

Studying the Anticancer Properties of Bone Marrow-Derived Mesenchymal Stem Cells against Hepatocellular Carcinoma Induced by N-Nitrosodiethylamine in Male Rats

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Received: 14.10.2021; Accepted: 18.11.2021; Published: 18.01.2022

Abstract: This study was initiated to emphasize the possibility that bone marrow-derived mesenchymal stem cells (BM-MSCs) could alleviate hepatocarcinogenesis *in vivo*. Forty rats were enrolled and distributed as follows; group 1 was set as a negative control, while all of groups 2, 3, and 4 were orally received N-nitrosodiethylamine (NDEA) for hepatocellular carcinoma induction (HCC). Group 2 was left untreated, whereas groups 3 and 4 were orally treated with BM-MSCs and doxorubicin (DOX), respectively. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γ -GT) activities were estimated. The study looked at serum alpha-fetoprotein (AFP), glypican-3 (GPC-3), signal transducer and activator of transcription 3 (STAT3), and suppressors of cytokine signaling 3 (SOCS3). The liver tissue sections were also examined histopathologically. Results: Serum AST, ALT, ALP, and γ -GT activity were all significantly higher in the HCC group, along with AFP, Gpc-3, and STAT3 levels associated with a significant reduction in SOCS3 level versus the negative control group. The current data indicated that BM-MSCs and DOX administration in HCC rats yielded a significant decline in serum levels of AFP, Gpc-3, and STAT3 along with significant enhancement in serum SOCS3 level. Histopathological examination revealed that treatment with BM-MSCs and DOX significantly reduced the disruption of hepatic tissue architecture caused by NDEA intoxication. Conclusion: The biochemical parameters and the structural organization of the liver were improved considerably in HCC groups treated with BM-MSCs or DOX. Finally, the current work demonstrated the anticancer activity of BM-MSCs against hepatocellular carcinoma in an experimental setting.

Keywords: Hepatocellular carcinoma; bone marrow-derived mesenchymal stem cells; rats.

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1. Introduction

Nitrosamines are created when nitrates or nitrites are combined with amines. Diethylnitrosamine (DEN) is a kind of nitrosamine known to cause liver cancer by creating DNA adducts [1]. The pathogenesis of hepatocellular carcinoma is further complicated by the induction of oxidative stress by DEN [2,3].

Hepatic injury is caused by DEN and other known hepatocarcinogens enhancing cell proliferation, which is accompanied by hepatocellular necrosis [4,5].

They are directed to the liver, where they are bio-transformed by the cytochrome p450-dependent mechanism CYP2E1, resulting in DNA-adducts [6,7]. Alcohol, phenobarbital, 2-

acetylaminofluorene, and DEN are all known to influence hepatic carcinogenesis, which is the sixth most common cancer [8]. Animal models are frequently employed in cancer research and are a significant tool for researching hepatic carcinogenesis. DEN has been shown to induce early oxidative stress, inflammation, and proliferation in rat models. [9,10].

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are adult, multipotent cells that can be detected in practically all postnatal organs [11]. BM-MSCs have been explored in several preclinical and clinical investigations as possible new therapeutic agents for treating immunological illnesses due to their immunomodulatory ability and aptitude for self-renewal and differentiation into tissues of mesodermal origin [12]. Through cell-to-cell contact or the production of soluble factors, BM-MSCs can alter the immune response and regulate the proliferation, activation, and effector function of T lymphocytes, professional antigen-presenting cells [dendritic cells (DCs), macrophages, and B lymphocytes], and natural killer (NK) cells. They are potential prospects for regenerative medicine and solid organ transplantation because of their ability to heal tissue and modulate the immune system [11].

Based on the previous background, the current study was undertaken to elucidate the antitumor potential of BM-MSCs against N-diethylnitrosamine-induced hepatocellular carcinoma in male rats with special concern on their mechanism of action.

2. Materials and Methods

2.1. Chemicals and drugs.

N-nitrosodiethylamine (NDEA; CAS no. 55-18-5) was supplied from Sigma-Aldrich Co. (St. Louis, MO, USA), while doxorubicin (Adricin) (Dox) was purchased from EIMC United Pharmaceuticals, Egypt. All the other chemicals were of high-purity grade following international standards.

2.2. Experimental animals.

A total of 40 mature male Wistar rats weighing 170–200 g were obtained from a breeding stock kept at the National Research Centre's Animal House in Giza, Egypt. Throughout the experiment, the animal housing was ventilated with a 12-hour light/dark cycle at an ambient temperature of 25°C–30°C, with free access to tap water and standard rodent food (Wadi El Kabda Co., Cairo, Egypt). Rats were given at least 10 days to adjust to their new surroundings before the trial began. The Ethical Committee of the Medical Research of the National Research Centre, Egypt, approved the housing and management of the animals and the experimental methodology, which followed the rules for animal research (approval no. 14023).

2.3. Preparation and isolation of BM-derived BM-MSCs.

The tibiae and femurs of 6-week-old male Wistar rats were flushed with phosphate-buffered saline (PBS) to extract bone marrow. Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) was used to plate bone marrow cells. A density gradient was used to separate nucleated cells, which were then resuspended in full culture media supplemented with 1% penicillin-streptomycin. Cells were incubated for 12e14 days at 37 °C in 5% humidified CO₂ as a primary culture or after establishing large colonies. When big colonies formed (80e90 percent confluence), the cultures were washed twice with

phosphate buffer saline (pH 7) and trypsinized for 5 minutes at 37 °C with 0.25 percent trypsin in 1 mmol EDTA. After centrifugation at 3000 rpm for 10 minutes, cells were resuspended in a serum-supplemented medium and incubated in 50 cm² culture flasks. The cultures that resulted were dubbed "first-passage cultures" [13]. Cells were recognized as BM-MSCs based on their morphology or fusiform shape, adhesion [14], and identification of CD73+, CD105+, and CD45-specific to BM-MSCs using Florescent Analysis Cell Sorting (FACS).

2.4. FACS analysis.

Cells were resuspended in wash buffer after brief centrifugation (BD Biosciences, Germany). For 45 minutes at room temperature, 300 ml of cell suspension was incubated with antibodies versus CD45, CD73, and CD105 conjugated with Allophycocyanin (APC), Cyanine 5 (CY5), Phycoerythrin (PE), and Fluorescein isothiocyanate (FITC) dyes, respectively. A FACS Caliber (BD Biosciences, Germany) was used for flow cytometry, and Cell Quest software was used for analysis.

2.5. Tracking of stem cells.

The 4th passage was used to collect BM-MSCs cells. The cells were then trypsinized and suspended as a single cell suspension. 2 x 10⁷ single cells were placed in a falcon tube, rinsed once with serum-free culture media, and centrifuged for 5 minutes at 400xg. Finally, cells were labeled with PKH26 fluorescent linker dye (per the manufacturer's instructions), and fluorescence microscopy was used to study them (Sigma-Aldrich, Saint Louis, USA).

2.6. Study design.

The male rats were randomly categorized into four groups (10 rats/group). (1) Negative control group (Con): The rats in this group were healthy and were given normal saline (0.9%) five times weekly orally throughout the trial. (2) HCC group: rats in this group were given NDEA (dissolved in 0.9 % normal saline) at a dose of 20 mg/kg body weight five times weekly for six weeks, according to Darwish and El-Boghdady [15]. (3) HCC+BM-MSCs group: in this group, HCC-bearing rats were injected with BM-MSCs i. v. by a single dose of 100 µl of a cell suspension containing allogenic BM-MSCs from rats at the moment of the boost (when BM-MSCs were collected) [16]. (4) HCC+Dox-treated group: in this group, HCC-bearing rats were intraperitoneally injected once a week for 5 weeks with Dox as a reference medicine at a dose of 0.72 mg/rat, which corresponded to a human dosage of 40 mg/m² according to Barnes and Paget [17]. Following the last treatment, the experimental rats' food was withheld for 12 hours, and blood samples were taken from the retro-orbital venous plexus in clean centrifuge tubes and allowed to coagulate at room temperature under diethyl ether anesthesia for biochemical tests; serum samples were separated by centrifugation at 1800g for 15 minutes at 4°C in a cooling centrifuge and kept at 20°C until analysis. The rats were sacrificed by exsanguination after the blood samples were collected, a middle abdominal incision was made, and the livers of each rat were rapidly taken out and cleaned in ice-cold saline. After that, a tiny portion of each liver was put in 10% formalin saline for histology slides.

2.7. Biochemical assays.

Hepatic total protein level was determined by the colorimetric method of Lowry *et al.* [18]. Hepatic alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity

were determined by the colorimetric method using the Salucea kit (The Netherlands) according to the method described by Young [19]. The activities of serum alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ -GT) were measured using a colorimetric kit bought from Reactivos GPL Co. Ltd (Barcelona), according to Tietz *et al.* [20]. The levels of serum alpha-fetoprotein (AFP), glypican-3 (GPC-3), signal transducer and activator of transcription 3 (STAT3), and suppressors of cytokine signaling 3 (SOCS3) were measured using ELISA kits purchased from Glory Science Co., Ltd (USA) and performed according to the manufacturer's instructions.

2.8. Histopathological investigation of liver sections.

The tissues were washed in flowing tap water after being fixed in formalin saline (10%) for 24 hours and then dehydrated with an ascending grade of ethyl alcohol (30, 50, 70, 90 %, and absolute). Specimens were cleaned in xylene for twenty-four hours and embedded in paraffin (melting point 58-60 °C). Using a slide sludge microtome, paraffin wax tissue blocks were made for sectioning at 4°C. Deparaffinized tissue slices were collected on glass slides and stained with hematoxylin and eosin. (H and E) stain [21] for histopathological examination through the electric light microscope.

2.9. Statistical analysis.

In the present study, all results were expressed as mean $\pm$ standard error of the mean. The statistical Package for the Social Sciences program, version 21.0, was used to compare the significance of every two groups. The difference was considered as statistically significant when $p < 0.05$. Percentage difference representing the percent of variation for the corresponding control group was calculated according to the following formula:

$$\% \text{ Difference} = (\text{Treated value} - \text{control value}) / \text{control value} \times 100$$

3. Results and Discussion

3.1. Cell surface marker expression analysis, characterization using flow cytometry, and tracking of stem cells.

Flow cytometry was used to examine the immunophenotype of BM-MSCs cells.

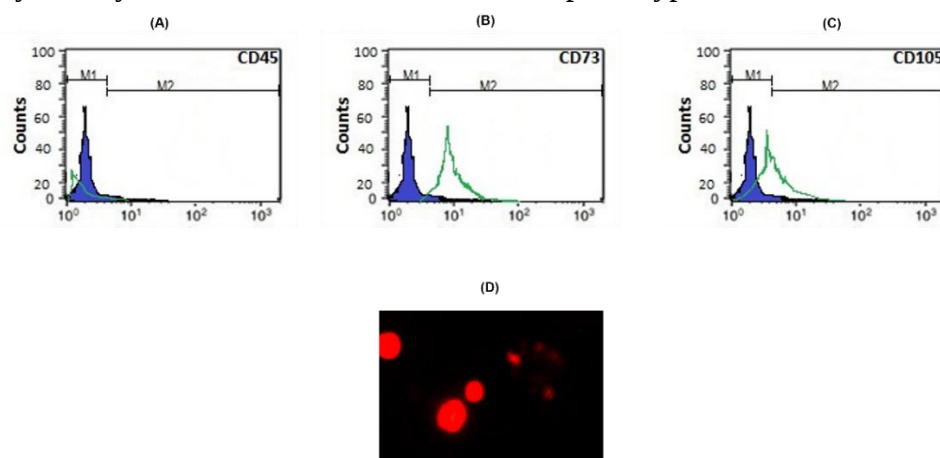


Figure 1. Flow cytometry was used to evaluate the immunophenotype of BM-MSCs. The hematopoietic marker CD45 was negative in BM-MSCs cells (A), while they were substantially positive for mesenchymal stem cell-specific markers such as CD73 (B) and CD105 (C). The green histogram represents the antibody labeled cells, whereas the blue histogram represents the isotype control. PKH26-labeled injected stem cells engrafted in liver tissue in the HCC+ BM-MSCs group (D).

The hematopoietic marker CD45 was negative in BM-MSCs cells (fig. 1A), but they were strongly positive for mesenchymal stem cell markers such as CD73 (fig. 1B) and CD105 (fig. 1C). The green histograms represent the antibody-labeled cells, whereas the blue histogram represents the isotype control. PKH26 was used to label BM-MSCs to track their engraftment in liver tissue (fig. 1D).

3.2. Effect of BM-MSCs and Dox on serum liver enzymes activity in HCC rat model.

The data in Table (1) illustrated the effect of treatment with BM-MSCs and Dox on serum liver enzymes (AST, ALT, ALP, and γ -GT) activity in HCC bearing rats. HCC group showed significant elevation ($P < 0.05$) in serum AST, ALT, ALP, and γ -GT activity concerning the negative control group. Treatment of the HCC group with BM-MSCs and Dox experienced a significant reduction ($P < 0.05$) in serum AST, ALT, ALP, and γ -GT levels compared to the untreated HCC group.

Table 1. Impact of BM-MSCs and Dox on serum liver enzymes activity in HCC rat model.

	AST (U/L)	ALT (U/L)	ALP (U/L)	γ -GT (U/L)
Con	204.32±13.22	95.14±9.26	264.91±12.31	35.22±3.62
HCC	355.21±11.14 ^a	171.33±12.41 ^a	362.04±16.02 ^a	75.61±5.42 ^a
HCC+BM-MSCs	252.03±9.66 ^b	132.61±7.61 ^b	295.52±8.61 ^b	53.11±4.22 ^b
HCC+Dox	223.42±7.25 ^b	115.71±6.52 ^b	276.42±15.03 ^b	43.62±3.70 ^b

Data are expressed as mean $\pm$ SE of 10 rats/group.

a Significant change at $p < 0.05$ compared to the negative control group.

b Significant change at $p < 0.05$ compared to the untreated HCC group.

3.3. Effect of BM-MSCs and Dox on serum AFP, GPC-3, STAT3, and SOCS3 levels in HCC rat model.

Table 2 represents the consequence of the treatment with BM-MSCs and Dox on serum levels of tumor markers in the HCC rat model. HCC group experienced significant increment ($p < 0.05$) in serum AFP, GPC-3, and STAT3 levels associated with a significant reduction ($p < 0.05$) in serum SOCS3 level in comparison with the negative control group. In contrast, the treatment of HCC-bearing rats with BM-MSCs or doxorubicin yielded a significant reduction ($p < 0.05$) in serum AFP, GPC-3, and STAT3 levels concomitant with significant elevation ($p < 0.05$) in the serum SOCS3 level relative to the untreated HCC group.

Table 2. Impact of BM-MSCs and Dox on serum AFP, GPC-3, STAT3, and SOCS3 levels in HCC rat model.

	AFP (ng/mL)	GPC-3 (pg/mL)	STAT3 (pg/mL)	SOCS3 (pg/mL)
Con	23.04±3.22	15.23±2.31	762.42±45.62	120.52±5.63
HCC	49.62±4.02 ^a	45.62±5.22 ^a	1456.63±83.71 ^a	65.63±4.21 ^a
HCC+BM-MSCs	34.71±3.11 ^b	26.71±3.82 ^b	982.72±63.81 ^b	105.13±6.72 ^b
HCC+Dox	32.34±2.64 ^b	22.36±1.54 ^b	853.24±46.21 ^b	109.43±7.42 ^b

Data are expressed as mean $\pm$ SE of 10 rats/group.

a Significant change at $p < 0.05$ compared to the negative control group.

b Significant change at $p < 0.05$ compared to the untreated HCC group.

3.4. Histological examination.

Figure 1 illustrates the histological features of the liver tissue from the negative control group stained with hematoxylin and eosin (H&E) showed normal histological architecture of the central vein and the surrounding hepatocytes. While an optical micrograph of the liver tissue section of rats in the untreated HCC group shows the portal area with fibrosis that has

spread and divided the hepatic parenchyma into dysplastic lobules with conspicuous nucleolus degenerated hepatocytes (Figures 2 and 3).

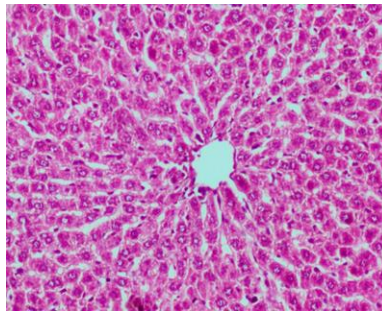


Figure 1. Photomicrograph of Liver from control rat showing normal liver architecture with normal central vein, hepatocytes, sinusoid, and kupffer cells. (H &E X 100).

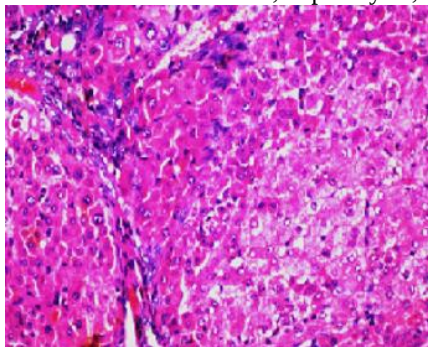


Figure 2. Photomicrograph of Liver from HCC untreated group shows a group of dysplastic hepatocytes with a prominent nucleolus. (H & E x 40).

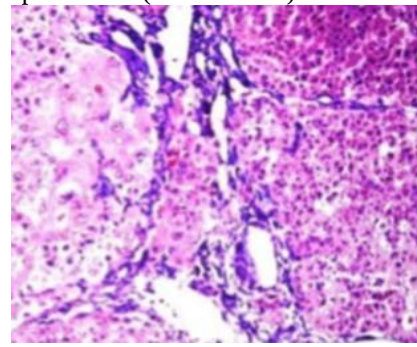


Figure 3. Photomicrograph of Liver from HCC untreated group shows fibrosis in the portal area and divided the hepatic parenchyma into dysplastic and degenerated hepatocytes (H&EX40).

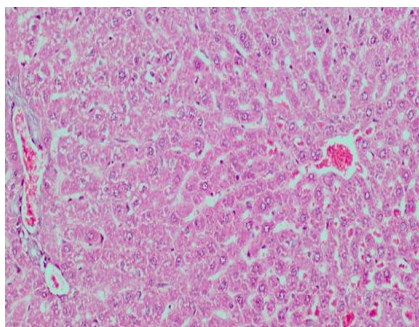


Figure 4. Photomicrograph of HCC rat treated with BM-MSCs showing congested central veins and blood sinusoids associated with vacuolization and degeneration of hepatocytes (H &E X 100).

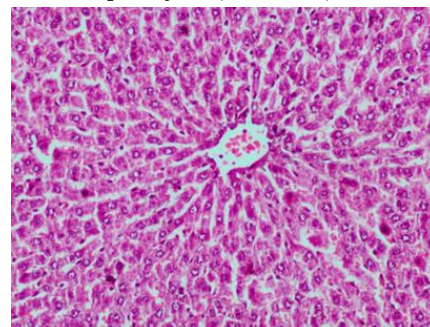


Figure 5. Photomicrograph of HCC rat treated with DOX showing prominent recovery in the form of reduced cytoplasmic vacuolization, normal central vein, and blood sinusoids (H &E X 100).

Whereas the microscopic investigation of the liver tissue sections of the BM-MSCs treated, the group showed congested central veins and blood sinusoids associated with vacuolization and degeneration of hepatocytes (Figure 4). The hepatocytes of the liver tissue section of the Dox-treated group showed prominent recovery in the form of reduced cytoplasmic vacuolization, normal central vein, and blood sinusoids (Figure 5).

3.5. Discussion.

The use of BM-MSCs in cell-based therapies for treating acute and chronic liver disorders has been extensively studied. Ischemia-reperfusion damage, acute failure, fibrosis, and hepatocellular carcinoma have all been demonstrated to be alleviated by BM-MSCs in studies [22]. The present setup indicated that administering BM-MSCs to experimental rats might modulate tumor marker activities and prevent HCC generated by NDEA. Current study

results demonstrated that the HCC group displayed significant elevation in serum AST, ALT, ALP and γ -GT activity compared to the negative control group. AST elevations are common in patients with cirrhosis and even in liver disorders where ALT levels are normally elevated [23]. Preoperative ALP levels may be used to monitor and predict recurrence in high-risk HCC patients, according to recent research [24]. Chemical carcinogens may cause a cascade of events that lead to the production of γ -GT [25]. As its activity corresponds with tumor growth rate, differentiation, and host survival, higher levels of γ -GT represent the development of carcinogenesis [26].

The current study's findings demonstrated that the treatment of the HCC group with BM-MSCs produced a significant reduction in serum AST, ALT, ALP, and γ -GT activity compared to the untreated HCC group. This finding suggests that BM-MSCs induced pronounced alleviation in the hepatocytes damage may be associated with antioxidant effect [27]. Numerous studies demonstrated that transplantation of stem cells had been determined to exert therapeutic effects in cases of liver fibrosis, after repeated partial hepatectomy, and significantly lowering levels of AST, ALT, and γ -GT [28]. Furthermore, BM-MSCs have been shown to limit the activation, proliferation, and death of hepatic stellate cells (HSCs) more effectively and maintain a better AST/ALT ratio [29,30]. The production of anti-inflammatory and angiogenic factors, the activation and upregulation of several antioxidant pathways, and the control of the apoptotic pathway are all processes by which BM-MSCs reduce liver fibrosis [31].

The metabolic activation of NDEA in the liver causes the production of reactive oxygen species (ROS), which cause oxidative stress. The production of DNA adducts, lipid and protein damage, and aberration of numerous signaling pathways, particularly those implicated in HCC, are all factors that contribute to hepatocarcinogenesis [32]. NDEA treatment considerably elevated serum AFP levels, according to the existing findings of the Song *et al.* study [33] who strongly supports this conclusion. AFP as a conventional diagnostic marker for HCC screening has been around for a long time [34]. AFP is a fetal glycoprotein synthesized in the fetal liver and yolk sac during pregnancy. Its serum level drops dramatically shortly after birth, and its synthesis in the adult liver ceases [35]. The overexpression of the AFP gene in NDEA-administered rats due to hepatocyte necrosis is most likely the cause of the observed rise in blood AFP levels [36]. Furthermore, the abnormal overexpression of nuclear factor kappa B (NF- κ B), which is known to be implicated in hepatocarcinogenesis, may be responsible for neoplastic hepatocytes' resynthesis of AFP [37]. NF- κ B regulates the expression of several genes involved in cell proliferation, inflammation, cell death resistance, and carcinogenesis, including cyclooxygenase-2 (COX-2) [38]. A former study of Cui *et al.* [39] demonstrated that the inflammation resulting from COX-2 upregulation can enhance AFP production by neoplastic hepatic cells.

On the opposite side, BM-MSCS administration in HCC group significantly dampened the elevation of the serum AFP level. The reduction of COX-2 gene expression by BM-MSCs, which is known to control AFP transcription, is thought to be how BM-MSCs might recover AFP serum levels. The action of NF-B, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) has been found to reduce the production of the COX-2 gene by BM-MSCs [40]. Furthermore, these researchers speculated that BM-MSC injection would cause a decrease in protein synthesis by hepatic tumor cells due to its anticancer efficacy, which could lead to tumor growth shrinkage and a decrease in AFP synthesis by tumor cells. This suggestion is

highly supported by Yaqub *et al.* [40], who demonstrated that BM-MSCS treatment attenuates the fibrotic and oxidative state.

Mamdouh *et al.* [41] found that giving Dox to HCC patients reduced the serum level of AFP. Such finding supports the current results, which showed that doxorubicin treatment of HCC group significantly reduced the AFP serum level. Also, the present study demonstrates that doxorubicin significantly decreased AFU serum levels in the HCC group. The capacity of doxorubicin to reduce tumor mass, resulting in lower protein synthesis by the tumor, could explain the decrease in AFP serum level Kusuzaki *et al.* [42] strongly support this suggestion.

GPC-3 is a heparan sulfate proteoglycan that regulates cell development and differentiation by coordinating signaling pathways [43]. GPC-3 serum level was found to be significantly enhanced in HCC group. This result is inconsistent with that of Suzuki *et al.* [44], and Chan *et al.* [45] suggested that the GPC-3 gene amplification in HCC could be owed to c-Myc gene overexpression. Because of its direct binding to the GPC-3 gene promoter, the transcription factor c-Myc has been discovered to be the major regulator of GPC-3 gene expression. Furthermore, STAT3 has been shown to play an indirect function in boosting GPC-3 expression *via* promoting the expression of c-Myc [44]. Overall, these findings suggest that c-Myc may promote neoplastic hepatocyte proliferation by upregulating the GPC-3 pathway and boosting its messenger RNA (mRNA) and protein levels [43]. Furthermore, Capurro *et al.* [46] indicated that GPC-3 can speed up the evolution of HCC by stimulating Wnt signaling and facilitating its binding to receptors.

In light of our results, treatment of HCC-rats with BM-MSCs experienced significant depletion in serum GPC-3 level. The current situation proposed that the observed decrease in GPC-3 serum level by BM-MSCs treatment could be attributable to its anticancer action, which inhibits tumor size and, as a result, suppresses tumor cell protein synthesis [47].

The current findings showed that NDEA administration elicited a significant rise in serum STAT3 levels. STAT3 is one of the most important signaling pathways in the injury-inflammation-regeneration cycle linked to chronic liver disease and the development of HCC [48]. High levels of interleukin-6 (IL-6) have been linked to the development of HCC by boosting the survival of hepatic cancer cells by activating STAT3 [49]. STAT3 is an intracellular signal transducer and transcriptional activator that is activated by proliferative stimuli such as cytokines like IL-6 or growth factors like epidermal growth factor (EGF). Different kinases, such as Janus kinases (JAK), phosphorylate STAT3 at its Tyr-705 position, followed by the homodimerization and nuclear translocation, and finally bind to its DNA regulatory sequences upstream its target genes, resulting in transcriptional regulation. STAT3 was discovered to enhance the expression of genes involved in cell proliferation, angiogenesis, and cell survival, including cyclin D1, c-Myc, and survivin. Moreover, numerous proteins, like tyrosine phosphatases (SHP1 and SHP2) and suppressors of cytokine signaling, have been shown to inhibit STAT3 activation [50].

On the other side, the treatment of HCC group with BM-MSCs caused a significant drop in serum STAT3 level. The antioxidant and antiproliferative properties of BM-MSCs could explain this impact [40,47]. Furthermore, IL-6/STAT3 communication between BM-MSCs can lower IL-17 levels and reduce liver fibrosis effects [51]. In macrophages, lymphocytes, fibroblasts, epithelial, and endothelial cells, IL-4 induces the Th2 phenotype and effects cell proliferation, death, and gene expression. IL-4 has two functional isoforms, and its effect is dependent on binding to the target's surface, which requires two types of receptors. IL-4 activates the M2 anti-inflammatory pathway in macrophages [52]. Moreover, IL4R

engagement could activate the tyrosine phosphorylation of STAT6, STAT3, STAT5, and STAT 1 [53]. Furthermore, downregulation of the JAK/STAT3 pathway by BM-MSCs can alleviate oxidative stress, enhance cell viability, and reduce neuronal apoptosis, resulting in a neuroprotective role in ischemic brain injury [54].

Tabulated results recorded a significant reduction regarding serum SOCS3 level in HCC-bearing rats. This result coincides with that of Ji *et al.* 2013 SOCS3 has been described as a negative regulator of the JAK/STAT pathway [55]. Many cancer types, including HCC, have been discovered to be epigenetically suppressed [56]. Furthermore, in more than 90% of HCC patients, Ogata *et al.* [57] reported a significant decrease in SOCS3 mRNA levels in malignant tissues compared to non-cancerous tissues. As a result, its downregulation could be linked to HCC progression and a bad prognosis. The abnormal mechanism of IL-6/STAT3 signaling in HCC could explain the decrease in serum SOCS3 levels. Since it was shown that an activated IL-6/STAT3 signal pathway can cause SOCS3 hypermethylation by increasing the activity of DNA methyltransferase-1 (DNMT1), cancer development and metastasis can occur [58].

In this study, treatment of the HCC group with BM-MSCs significantly amplified serum SOCS3 level. BMSCs increased SOCS3 protein expression and the Bcl-2/Bax ratio while decreasing STAT3 and cleaved caspase-3 proteins. Furthermore, through inhibiting the JAK/STAT3 pathway, BMSCs can minimize neuronal death in hypoxia-induced PC12 cells and reduce oxidative stress and cell viability [54].

The present results revealed that the treatment of the HCC group with DOX showed significant depletion in serum GPC-3 and STAT3 levels associated with significant elevation in serum SOCS3 level with the untreated HCC group. These results could be attributed to doxorubicin's antiproliferative and apoptotic properties [59]. The intimate mode of action of doxorubicin is confounded and remains poorly unknown, although it is thought to interact with DNA by intercalation and suppression of macromolecular biosynthesis. Li *et al.* [60] In human HCC cell lines Hep3B, HepG2, and HuH-7, doxorubicin can suppress the expression of epithelial cell adhesion molecule (EpCAM) and reduce EpCAM-positive cell numbers. The liver, kidney, pancreas, fetal lung, skin, germ cells, and adult epithelia express EpCAM. EpCAM activates the proto-oncogene cycles A/E and c-Myc, critical for cell cycle and proliferation. In ovarian, breast, esophageal squamous cell carcinoma, colon, and squamous head and neck carcinoma cells, overexpression of EpCAM was connected to cancer aggressiveness and poor survival. EpCAM's function and regulation mechanism in HCC remains unknown. Li *et al.* [60] demonstrated that EpCAM is the target of doxorubicin.

The liver tissue sections of rats in the HCC group showed typical anaplastic features on histological evaluation. Such findings are consistent with those reported in Seufi *et al.* [61] and Darwish and El-Boghdady's [15] studies.

Moreover, the liver tissue sections obtained from the BM-MSCs control group revealed no histological alteration. Such a finding is in harmony with Chen *et al.* [62].

Histological investigation of the liver tissue section obtained from the doxorubicin-treated group showed a low-grade anaplastic activity. This finding agrees with that of Alhusaini *et al.* [63].

4. Conclusions

The current setting provided experimental evidence that BM-MSCs have promising anticancer potential in rats with HCC. Its hepatoprotective qualities, powerful antiangiogenic activity, and effective antiproliferative potential could contribute to this effect.

Funding

This research received no external funding.

Acknowledgments

Asmaa Magdy Zaazaa: study design, biochemical analysis, histological evaluation, statistical analysis, English writing and grammar correction, and manuscript writing. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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