0.02 percent EDTA for 2 minutes at 37 °C to detach cells from the flasks.After 2 min CM was added to stop trypsin reaction, then after centrifugation at 2500 rpm for 10 min CM and trypsin were removed and BM-MSCs were dissolved in PBS. This culture was called primary culture or passage (0) culture. they were sub cultured. The resulting culture was referred to as passage (1) culture. the viability test were occurred by Trypan blue. Counting the cells via hemocytometer (LEICA Light microscope) and each rat injected intravenously with 1.5×10^6 cells per mL which suspended in 1.0 ml of PBS . [15,16].

The Experimental groups :

Group 1:(Control group): N=5

Rats in this group were fed ad libitum until the completion of the experiment, after which liver specimens and blood samples were obtained for biochemical examination and histological study.

Group2: (CCL4 group): N=10

To induce liver damage, rats in this group were given an intraperitoneal (IP) injection of carbon tetrachloride (CCL4) at a dose of 0.5 mg/kg twice weekly for two weeks. blood samples was obtained during

the investigation for biochemical examination and liver specimens was obtained at the end of the experiment for histological study. [4,16]

Group3: (Curcumin treated group) N=10

To induce liver damage, rats in this group were given an intraperitoneal (IP) injection of carbon tetrachloride (CCL4) at a dose of 0.5 mg/kg twice weekly for two weeks and subsequently treated with Curcumin (CUR) (400 mg/kg) once a day over 6 days orally in gavage [17] . blood samples was obtained during the investigation for biochemical examination and liver specimens was obtained at the end of the experiment for histological study

Group 4:(Bone Marwa Mesenchymal Stem Cells treated group) N=10

To induce liver damage, rats in this group were given an intraperitoneal (IP) injection of carbon tetrachloride (CCL4) at a dose of 0.5 mg/kg twice weekly for two weeks and subsequently treated with mesenchymal stem cells (BM-MSCS), was injected (IP) once does . then blood samples were collected along of experiment[15]. At the end, rats were sacrificed . Blood was collected from the retro orbital sinus for the biochemical analysis. All rats were euthanized, liver tissues were harvested and handled for histological examination

Biochemical analysis:

Blood was collected in two vacuum tubes, first one having EDTA to HB Conc ,WBCs Count and PLT Count analysis and second tube having gel material to measure the activites of Serum aspartate aminotransferase (AST) ,alanine aminotransferase (ALT) ,Serum Alkaline phosphatase (ALP) , and levels of Serum Total bilirubin (T.bilirubin),Total Protein(T.P),Total antioxidant(TAC),Malondialdehyde (MDA).

Histological study:

Liver tissues were collected and preserved in 10% buffered formalin. We generated 5-μm paraffin slices and stained them with H&E, MTC, and PCNA immunohistochemistry.

Statistical methods:

All analyses were conducted using Microsoft excel and the statistical package of social science (SPSS) version 16 . All analyses were performed using the approach demonstrated by [18,19] . The data were presented as mean ± standard error (SE). P-values less than 0.05 were deemed statistically significant

Result :

The body weight change :

The result of the body weights (BWs) were recorded as mean± SD during the first day and at the finally day of the experiment. all groups showed increase in their BWs compared to the beginning of the experiment. when compared with the control gp at the end of experiment , there was highly significant decrease in BWs in the CCL_4 gp and there was a significant decrease in BWs in Cur Tr gp . and non significant in BM-MSCs Tr gp and when compared BWs with CCL_4 gp at the end of experiment, showed highly significant increased BWs in Curcumin and BM-MSCs treated groups. Figure (3).

Biochemical result:

CCL_4 gp showed highly significant rise in ALT, AST, ALP, MDA and WBCs count with (p<0.001) when compared with control gp and showed highly significant reduction in T.P,TAC ,HB concentration and PLT count with (p<0.001) when compared with control gp.

Cur tr gp showed highly significant rise in ALT, AST, ALP, MDA and WBCs count with (p<0.001),showed highly significant reduction in T.P , HB concentration and PLT count with (p<0.001), significant rise in total bilirubin with (P=0.027) and nonsignificant in TAC with (P >0.05) when compared with control group. But in compared with CCL_4 showed highly significant rise in ALT,AST, total bilirubin, ALP,MDA and WBCs count with (P<0.001) and showed highly significant reduction in T.P,TAC ,HB concentration and PLT count with(P<0.001).

BM-MSCs tr gp showed highly significant increase in MDA and WBCs count with (p<0.001) . but showed highly significant reduction in HB concentration and PLT count with (P<0.001), showed significant rise in ALT, AST, T.P and ALP with (P≤0.05) , nonsignificant in T .bilirubin and TAC with(P>0.05) when compared with control gp. But in compared with CCL_4 gp showed highly significant rise in ALT,AST, total bilirubin, ALP,MDA and WBCs count with (p<0.001) and showed highly significant reduction in T.P,TAC ,HB concentration and PLT count with (p<0.001).

Histopathological examination :

Hematoxylin and eosin (H&E) Liver tissue was taken and fixed in 10% (buffered formalin). Liver slices were dewaxed with xylene then dehydrated with alcohol, rinsed with water, stained with hematoxylin (Merck Millipore, Germany) , washed fast, and differentiated with 1% acid alcohol before washing . The slides were stained with eosin solution for 2-3 minutes, then cleaned and mounted. Photographs were collected.

I-Examination of H & E staining

Control groups had very same histological texture. Hepatocytes were created in the form of branching and anastomosing strands emanating from the central veins and divided by blood sinusoids surrounded by endothelial cells. The hepatocytes had acidophilic cytoplasm with core spherical vesicular nuclei, whereas some were binucleated. (Fig. 4a). CCL_4 gp , numerous of the hepatocytes have cytoplasmic vacuoles and deeply acidophilic cytoplasm ,widely and congested central vein surrounded with large amount of leukocytic inflammatory cells and increasing thickness of each central and portal vein , and deeply stained nuclei (Fig. 4b). On the other hand, in the cur gp the liver showed some improvements as decreasing leukocytic inflammatory cells However, several hepatocytes had deeply nuclei and vacuolated cytoplasm (Fig. 4C). After treatment with BM-MSCs, Hepatocytes showed remarkable improvement, appearing essentially identical to those in the control groups. However, a few hepatocytes seemed to have somewhat vacuolated cytoplasm(Fig. 4d).

II- Masson's trichrome staining

Masson's trichrome stained slices in control group revealed that the hepatocyte parenchyma was supported by a stroma of extremely fine collagenous fibers. Little collagenous fibers rounded central veins were also visible in the portal

region (Fig. 5a). In CCL_4 injected gp, the stroma was in good form. There was thick connective tissue and collagen fibers appeared thicker around core veins, hepatocyte cords, and portal locations. (Fig. 5b). while in the Cur Tr gp some collagen fibers were noticed (Fig. 5C). In BM- MSCs Tr gp, limited collagen fibers were noticed (Fig. 5d)

III- Immune sections staining for PCNA

In normal (control) group PCNA showed positive nuclear reaction in few hepatocytes (Fig. 6a). but tiny positive nuclear reaction in CCL_4 group (Fig. 6b) and some positive nuclear reaction in Cur Tr gp (Fig. 6C). While in BM-MSCs Tr gp the positive nuclear reaction of PCNA were numerous (Fig. 6d).

Discussion:

Liver toxicity is a complex and detrimental condition that can arise from various factors [20] Toxic substances and drugs are a major cause of hepatotoxicity. [21].CCL4 was employed in our investigation to generate liver damage because of the several experimental animal models of liver toxicity, the CCL4 model was the most closely mimicking human liver toxicity.[22]

Curcumin's biological actions include anticancer, anti-inflammatory, and pro-apoptotic activity in cancer cells. [8].Curcumin can also decrease liver toxicity and toxicity induced by other agents. [23]

BM- MSCs have metabolic regulating, anti-apoptotic, antioxidant, and immunomodulatory properties that aid the restoration of liver structure in the experimental model of hepatotoxicity [24]

The rats administrated CCl4 at the end of experiment demonstrated a substantial decrease in body weight when compared to the control group and treated groups. this results in harmony with [25]

Liver enzymes such as ALT, AST, and ALP are significantly blood indicators of liver damage [26].Hepatotoxicity produced by CCl4 injection resulted in significantly higher levels of blood liver enzymes AST, ALT, and ALP when compared to the control group. Our results were consistent with an earlier research by Khalil et al[16], EL Sayed et al ., [27] , Foaud et al ., [28] and Kepekçi et al ., [29], who demonstrated that injecting CCL4 IP into rats (0.5 mg/kg twice a week for 8 weeks) caused liver fibrosis, as well as a rise in ALT, AST and ALP levels.This rise might be related to the release of these enzymes from the cytoplasm. They quickly localized into the blood after cellular injury and represent the levels of liver cell membrane damage[29] However, Cur Tr gp demonstrated a decrease in serum ALT, AST, and ALP levels when compared to CCL4 gp. The results of Zhao et al ., [17] confirm these findings. They demonstrated that curcumin treated group showed decreased in serum ALT, AST and ALP levels in the CUR-L(400 mg/kg) and CUR-H (1200 mg/kg) groups. and agree also with Rivera et al ., [30] Curcumin treatment (100 or 200 mg/kg) effectively reduced liver enzyme increases. Additional Our results of BM-MSCs Tr gp were showed Serum ALT, AST, and ALP levels decreased in comparison to CCL4 gp. These results were agreed with prior research by Aziz *et al*.,[31], Idriss *et al*.,[32]and Li *et a*l., [33] ,They shown that BM-MSCs ameliorated serum of ALT, AST, and ALP levels of treated groups when compared to CCL_4 gp.

The level of bilirubin dramatically increased in CCl_4 gp, compared to control gp. These findings are consistent with findings of Barghi et al ., [34] and Fattah et al ., [35] They demonstrated a considerable rise in the level of total bilirubin in rats injected with CCL_4. We showed also serum total bilirubin level decreased in Cur Tr gr when compared with CCL_4 gp. these results agree with Hussein et al ., [36] who showed Curcumin's impact on hepatotoxicity caused by thioacetamide (TAA) in rats was demonstrated by a reduction in blood total bilirubin levels.as well as , we have found that treatment of rats with BM-MSCs improved the CCl4-altered serum bilirubin levels towards normal value. These findings are consistent with those of Fattah et al ., [35] and Zhang et al.,[37] They observed comparable findings when treating CCl4-intoxicated and cirrhotic mice with MSCs.

serum T.P level reduced in hepatotoxicity rats a consequence of the decreased ability of liver cells to produce proteins[38] . T.P level In CCL_4 gp showed decreasing in as compared to control gp . These findings were in line with those of Khedr and Khedr., [38], EL Sayed et al .,[27]who showed albumin was decreased significantly in albino rats injected with CCL4 and Cho KA et al .,[39] and Ayatollahi et al ., [40] who showed albumin was decreased significantly in mice which injected with CCL4 When the liver was damaged, The liver's capacity to produce ALb is severely diminished. [41]but In Cur Tr gp showed increase in serum T.P level as compared to CCL_4 gp . our results agree with Hussein *et al* ., [36] who say in Curcumin treated group (100 mg/kg b.wt/daily, orally) Albumin and total protein levels increased significantly as compared to thioacetamide (TAA) gp (50 mg/kg /b.wt twice a week, i.p. for three weeks to induce liver damage) .and Khedr and Khedr., [38] who say Curcumin (300 mg/kg, three times per week) treatment enhanced plasma total protein levels in comparison to CCl4 gp (1 mL of CCl4/corn oil (1:1, v/v)/kg, i.p. injection twice weekly).In our BM-MSCs treated group showed Significant increase in serum total protein levels as compared to CCL4 gp. In hepatotoxic rats, BM-MSCs were able to restore serum protein levels. our results in line with Fattah et al ., [35] who showed Alb was increased in MSCs treated rats and Khalil et al ., [16] They demonstrated that the group treated with BM-MSCs had considerably greater blood albumin levels than the CCL4 gp.

Oxidative stress and excessive ROS generation by chemicals and poisons, such as CCL4, cause oxidative damage to DNA, proteins, and lipids. This molecular damage may cause membranes, enzymes, and proteins to disorganize, malfunction, and eventually be destroyed[42,43]. The metabolism of CCl4 in the liver promotes lipid peroxidation and free radical production, which causes inflammation [44]. CCl4 injection caused oxidative damage to the liver tissues, as evidenced by higher levels of MDA, the lipid peroxidation marker, and TAA, coupled by a substantial decrease in CAT enzyme as compared to the control group. According to Abd El-Monem et al., [45], our investigation found a substantial rise in serum lipid peroxidation (MDA) and a significant decrease in serum total antioxidant (TAC) in CCL4

compared to control gp, which might be attributed to CCL4-induced free radical formation.These findings support those of Abd El-Monem et al., [45] They discovered a significant rise in MDA and a substantial decrease in CAT when compared to the control group. And Fattah et al.,[35] who had a considerable rise in serum LPO and a reduction in serum GPx and GST when compared to the control gp .And showed in cur and BM-MSCs Tr gps Serum MDA decreased significantly, whereas serum TAC increased significantly. These may be due to antioxidant activity of Curcumin and BM-MSCs . these data are supported by the result of Fattah et al., [35] They shown that Nano-Cur, alone or in combination with BM-MSCs, dramatically decreased lipid peroxidation. Cur also enhances antioxidant enzyme levels and reduces lipid peroxidation products including lipid hydroperoxide and MDA. Our findings are consistent with those of Alzahrani et al., [46], who validated MSCs' capacity to generate a highly significant rise in blood antioxidant enzyme levels, followed by a highly significant drop in MDA when compared to the CCL4 group, but no significant difference when compared with the control gp. and agree with Abd El-Monem et al., [45] that curcumin causes a considerable rise in blood antioxidant enzyme levels while decreasing MDA levels.

lipid peroxidation was produced in experimental animals by CCl_4. It has been suggested that use of CCL_4 results in hepatotoxicity. that the formation of lipid peroxides causes red blood cell hemolysis. This might be one of the explanations for a lower RBCs count and Hb concentration. Another factor is erythropoietin levels. Erythropoietin, a glycoprotein hormone secreted primarily by the kidney and liver ,which regulates the synthesis of red blood cells. in the case of hepatotoxicity The capacity to release erythropoietin in response to anemia decreased.[47]

White blood cells supply the body with defenses against cancers, viruses, germs, and parasites [48]. Intoxication with CCl_4 generated a significantly substantial rise in WBCs count, which might be attributable to the immune system's protective response[48] .Our study displayed that in compared to the normal control, CCL4 showed a large drop in HB concentration and PLT count, as well as a significant rise in WBCs count. these findings concur with those of Asmaa *et al* .,[49] They shown results clearly demonstrated that CCl_4 administration caused pancytopenia (reduction in the blood elements) as evidenced by microcytic hypochromic anemia and thrombocytopenia (low levels of platelets) as showed by a significant reduction in RBCs count, haematocrit ratio, PCV values and Hb concentration .They founded also leucocytosis (rise white blood cell counts). In our BM-MSCs and Curcumin treated groups we demonstrated a highly significant rise in HB concentration and PLT count, as well as a highly significant drop in WBCs count, as compared to CCL4 gp. This result consistent with Abubakar et al ., [50] They showed Cur treatment has ameliorative effect on hematological of rats with hepatorenal toxicity inducded by lead. and Sandhi et al ., [51]who showed using the stem cell therapy to tread cirrhosis(in Male patient, 50 years old) has ameliorative effect on HB concentration and PLT count .

In our histology study of H & E Examination showed in CCL_4 gp most of the hepatocytes were vacuolated ,congestion blood vessels , huge leukocytic inflammatory cells and increasing thickness of each central and portal vein . In Masson's Trichrome There was dense connective tissue and a noticeable increase in collagen fibers surrounding the central veins, between the hepatocyte cords, and in portal locations. PCNA-immunostained slices indicated a small positive nuclear response.

But histology study of H & E Examination showed in Cur, BM-MSCs Tr gp improvement by decreasing leukoctic inflammatory cells and vacuolated cytoplasm but BM-MSCs Tr gp exhibited substantial improvement in hepatocytes, appearing virtually identical to normal gp of rat . Only a few hepatocytes showed mild vacuolated cytoplasm . Masson's trichrome showed in Cur Tr gp some collagen fibers but it was decreased compared to CCL_4 gp. In BM-MSCs Tr gp few collagen fibers were detected which are equivalent to those in the control group. Immune-stained slices for PCNA shown some positive nuclear reaction in cur Tr gp. BM-MSCs Tr gp had a large number of positive PCNA hepatocytes.

Our results were consistent with Ahmed et al., [15], who demonstrated in CCL4 gp The majority of the hepatocytes exhibited several, massive cytoplasmic vacuoles. Few liver cells have acidophilic cytoplasm and deeply pigmented nuclei. Few hepatocytes in BM-MSCs Tr gp exhibit granular acidophilic cytoplasm and vesicular nuclei. Few cells have cytoplasmic vacuolation. Stroma was well defined in Masson's trichrome stained slices. CCL4 gp showed an increase in thick connective tissue capsule and many collagen fibers around the major veins. A small number of collagen fibers were found in BM-MSCs. PCNA positive hepatocytes were greater in BM-MSCs gp as compared to the control and CCL4 gps. Our results also agree with **Khalil *et al* ., [16]** They shown that Microscopical investigation of liver sections of rats in BM-MSCs Tr gp revealed normal tissue and reduced the degree of histopathological alterations generated by CCl4. And in Masson's trichrome collagen was showed surrounded the central vein and extending to the portal region of liver tissue in CCL_4 gp. And in BM-MSCs Tr gp showed normal tissue normal tissue with no collagen detected. Khedr and Khedr., [38] studied anti-inflammatory and antioxidant properties of Curcumin on CCl_4 (1 mL of CCl4/corn oil i.p. twice weekly) – induced Liver Fibrosis in Rats .there histological study agree with our study .they were showed in CCl_4 tr gp revealed necrosis in a significant region of liver tissue, which was accompanied by neutrophil infiltration in Cur Tr gp shown improved necrosis and inflammatory cellular infiltration while lowering fibrous tissue.

Conclusions : The previous results showed Bone marrow derived mesenchymal stem cells can return construction and job of liver plus significantly reduced the caused liver damage in rats injected by CCL_4, The re-generative abilities and resolve of hepatotoxicity of BM-MSCs were more significantly effective than of curcumin this determined by the biochemical analyses , Histopathological examination and Immunohistochemistry presented

supporting evidence . BM-MSCs treatment may offer hope to hebetic patients . Further research into the differentiation of BM-MSCs into hepatocytes and their application in the treatment of liver disease was advised.

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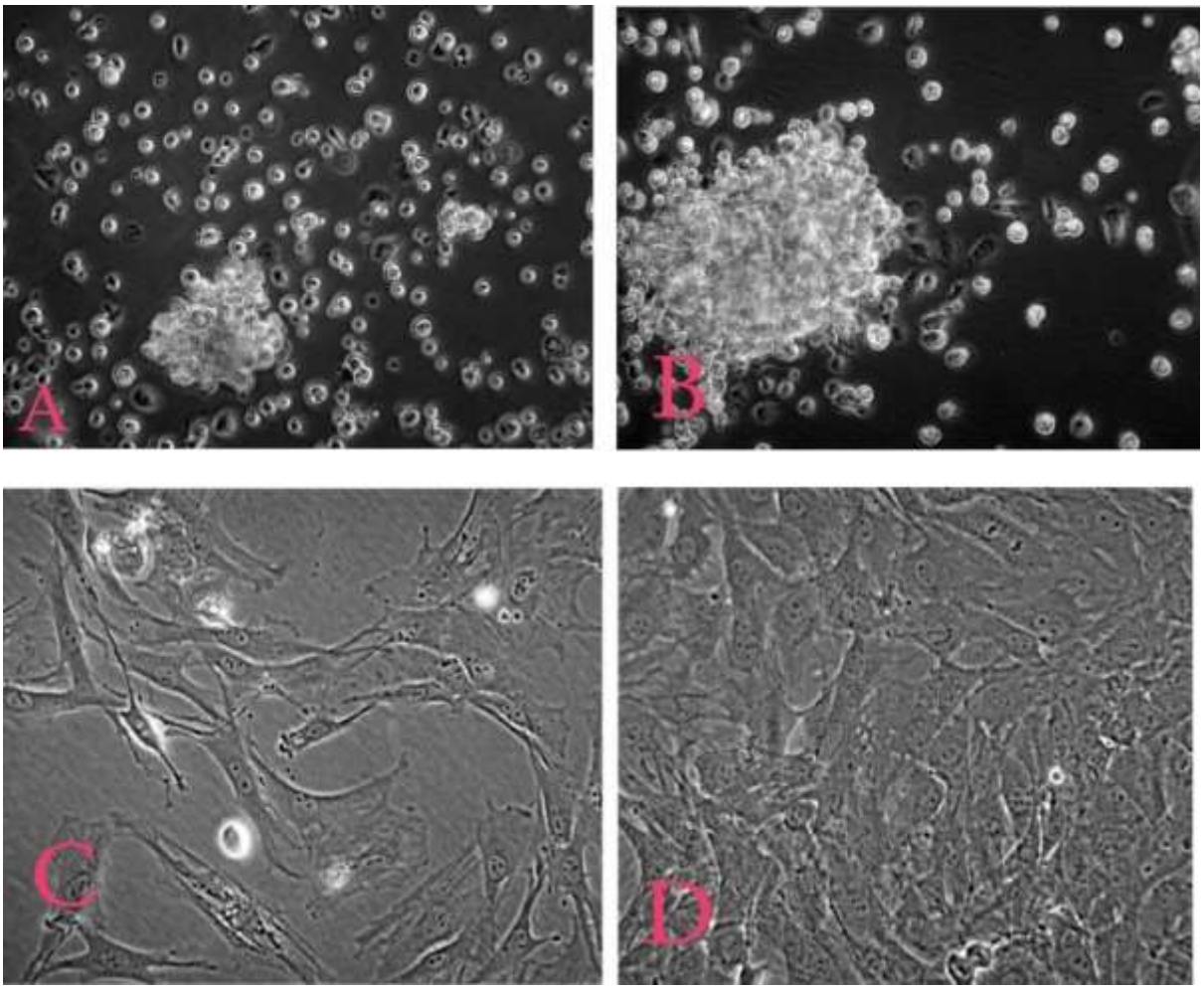
The table (1) :Biochemical results of different gps at the end of experiment:

	Control gp	CCL_4 gp	Cur tr gp	BM-MSCs tr gp
ALT(Units/ml)	**21.56±1.28**	**149.8±5.1** ***P*^{a}=<0.001**	**40.18±0.75** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**28.6±2.75** ***P*^{a}=0.001** ***P*^{b}= <0.001**
AST(Units/ml)	**29.38±3.32**	**196.7±2.33** ***P*^{a}=<0.001**	**37.5±2.5** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**37.5±2.58** ***P*^{a}=0.003** ***P*^{b}= <0.001**
T.bilirubin(g/L)	**0.05±0.027**	**3.78±0.16** ***P*^{a}=<0.001**	**0.086±0.11** ***P*^{a}=0.027** ***P*^{b}= <0.001**	**0.068±0.013** ***P*^{a}=0.221** ***P*^{b}= <0.001**
T.protein(g/L)	**8.62±0.48**	**2.12±0.13** ***P*^{a}=<0.001**	**6.76±0.29** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**7.7±0.29** ***P*^{a}=0.006** ***P*^{b}= <0.001**
ALP(U/L)	**135.4±3.2**	**342.2±3.56** ***P*^{a}=<0.001**	**152.8±4.76** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**145.82±3.29** ***P*^{a}=0.001** ***P*^{b}= <0.001**
TAC(mM/L)	**0.5±0.064**	**0.19±0.18** ***P*^{a}=<0.001**	**0.45±0.027** ***P*^{a}=0.132** ***P*^{b}= <0.001**	**0.49±0.030** ***P*^{a}=0.221** ***P*^{b}= <0.001**
MDA(nmole/mL)	**4.44±0.36**	**11.32±0.25** ***P*^{a}=<0.001**	**6.28±0.13** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**5.58±0.2** ***P*^{a}=<0.001** ***P*^{b}= <0.001**
HB(gm/dl)	**15.17±0.12**	**8.24±0.23** ***P*^{a}=<0.001**	**13.9±0.22** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**14.4±0.245** ***P*^{a}=<0.001** ***P*^{b}= <0.001**
WBCs×10^6/mm^3	**8.29±0.042**	**14.3±0.16** ***P*^{a}=<0.001**	**9.21±0.25** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**8.76±0.16** ***P*^{a}=<0.001** ***P*^{b}= <0.001**
PLT×10^3/mm^3	**278.2±2.95**	**236.2±1.3** ***P*^{a}=<0.001**	**261.8±2.39** ***P*^{a}=<0.001** ***P*^{b}= <0.001**	**268.6±2.3** ***P*^{a}=<0.001** ***P*^{b}= <0.001**

P^a: *P* value versus control group, *P*^{b}: P value versus CCL_4 gp, P>0.05 non-significant , P ≤0.05 significant and *P*<0.001 highly significant



Figure(1)photomicrograph showing growth of rat BM-MSCs

(A) **Day(0) from culture**

(B) **Day(3) of the primary culture showing most of the cells appear rounded in shape with variable sizes after changing media.**

(C) **Day (10)of the primary culture showing most of the attached cells had processes, where some cells appeared spindle-shaped while others showed some cell rounded.**

(D) **Day(14) of the primary culture showing colonies of attached cells with vesicular nuclei and interdigitating processes.**

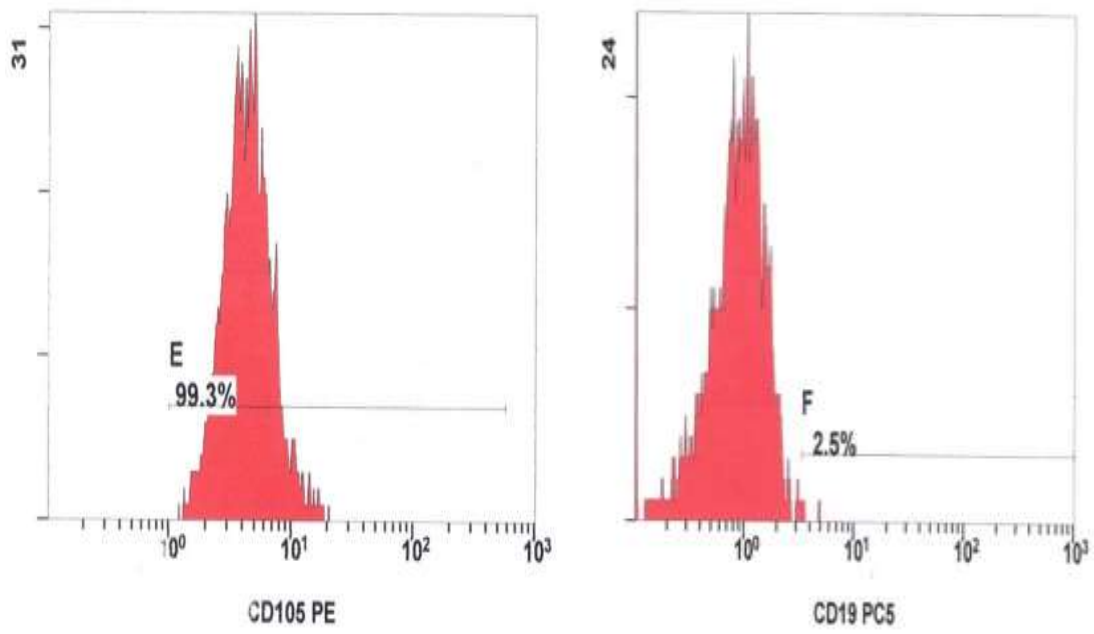


Figure (2): Flow cytometric analysis of cell – surface antigens of rat BM-MSCs a positive reaction CD105 and a Negative CD19

The body weight change :

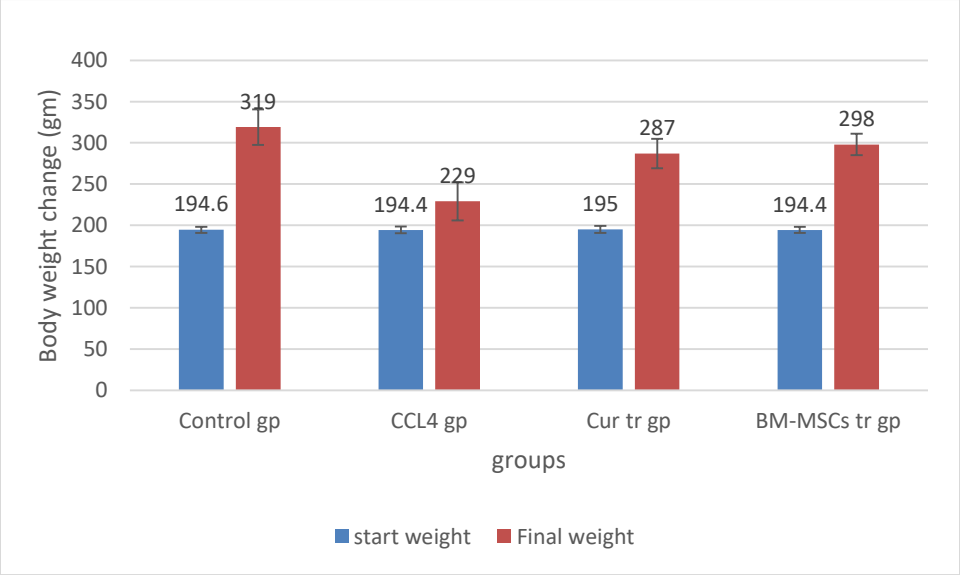


Figure (3): Mean ±SD of body weight (gm) in different groups .

Histopathological examination :

I-Examination of H & E staining

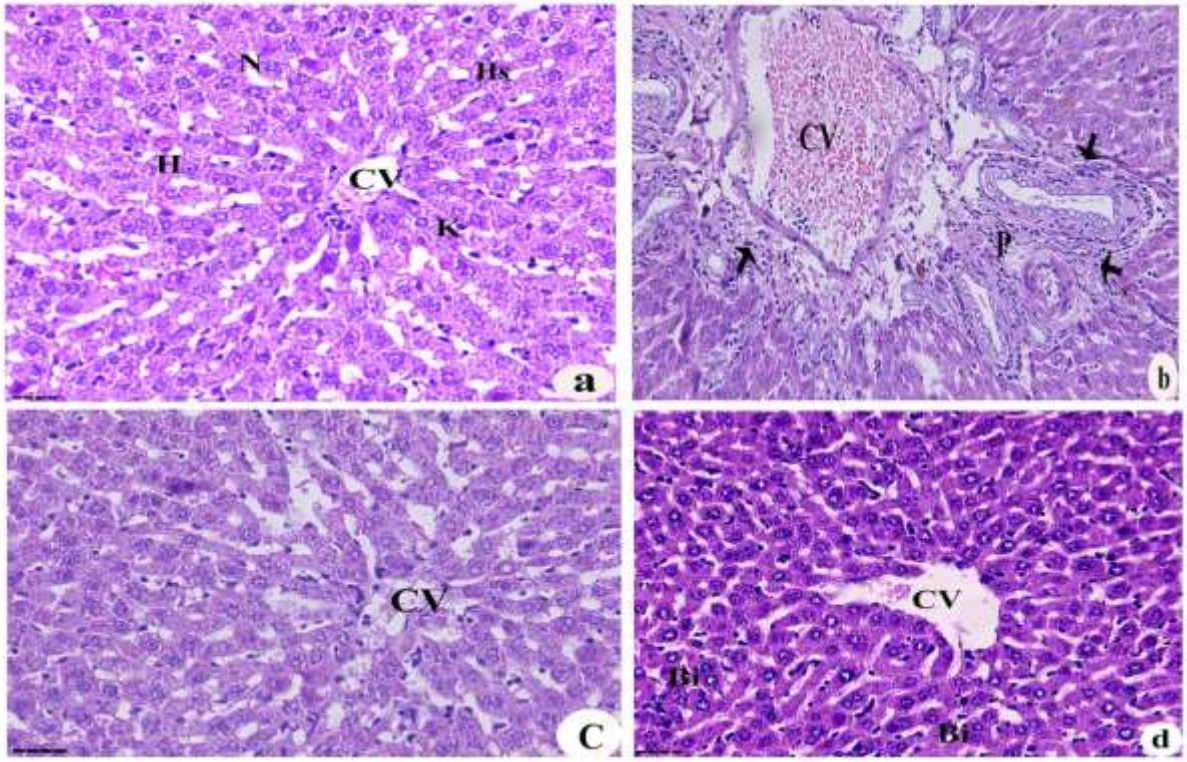


Figure. 4. (a) Showing radiating hebetic strands (Hs)from the central vein (CV.). The hepatocytes(H) feature central, rounded, nuclei (N), acidophilic cytoplasm with kipper cells (k). Some of the cells appear to be bi-nucleated (▲). (b) Most of the hepatocytes are vacuolated and degenerated and huge leukoctic inflammatory cells (↑) in ccl_4 gp. while some improvements showed in Cur Tr group (c)but marked improvements showed in BM-MSCs Tr gp (d)

II- Masson's trichrome staining

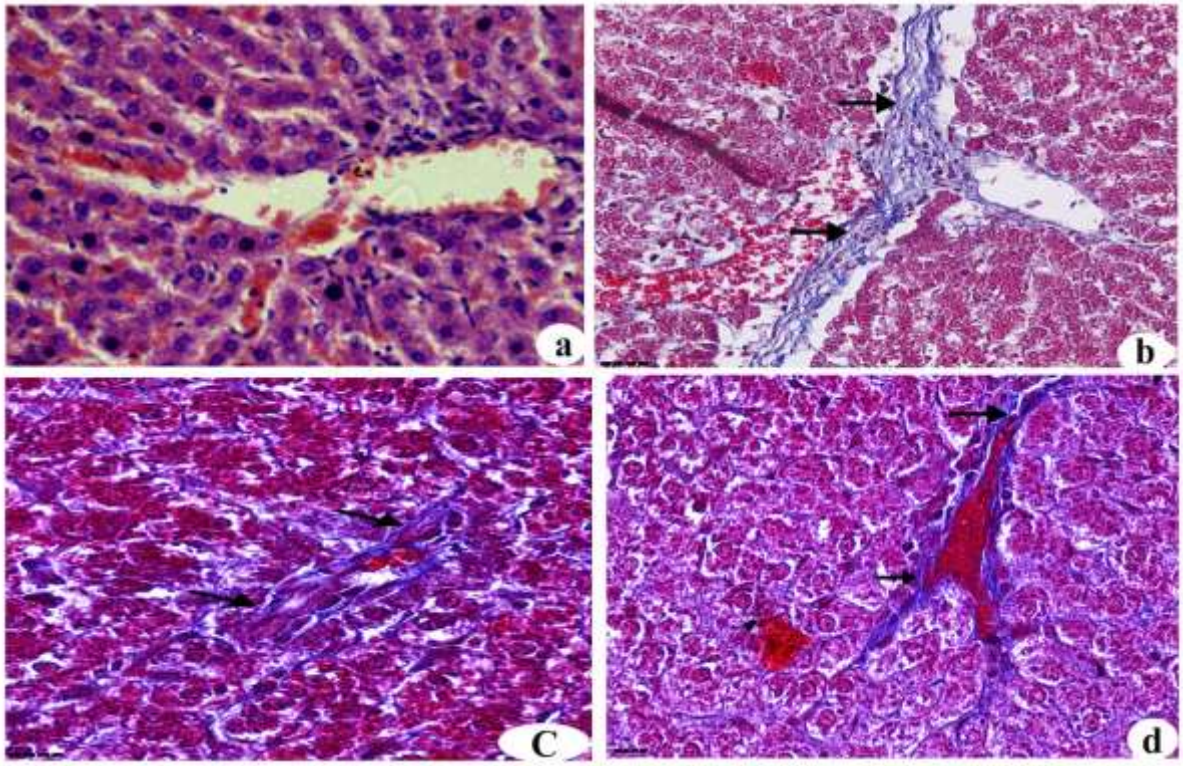


Figure. 5. (a) There is little collagen fibers (↑) a rounding central vein (V), at portal zone (P) but ccl_4 gp Showing many collagen fibers (↑)a rounding the central veins (V), in portal zone (P). (c) showing marked decreasing in collagen fibers in Cur Tr gp (d) Showing The collagen fibers of the BM- MSCs Tr gr are comparable to those of the control group.

III- Immune sections staining for PCNA

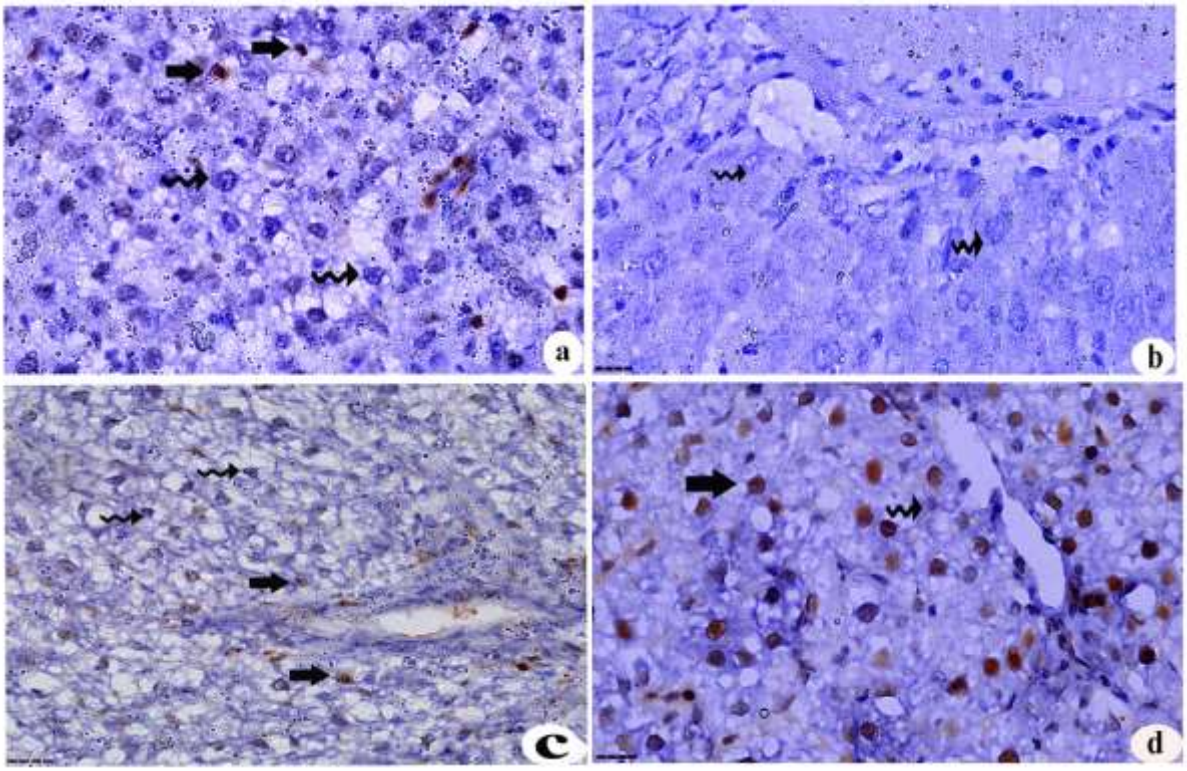


Figure. 6. control gp (a) There is Few hepatocytes with neucler positive immunological reactivity to PCNA (bent arrowe) (b) CCL4 gp There is no positive immunological response for PCNA. (C) There is some increase in PCNA-positive hepatocytes. in Cur Tr gp. (d) BM-MSCs Tr gp show a high number of PCNA-positive hepatocytes.