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Anti-cancer potential of liver mesenchymal stem cell conditioned media against Ehrlich ascites carcinoma bearing mice

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Abstract

This research evaluates the antitumor outcome of liver mesenchymal stem cell conditioned media (L-MSCs-CM) versus Ehrlich ascites carcinoma-bearing mice. A total of forty mice were categorized into four groups normal control group, and the further 3 groups that were injected intraperitoneally with 1×10^6 EAC cells, including the Ehrlich ascites carcinoma group, Methotrexate group that administrated with methotrexate (MTX), and liver mesenchymal stem cell treated group mice that was administrated with L-MSCs-CM. The findings demonstrated that the injection of L-MSCS CM revealed a significant reduction in EAC cell count and tumor ascites volume. Furthermore, a significant reduction in malondialdehyde level and expression of Bcl-2 in the liver and kidney tissue contrasted to the EAC group. Furthermore, there was a significant elevation in total antioxidant capacity and caspase-3 expression level in the liver and kidney contrasted to the EAC group. Many histopathological alterations were observed within the hepatic and renal tissue of the EAC group on the other hand, treatment with L-MSCs-CM restore the hepatic and renal tissue's normal histological architectures. The results indicated that L-MSCs-CM has an antitumor effect against EAC through inhibiting angiogenesis and inducing apoptosis.

Keywords: liver, Mesenchymal stem cells, Cancer, Oxidative stress, Apoptosis, BCL-2, Caspase-3

1. Introduction

Cancer is the most frequent health problem that continues to threaten current daily life in both developing and developed countries as a consequence of both external factors (e.g., carcinogenic food, alcoholic drinking, and radiation) and internal factors (e.g., mutation, hormonal and immune disturbance) [1][2]. Cancer cells are developed as a result of dysfunction in normal cell cycle regulation mechanisms, leading to unnecessary cell proliferation and/or inhibited cell removal [3]. Cancer cells also have the ability to spread to distant sites far from their origin [4]. Several therapeutic approaches are used for cancer treatment, including radiotherapy, chemotherapy, and surgery. Although chemotherapy is currently reflected an effective medication procedure, the drugs' hepatotoxic, renal toxicity, and other negative effects inhibit its success. [5]. Methotrexate (MTX) is a highly effective chemotherapy medication, although substantial doses are required to obtain the desired therapeutic effect. These high doses are associated with severe adverse effects, particularly delayed neurotoxicity [6]. Furthermore, some cancer cases acquire resistance to MTX therapy, particularly when high dosages are administered over a lengthy period of time [7]. Ehrlich ascites carcinoma (EAC) is an undifferentiated tumour with an extreme transplant ability, no regression, a shorter life span, 100% malignancy, rapid proliferation, originally hyper-diploid, and defined by the lack of TSTA (tumor-specific transplantation antigen) [8]. The EAC cell line is mostly utilized to validate the antitumor effects of various agents [9]. Mesenchymal stem cells (MSCs) are multipotent, undifferentiated cells that can differentiate into various cell types, including adipocytes, neuron-like

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cells, osteocytes, etc. [10]. Recent studies have examined the involvement of MSCs in cancer, blood problems, heart failure, genetic, and neurological disorders [11-12]. The considerable impact of stem cells is probably influenced by a paracrine process characterized as stem cell secretome or conditioned medium (CM) [13]. In CM or secretome, stem cells release soluble proteins, extracellular vesicles (EV), and lipids throughout culture [14] [15]. These extracellular vesicles (EVs) are also known as cell-derived vesicles, microvesicles, shedding vesicles, microparticles, and exosomes [16]. MSC-EXs are natural nanovesicles that have a unique antitumor impact and are characterized by good biocompatibility, low immunogenicity, and low cytotoxicity [17]. MSCs have been shown in several studies to have an antitumor effect in various malignant tumours. CM-MSCs inhibit ovarian tumour cells (SK-OV-3) through the suppression of insulin-like growth factors, Interleukin-8 and Vascular endothelial growth factor [18]. MSCs can also prevent the multiple myeloma cells' proliferation via the Fas/Fas-L signalling cascade, as per Atsuta et al. [19]. Therefore, we designed this study to evaluate the potential of secretomes harvested from liver mesenchymal stem cells on Ehrlich ascites carcinoma

2. Materials and methods

2.1. Chemicals and drugs

Trypan blue was obtained from El-Gomhouria Company, Cairo, Egypt. MTX was gathered from Sandoz Limited, a Novartis division, UK. Kits used in biochemical measurements were obtained from Biodiagnostic Company, Egypt, and ELISA kits were obtained from Cloud Clone Corp Company (USA).

2.2. Experimental animals

Swiss albino mice (female 25-30 g weight) were purchased from Abo Rawash culture, Giza, Egypt. Mice were confined in steel mesh cages (in animal house, faculty of science, Zagazig University). Mice were kept in a pathogen-free, climate-controlled, and pathogen-free animal housing with a 12:12h light/dark cycle and ad libitum available to food and drink. (Approval number ZU-IACUC/1/208/2019) The Zagazig University Ethical Committee approved the experimental design and animal care.

2.3. Tumor cell line

EAC cells were firstly gathered from the National Cancer Institute in Cairo, Egypt (only for the first transplantation). The EAC cells were preserved in vivo in Swiss Albino mice via serial (I.P.) injection of 2×10^6/mouse every 10 days [20].

2.4. Preparation of stem cell-conditioned media

Liver MSCs (L-MSCs) were separated from Sprague Dawley rats utilizing the technique previously reported [21] [22], with a slight modification. Cells were cultured at 10,000 cells per ml after 3–4 passages, and the culture medium was then incubated (CO_2 incubator at 37° C) for 1 day. After being thoroughly rinsed three times with phosphate-buffered saline (PBS), the liver mesenchymal stem cells were incubated for 24 hours in a basal medium devoid of serum. The supernatant was then removed and subjected to differential ultracentrifugation before being concentrated at 4° C. The acquired MSC-CM was stored in aliquots at -80° C under sterile conditions until utilization.

2.5. Study design

The study included 40 adult female Swiss albino mice measuring 20-30 g, which were categorized into four groups, each group 10 mice [23]:

Group 1: negative control group, 10 mice underwent an intravenous injection of saline.

Group 2: Ehrlich Carcinoma Cells group (positive group), EAC cells were injected into the mice's peritoneal cavity via serial (IP) transplantation of 1×10^6 cells.

Group 3: Methotrexate group, EAC cells were injected into the mice's peritoneal cavity via serial (IP) transplantation of 1×10^6 cells and then medicated with Methotrexate (2.5 mg/kg / I.P) every day for 9 days which start following the appearance of tumor ascites day 7.

Group 4: Treated group, EAC cells were injected into the mice's peritoneal cavity via serial (IP) transplantation of 1×10^6 cells and then were treated with liver mesenchymal stem cells conditioned media (2ml / kg / I.p) every day for 9 days which start after the appearance of tumor ascites day 7.

All animals were euthanized 10 days later at the end of the experiment. Biochemical and histopathological investigations were performed on blood, and tissue samples.

2.6. Collection and preparation of samples

Under light anaesthesia, blood samples were collected from the retro-orbital venous plexus at the conclusion of the experiment for biochemical analysis. EAC cells were collected from each mouse's peritoneal cavity in a centrifuge tube containing heparinized saline for tumour cell viability and counting assay.

Samples of kidney and liver tissues were cut in half. The first half was kept in formaldehyde for histopathological evaluation. While the other half was gathered in a centrifuge tube comprising phosphate buffer saline (PBS) to prepare tissue homogenate for apoptosis assay (Caspase-3 and Bcl-2) and oxidative stress parameters (TAC and MDA).

2.7. Viability and counting of EAC cells

The viability test and cell count were estimated by the trypan blue exclusion procedure [24]. The cell count was measured by the Thoma cell counting chamber and adjusted to $1x10^6$ cells/ml.

2.8. Evaluation of oxidative stress markers

2.8.1. Approximation of Total Antioxidant Capacity (TAC)

TAC was estimated in kidney and liver tissue homogenates by using a biodiagnostic kit as per the technique of Koracevic et al. [25].

2.8.2. Measuring lipid peroxide malondialdehyde (MDA)

MDA was determined in liver and kidney tissue homogenates by using a biodiagnostic kit according to the method of Satoh K [26].

2.9. Apoptotic markers

2.9.1. Quantitative determination of caspase-3 activity

Caspase-3 activity determination was done as an indicator for apoptosis in kidney and liver homogenate; the activity of caspase-3 was measured via the colorimetric caspase-3 kit as per the manufacturer's guidance (R & D Systems, Inc.) as per the procedure of Casciola et al. [27].

2.9.2. Quantitative determination of BCL-2 activity

The quantitative sandwich enzyme immunoassay technique was used for the determination of anti-apoptotic marker Bcl-2 in liver and kidney homogenate as per the method of Reed et al. [28].

2.10. Laboratory Biochemical Investigations:

Profiles of liver and kidney function are determined. Using an automated auto analyzer, the levels of albumin [29], total protein (T.P) [30], aspartate aminotransferase (AST) [31], and alanine aminotransferase (ALT) [32] were evaluated as liver damage markers. As kidney damage markers, urea [33] and creatinine [34] levels were evaluated utilizing assay kits provided by German diagnostic companies.

2.11. Statistical analysis

The statistical evaluation was carried out using the Package for Social Sciences (SPSS version 25) [35]. The data was presented as mean SE.

3. Results

3.1. The impact of liver stem cells conditioned media treatments on EAC cells count

After the inoculation of Ehrlich Ascites Carcinoma cells intraperitoneal, ascites fluid accumulated in the experimental mice's peritoneal cavity. Untreated EAC-bearing mice indicated a significant *($p < 0.001$)* rise in viable cell count, while treatment with (L-MSCs) CM demonstrated a marked reduction in count contrasted to the positive group, while in the MTX group, the side effect of the drug increased mortality of mice five mice from ten 50% before ascites appeared Table (1) figure (1)

Table (1): The change in EAC cells count among the studied groups

EAC cells count (10^6)	Positive Control	MTX treated group	Liver MSCs Media	Anova test	*p*-value
Mean ± SE	21.3±1.28	No EAC	$3.6±0.9^c$	81.8	<0.001
% change	----------	No EAC	-83.09%		
T-test			<0.001		

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by Tukey's post hoc test. Significant differences *($p<0.05$)* are represented by values with different superscript letters. $^a p < 0.05$, $^b p < 0.01$, $^c p < 0.001$ contrasted to the positive control group. The percent change of all treated groups was computed as per positive control

3.2. **The impact of liver stem cell conditioned media (L-MSCs-CM) on tumor volume**

The increased ascites fluid volume of tumor-bearing animals was found to be decreased on treatment with (L-MSCs) CM and MTX groups, respectively contrasted to the positive control group *($p <0.001$)* table (2) figure (1)

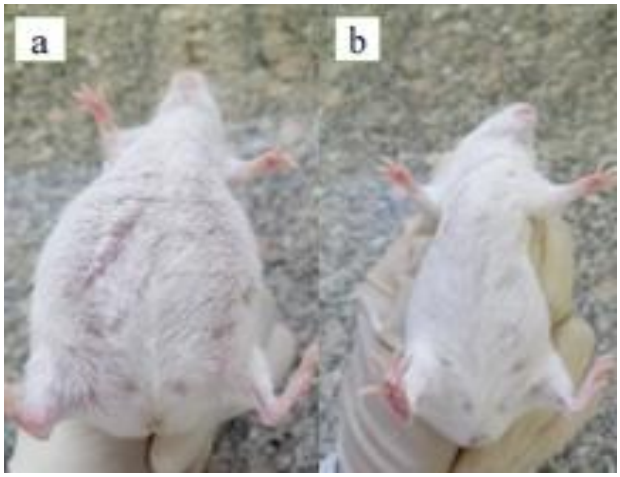

Figure (1) Shows the changes in ascites volume (a) EAC untreated group (b) L-MSCs CM treated group

Table (2): The change in EAC ascites volume among the studied groups

EAC cells volume (ml)	Positive Control	MTX treated group	(L-MSCs) CM treated group	Anova test	*p*-value
Mean ± SE	7.75±0.6	No EAC	1.72±0.28 [c]	54.230	<0.001
% change P	----------	No EAC	-77.8%		
T-test	----------	No EAC	<0.001		

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by the Tukey's post hoc test. Significant differences *($p < 0.05$)* are represented by values with various superscript letters. *[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$* contrasted to the positive control group. The percent change of all treated groups was estimated as per positive control.

3.3. **Elevation of oxidative stress markers**

3.3.1. The impact of liver stem cell conditioned media on hepatic and renal total antioxidant capacity (TAC)

Figure (2) summarizes (TAC) levels in hepatic and renal tissue of all studied groups. TAC level was significantly decreased in the positive control group when contrasted to the negative control *($p < 0.001$).* When contrasted to the positive control, the undesirable decrease in TAC was significantly improved in the stem cell CM and MTX groups *($p < 0.001$).*

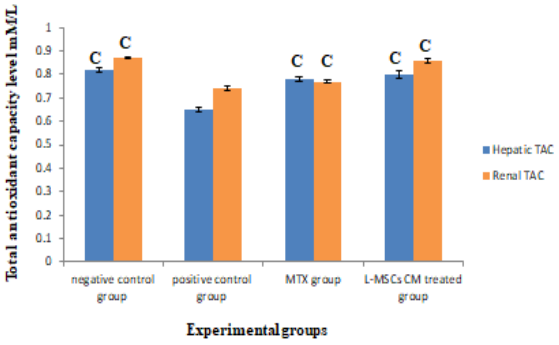

Figure (2) TAC level among experimental groups

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by Tukey's post hoc test. Significant differences *($p < 0.05$)* are represented by values with various superscript letters. *[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$* contrasted to the positive control group. The percent change of all medicated groups (negative control group, MTX group and L-MSCs CM group) was estimated as per positive control.

3.3.2. The impact of liver stem cells conditioned media on hepatic and renal MDA levels

Figure (3) summarizes (MDA) level in hepatic and renal tissue of all studied groups. MDA levels in the positive control group were substantially higher than in the negative control group *($p < 0.001$).* The undesirable increase in MDA was significantly reduced in the stem cell CM and MTX groups when contrasted to the positive control. *($p < 0.001$).*

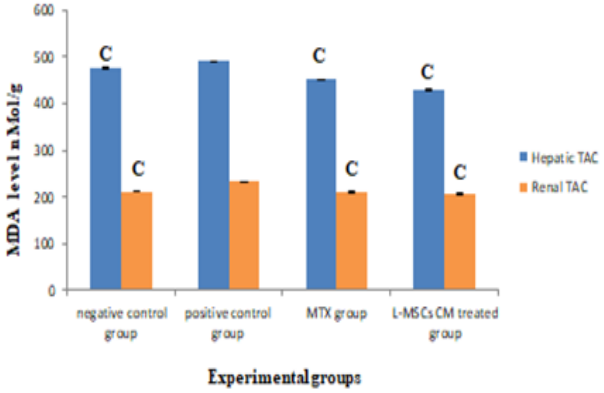

Figure (3) MDA among experimental groups

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by Tukey's post hoc test. Significant differences *($p < 0.05$)* are represented by values with various superscript letters. *[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$* contrasted to the positive control group. The percent change of all treated groups (negative control group, MTX group and L-MSCs CM group) was estimated as per positive control**.**

3.4. Apoptotic markers:

3.4.1. Apoptotic marker caspase-3 in liver and kidney homogenate (p mol/l)

The hepatic and renal expression of caspase-3 in the positive control group was significantly less than in

the negative control group *(p < 0.001)*. As shown in figure (4), the under-expression of caspase-3 was markedly increased after treatment with (L-MSCs) CM and MTX when contrasted to the positive control *(p < 0.001)*.

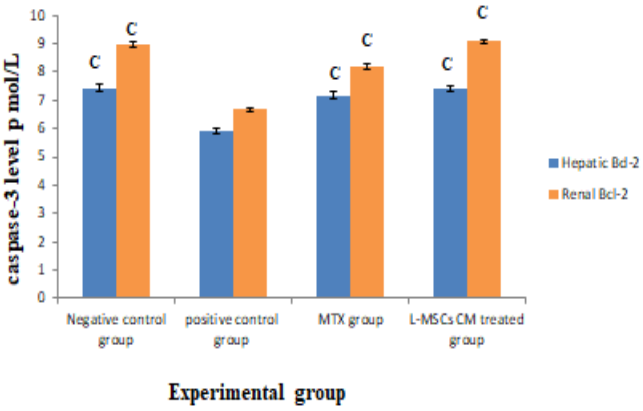

Figure (4) Caspase-3 level among different groups

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by Tukey's post hoc test. Significant differences *(p < 0.05)* are represented by values with various superscript letters. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ contrasted to the positive control group. The percent change of all medicated groups (negative control group, MTX group and L-MSCs CM group) was calculated as per positive control

3.4.2. **Anti-apoptosis marker Bcl-2 in liver and kidney homogenate (pg/ml)**

The hepatic and renal expression of caspase-3 in the positive control group was significantly higher than in the negative control group *(p < 0.001)*. As shown in figure (5), the increase in Bcl-2 levels was markedly decreased after treatment with (L-MSCs) CM and MTX when contrasted to the positive control *(p < 0.001)*.

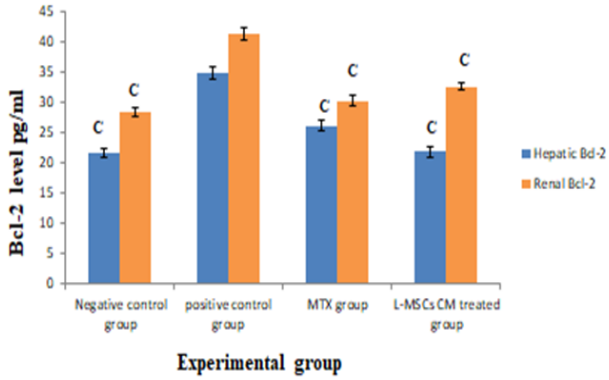

Figure (5) Bcl-2 level among different groups

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by the Tukey's post hoc test. Significant differences *(p < 0.05)* are represented by values with different superscript letters. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ contrasted to the positive control group. The percent change of all medicated groups (negative control group, MTX group and L-MSCs CM group) was estimated as per positive control.

3.5. Effect of treatment with liver stem cells conditioned media and MTX on serum liver function parameters

Table (3) demonstrates the impact of L-MSCs-CM on the liver function parameters' results serum ALT and AST activities. ALT and AST activities were significantly higher in the positive control group when contrasted to the negative control group *(P< 0.001)*, but significantly improved in the stem cell conditioned media and MTX groups when contrasted to the positive control group *(P < 0.001)*. While albumin and (T.P) levels were significantly lower in the positive control group when contrasted to the negative control group *(P < 0.001)*, their levels was significantly improved *(P < 0.001)* in the MSCs CM medicated group and MTX treated group when contrasted to the positive control group

Table (3) liver function parameters among the studied groups

Groups	Negative control	Positive Control	Methotrexate Groups	(L-MSCs) CM treated group	Anova test	*p*-value
ALT U/L	**48.57±0.529^{c}**	**57.02±0.65**	**50.4±0.74^{c}**	**48.92±0.49^{c}**	**41**	**<0.001**
% change	**-14.81%**	**----------**	**-11.6%**	**-14.2%**		
AST U/L	**167.4± 2.2^{c}**	**270.4± 1.96**	**250.6± 1.8^{c}**	**175±2.32^{c}**	**628.9**	
% change	**-30.09%**	**----------**	**-7.322%**	**-36.39%**		
Albumin g/dl	**2.96± 0.037^{c}**	**1.81± 0.04**	**2.34 ±0.039^{c}**	**2.73± 0.039^{c}**	**161.1**	
% change	**63.5%**	**----------**	**29.28%**	**50.8%**		
Total protein g/dl	**4.53± 0.036^{c}**	**4.07± 0.047**	**4.38± 0.083^{b}**	**4.49± 0.06^{c}**	**12.21**	
% change	**11.3%**	**----------**	**7.6%**	**10.31%**		

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by Tukey's post hoc test. Significant differences *($p < 0.05$)* are represented by values with various superscript letters. *$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$* contrasted to the positive control group. as per the positive control, the percent change of all medicated groups was calculated.

Table (4) shows the results of serum urea and creatinine levels for all studied groups. Urea and creatinine levels in the positive control group were substantially higher than in the negative control group. *($p < 0.001$),* When contrasted to the positive control group, their levels were significantly less in the stem cell conditioned media and MTX groups. *($p < 0.001$).*

3.6. Effect of treatment with stem cells conditioned media and MTX on serum kidney function parameters

Table (4) kidney function parameters among the studied groups

Groups	Negative control	Positive Control	Methotrexate Groups	(L-MSCs) CM treated group	Anova test	*p*-value
Urea mg/dl	26.0±0.49^{c}	50.9±1.73	25.7±0.53^{c}	26.4±0.49^{c}	163.097	<0.001
% change	-48.9%	----------	-49.5%	-48.13%		
Creatinine mg/dl	0.44± 0.016^{c}	0.53± 0.026	0.34± 0.016^{c}	0.42± 0.024^{c}	13.29	
% change	-16.98%	----------	-35.84%	-20.75%		

Note: the values are described in mean ±SE, n = 10. The one-way ANOVA test was utilized for statistical analysis, accompanied by the Tukey's **post hoc test.** Significant differences *($p < 0.05$*) are represented by values with various superscript letters. *$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$* contrasted to the positive control group. as per the positive control, the percent change of all medicated groups was calculated.

3.7. Histopathological studies

3.7.1. Histopathological results in kidney sections demonstrated normal renal parenchyma; normal glomeruli, and normal renal tubules in the negative control group (Figure 6 (a)). Conversely, the positive control group shows massive renal degradation and necrosis; the glomerular epithelium and the renal tubular epithelium's vacuolar degeneration (Figure 6 (b)). The kidney from the MTX group displayed a result nearly resembling a positive one: the vacuolation of the glomerular epithelial and the desquamation of renal tubular epithelium with the formation of luminal renal casts (Figure 6 (c)). Unlike the MTX group, the L-MSCs CM treated group (Figure 6 (d)) showed marked regeneration to normal renal parenchyma; normal glomeruli and normal renal tubules were identical to the negative control group.

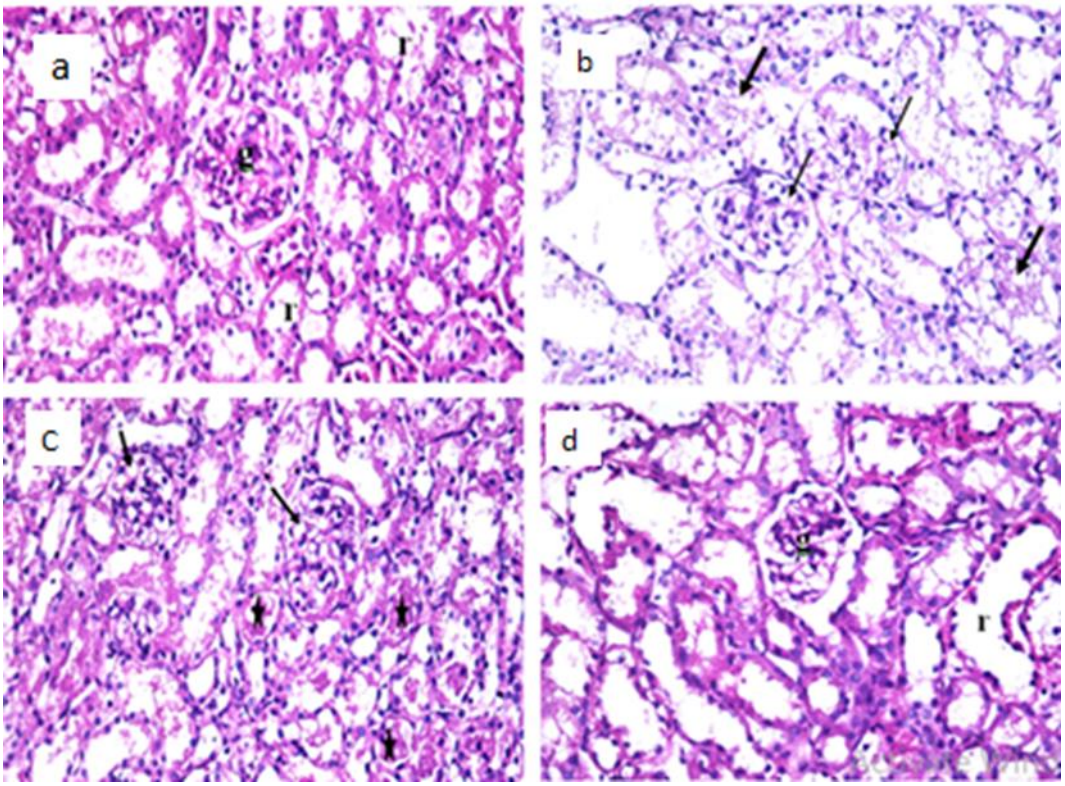

Figure (6) histopathological examination of kidney sections from all groups studied: (a) A negative control group kidney section revealed normal glomeruli (g) and renal tubules (r) (score lesion: 0). (b) A positive group kidney section showed renal degradation and necrosis, as well as vascuolar degeneration of the glomerular epithelium (thin arrows) and renal tubular epithelium (thick arrows) (score lesion: ++++). (c) A kidney section from the MTX-treated group showed glomerular epithelial vacuolation (arrows) and renal tubular epithelial desquamation with the formation of luminal renal casts (*) (score lesion: +++). (d) Kidney sections from the (e) L-MSCs CM-treated group revealed normal renal parenchyma, similar to the control group (score lesion: +) (H & E staining X400).

3.7.2. In the negative control group, histopathological results in liver sections revealed normal renal hepatic parenchyma, normal organized hepatic cords, blood sinusoids, and central vein (Figure 7 (a)). The positive control group, in contrast, showed hepatocyte necrosis with hyperchromatic nuclei, widening in blood sinusoids, and pericentral leucocytic cell infiltration in the portal tract (Figure 7 (b)). MTX group displayed a result nearly resembling a positive one with diffuse hepatocytes swelling and vacuolation with focal mononuclear cell infiltration (Figure 7 (c)). In contrast to the MTX group L-MSCs, the CM treated group (Figure 7 (e)) demonstrated marked regeneration to normal hepatic parenchyma with normal organized hepatic cords; blood sinusoids and central vein, as well as marketed regeneration of the leucocytic cell infiltration identical to the negative control group.

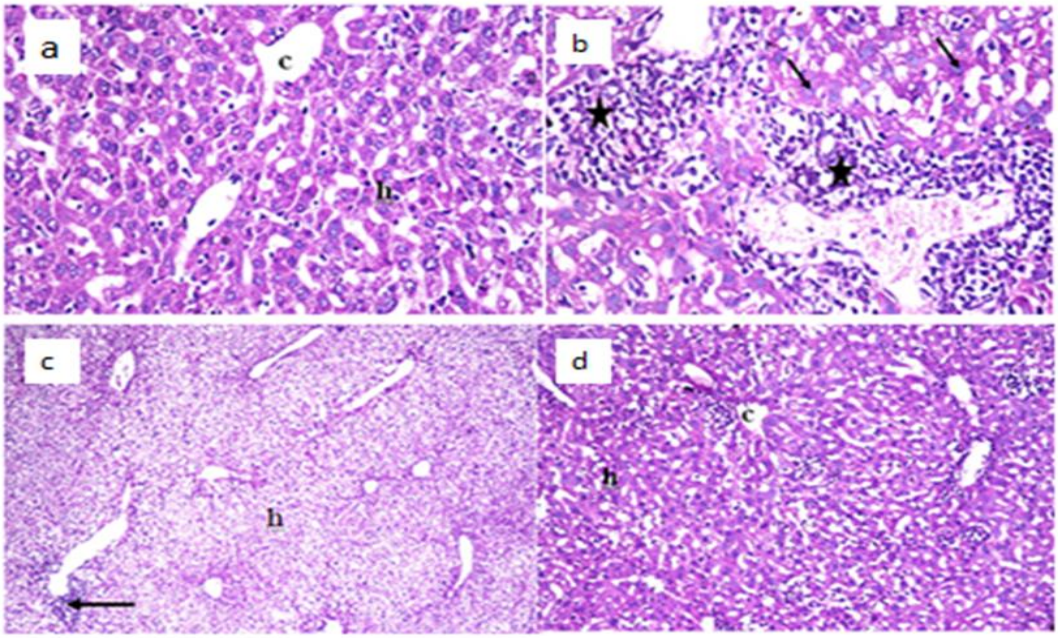

Figure (7) Histopathological examination of liver sections from all groups studied: (a) liver section from a negative control group showed normal polygonal hepatocytes (h), blood sinusoids, and central vein (c) (score lesion: 0) (b) A positive group liver section displayed hepatocyte necrosis with hyperchromatic nuclei (arrows) and pericentral leucocytic cell infiltration (*) (score lesion: ++++). (c) A liver section from the MTX group showed hepatocyte sewling and vacuolation (h), as well as focal mononuclear cell infiltration (arrow) (score lesion: +++). Section from the (d) L-MSCs CM-treated group demonstrated normal hepatic parenchyma with normal organized hepatic cords (h), blood sinusoids and central vein (c) with marked regression of leucocytic cell infiltration similar to the negative group (score lesion: +) (H & E staining X400).

4.Discussion

Cancer is among the top global causes of illness and mortality. In contrast to other therapeutic approaches like as radiation, chemotherapy, and surgery, these techniques are typically constrained by metastatic repetition, drug resistance, off-target effects, and comorbidities. [36]. Stem cells may self-renew, develop into numerous cell types, and create single-cell-derived clonal cell populations, making them a distinct population [37]. MSCs stem cell anticancer applications have recently received a lot of attention. By homing to and targeting primary and metastatic cancerous cells, MSCs can start serving as novel delivery targets [38]. The ongoing research was done to evaluate the antitumor impact of liver MSCS conditioned media versus Ehrlich Ascites Carcinoma (EAC) bearing mice. There was a significant decrease in the tumour ascites volume. Mice injected with EAC cells and medicated with L-MSCS CM demonstrated a significant reduction in the ascetic fluid (tumor volume) and viable tumour cell count as contrasted to mice in the untreated EAC group, and no EAC cells appear in MTX group [39]. Our findings were in consistent with the study of Borghese et al [40] they noted a significant inhibition in tumour cell viability. Increased ascites volume due to this fluid is assumed to be the tumour cells' primary source of nutrition [41] [42]. It is possible to conclude that L-MSCs CM reduced the tumour ascites volume throughout, preventing its growth, indicating that L-MSCs CM has an antitumor effect against EAC-bearing mice.

Cells become more sensitive to free radicals when the antioxidant defense mechanism is out of balance [43]. Excessive production of free oxygen species and low levels of antioxidants throughout cancer play a vital function in tissue damage and apoptosis, which destroys DNA, RNA, lipids, and proteins [44] [45]. Lipid peroxidation is a spontaneous catalytic free radical chain propagating reaction that has been linked to cell pathology [46]. MDA, a lipid peroxidation end product, is shown to be elevated in tumour tissues than in healthy tissues [47]. Our results demonstrated a significant increase in MDA levels 490.6 n Mol/g with

decreased TAC 0.65 mM/l in EAC bearing mice, while in the medicated mice, MDA levels were significantly reduced to 450.7 and 429.3 n Mol/g in MTX group and L-MSCs CM group respectively, and TAC improved 0.78 and 0.801 mM/l in MTX group and L-MSCs CM group respectively. Our findings are consistent with the study of Chen et al. [48], who noted a significant reduction in MDA and elevation in antioxidants after treatment with MSCs-CM.

Apoptosis is a mechanism of programmed cell death that regulates the presence and development of cancer cells [49]. [50] Aspartate-specific protease stimulation of the death receptor (extrinsic cascade) and mitochondrial caspases (intrinsic cascade) can induce apoptosis. Crucial regulators of apoptosis are the Bcl-2 family members, which are categorized into two groups: pro-apoptotic, which indicates apoptosis, and anti-apoptotic, which inhibits it. These parameters and mechanisms involved in cancer can induce apoptosis. Our results demonstrated the induction of apoptotic pathways and the suppression of anti-apoptotic markers in the mice's liver and kidney tissues medicated with L-MSCs CM. In the liver and kidney tissue of medicated groups, there was a significant increase of apoptotic marker caspase-3. This elevation of apoptotic marker caspase-3 was also evidenced by the downregulation of anti-apoptotic Bcl-2 in the treated group with L-MSCs CM in the liver and kidney tissues of mice compared with untreated EAC-bearing mice. A major factor in the activation of the apoptotic marker caspase-3, which led to the downregulation of the AKT protein and the induction of apoptosis, is the reduction of bcl-2. These results corroborate a prior work which demonstrated that conditioned media from human foetal dermal mesenchymal stem cells indicates death of A375 melanoma cells via anti-apoptotic Bcl-2 downregulation [51]. MSC-conditioned medium can promote apoptosis in human U251 cell lines by raising the expression of the apoptotic gene caspase-3 and lowering the anti-apoptotic genes' expression, according to a separate study [52]. Our research suggested that L-MSCs CM have anticancer properties by enhancing caspase-3 activation and lowering Bcl-2 activation.

Regarding the biochemical results, it demonstrated a significant reduction in albumin and overall protein levels in the positive EAC untreated group contrasted to the treated groups. This reduction may be attributable to an increase in the meiotic cell division of neoplastic cells with high blood fluid evacuation and capillary permeability, allowing plasma proteins to escape into the peritoneal cavity [53]. Furthermore, overall proteins and albumin levels in animals with liver disease may be lower [54]. Albumin and total protein levels increased in treated groups towards a normal level which indicate the anti-tumor effect of L-MSCs which induce apoptosis and reduce EAC cells growth which decreases ascites in treated mice. Generally, liver damage caused by tumours is a result of disruptions in liver cell metabolism, which lead to alterations in blood enzyme activity. Due to liver damage and alterations in membrane fluidity, serum ALT and AST values may be increased, reflecting the severity of EAC-cells [55] [56]. In addition to liver function, BUN and creatinine are two common test indices for kidney function and structural integrity. Significant elevations in BUN and creatinine could be attributed to the presence of renal damage as a result of cancer cell invasions. The recent study showed a significant elevation in urea and creatinine levels in the EAC group compared to treated group with L-MSCs CM, which showed a significant reduction toward normal levels in the negative control group. Our findings are consistent with the results of [57][58], that reported a significant decrease in the kidney function parameters BUN and creatinine following treatment with MSCs conditioned media [59][60].

Our work was limited by the fact that it was a single-center experiment that required evaluation in additional centres. In addition, we examined the impact of L-MSC-CM as a whole, despite the fact that it is a combination of diverse exosomes, and the results of our research did not identify the specific exosomes or active ingredients responsible for the anti-tumor activity.

5. Conclusion

Dependent on the findings obtained from the study, conditioned media from liver mesenchymal stem cells (L-MSCS CM) has a therapeutic effect on oxidative stress, apoptotic markers Caspase-3 and Bcl-2 expression level, biochemical and histopathological abnormalities in Ehrlich ascites carcinoma bearing mice through inhibiting angiogenesis, suppressing the signaling of Wnt and AKT, and stimulating apoptosis, so liver stem cell-conditioned media showed a significant anti-tumor effect versus Ehrlich ascites carcinoma (EAC) bearing mice.

6. Conflicts of interest

"No conflicts need to be declared."

7. Acknowledgement

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