**IN VITRO AND IN VIVO EVALUATION ACTIVITIES OF EACH MESENCHYMAL STEM CELLS, DOXORUBICIN AND LPS OR COMBINED ON BREAST CARCINOMA**

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**ABSTRACT**

**Aim:** We aimed to explore *in vitro* and *in vivo* activities by one or combined therapy of hMSCs, doxorubicin or LPS against breast cancer MCF7. **Methods:** MCF7, hMSCs and coculture were cultivated, harvested and exposed to either or doxorubicin or LPS in groups then examined by Flow Cytometer for *in vitro* examinations. Cell proliferation was investigated by MTT assay and both PJNK and IL-6 levels by ELISA. *In vitro* genes’ expression was examined by qRT-PCR for TLR4, VEGF, NF-ƙB, IL-8 and IL-1. *In vivo* cancer induction was performed for 70 albino mice that exposed to treatments before and followed by pathological examination and measuring the same genes’ expression. **Results:** *In vitro* results showed significant reduction in MCF7 proliferation, PJNK, IL-6 and genes’; TLR4, VEGF, NF-KB, IL-8 and IL-1; expression for groups’ hMSCs and/or doxorubicin but LPS was an exception in PJNK and IL-6 levels elevation. *In vivo* results supported the previous by reduction in cancer cells number when mixed with hMSCs. Similar genes expression reductions were noticed for hMSCs and/or doxorubicin but LPS also appeared as exception in genes’ expression elevation due to its antigenic properties. **Conclusions:** Each of hMSCs, doxorubicin or LPS was effective alone but appeared more effective when combined together. hMSCs combined with doxorubicin therapy achieves the highest benefits. Even if LPS showed some antigenic properties but it stills reduced MCF7 proliferation.
Keywords MCF7, hMSCs, doxorubicin, LPS.

INTRODUCTION
Breast cancer is the most common cause of cancer death among women worldwide, accounting for 458,000 deaths each year [1] and is strongly related to age with only 5% of all breast cancers occurring in women under 40 years old. It is higher in developed countries, and on the rise in developing countries [2]. However, women in developing countries tend to present with more advanced breast cancer upon initial diagnosis as compared to women in developed countries and the majority of patients are diagnosed at advanced disease stages at stages III and IV [3]. Breast cancer screening test; clinical and self-breast, mammography, genetic screening, ultrasound, and magnetic resonance imaging; is investigated in an attempt to achieve an earlier diagnosis under the assumption that early detection will improve outcomes [4]. The management of breast cancer is depending on various factors, including the stage of the cancer that is usually treated with surgery and then possibly with chemotherapy or radiation, or both. A multidisciplinary approach is preferable. Hormone positive cancers are treated with long term hormone blocking therapy. Treatments are given with increasing aggressiveness according to the prognosis and risk of recurrence [5].

Stem cells are considered the new line of therapy. Traditional treatments are effective against non-metastatic forms and stem cell transplantation can increase survival in patients [6]. High dose chemotherapy with stem cells support has improved the disease free survival in metastatic stage [7]. In reduced intensity conditioning regimens, allogeneic human stem cells transplantation has proven to be effective in persistent and progressive metastatic stage, decreasing relapse, while with myeloablative conditioning regimens may provide cytoreduction and eradication of disease with a cancer free-graft and an immune-mediated graft-versus-tumor effect mediated by the donor's immune cells [8]. Umbilical cord derived human mesenchymal stem cells (hMSC) have been shown to be capable of attenuating the growth of tumor cancer cell lines in vitro and in vivo [9]. These tumoricidal effects are produced by secretory proteins/peptides which are produced by either the hMSC or the tumor cells in response to the hMSC secretory products [10]. hMSC produced secretory proteins induces cell death of cancer cells and cell cycle arrest because of stimulating caspase activities and arrest the cell cycle even in the absence of direct contact with cancer cells [11]. Additionally, hMSC stimulates immune reaction through infiltrating lymphocytes T cells and monocyte chemotactic protein-1 that induces migration of lymphocytes to cancer cells [12].
Many factors share in the etiology and progression of breast cancer. Toll-like receptors (TLRs) are widely expressed on tumor cells and play important roles in the initiation and progression of cancer that can promote inflammation and cell survival in the tumor microenvironment. Multiple links between TLR4 and breast cancer have been identified through activation the migration, invasiveness and angiogenic potential of cancer cells at primary site or metastatic locations. TLR4 exert a key role in the antigens secretion from cancer cells succumbing to chemotherapy and radiotherapy. TLR4 is a critical player in breast cancer that warrants further investigation for the development of novel therapeutic modalities [13,14].

Nuclear factor kappa B (NF-κB) incorrect regulation has been linked to breast cancer cell survival and inhibition its apoptosis [15,16].

C-Jun N-terminal kinases (JNKs) play a role in T cell differentiation and the cellular apoptosis pathway [17]. JNK1 is involved in apoptosis, cell differentiation and proliferation, inflammatory conditions and cytokine production mediated by AP-1 (activation protein 1) [18].

Vascular endothelial growth factor (VEGF) is a key regulator of cancer angiogenesis, thus its expression has been correlated with a poor outcome in the pathogenesis of breast cancer [19-20].

The Interleukin family group of 11 cytokines plays a central role in the regulation of immune and inflammatory responses [21]. IL-1, IL-6 and IL-8 are known to be up regulated in breast carcinoma and have been implicated as a factor in tumor progression via the expression of metastatic, angiogenic genes and growth factors [22-24].

We aimed to study effect of coculture of umbilical cord hMSCs with human breast cancer cells MCF7 in vitro and in vivo on breast carcinoma proliferation. In addition, we aimed to investigate and compare the impact effects of TLR4 agonist (LPS) and doxorubicin anticancer drug alone or mixed with the hMSCs, MCF7 and hMSCs+MCF7 on breast carcinoma proliferation, JNK phosphorlyation, IL-6 level and some related genes; TLR4, VEGF, NF-KB, IL-8 and IL-1; expression.
MATERIALS AND METHODS
This work was performed at the unit of Biochemistry and Molecular Biology at the Medical Biochemistry department, faculty of Medicine, Cairo University, Cairo, Egypt. The work was in collaboration with gynecology and obstetrics department, faculty of medicine, Cairo University. The design of this work was divided into in vitro and in vivo evaluation.

In vitro evaluation
Coculture of hMSCs and MCF7
Human umbilical cord specimens were obtained using protocols approved by the ethical committee of faculty of Medicine, Cairo University, by collaboration with the Labor and Delivery nursing staff. After obtaining patient’s own informed consent, 4 fresh cord samples of women with healthy pregnancies was retrieved during caesarean deliveries.

Wharton jelly was harvested from term deliveries at the time of birth. hMSC was isolated by collagenase II enzyme (IgG, C. histoliticum, Biological life science, USA) , digested and maintained in 2% fetal bovine serum and 1x Pen/Strep (Invitrogen, CA, USA). Cells were incubated at 5% CO₂, 37° C until cells will reach 70%–80% confluency, washed with phosphate buffer saline and trypsinized with 0.25% trypsin for 5 minutes at 37° C. After centrifugation, cell pellets were resuspended with medium and incubated as first-passage cultures [25].

1x10⁵ cells were incubated with 10 µL of monoclonal antibodies: CD34 PE, CD 29 PE and CD 44 PE (Beckman coulter, USA) at 4° C in the dark, same species isotypes served as a negative control. After incubation, 2 ml of PBS containing 2% FCS solution were added, centrifuged and cells were resuspended with fresh medium. Cell analysis was performed using CYTOMICS FC 500 Flow Cytometer (Beckman coulter, FL, USA) and analyzed using CXP Software version 2.2.

Human breast adenocarcinoma (MCF7) cells were obtained from (VACSERA, Egypt). The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) in 5% CO₂ at 37° C. By day 3, cells were trypsinized, centrifuged and resuspended with fresh medium. Three 96-well culture plates were used for cell proliferation assay, the assessment of JNK phosphorylation and IL-6 levels and genes qRT-PCR.
Each plate was then divided to nine groups

Three groups were cultured by hMSCs, the rest of groups were cultured by MCF7 and incubated 24 h. After that, MCF7 cells were co-cultured with hMSCs and incubated for another 24 h also LPS as TL4 ligand and doxorubicin (Sigma Aldrich, USA) were added as the following.

- 1st group contained 10^4-10^6 hMSCs only.
- 2nd group contained 10^4-10^6 hMSCs + (1 µg/mL) LPS [26].
- 3rd group contained 10^4-10^6 hMSCs + 20 nM of doxorubicin [27].
- 4th group contained 10^4-10^6 MCF7 cell line only.
- 5th group contained 10^4-10^6 MCF7+ (1 µg/mL) LPS.
- 6th group contained 10^4-10^6 MCF7+ 20 nM doxorubicin [27].
- 7th group contained coculture of 10^3-10^5 of each hMSCS and MCF7 cells.
- 8th group contained hMSCs + MCF+LPS.
- 9th group contained hMSCs + MCF7+doxorubicin.

**Cells proliferation MTT assay:** The measurement of cell proliferation was done by TACS MTT cell proliferation kit (Trevigen Inc., Wiesbaden-Nordenstadt, Germany). Cells were seeded in 96-well microtiter plates at the required concentration of 1-2x10^4 cells/well and after overnight incubation the cells were exposed to the treated media. After 24 h cells incubation, 10 mL of tetrazolium compound, MTT (3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyl-tetrazolium bromide) was added to the wells and the cells were incubated 2–4 h at 37° C. MTT was reduced by metabolically active cells to insoluble purple formazan dye crystals. When purple precipitate was clearly visible under the microscope, 100 mL of detergent reagent was added to all wells, including control wells. The covered plate was left in the dark at 18–24° C for overnight. After that, plate cover was opened and the absorbance was measured of the wells, including the blanks, at 570 nm with a reference wavelength of 650nm. The absorbance was blotted against cell number/ml and 8 wells were used for each group. Cell proliferation was assessed as the percentage of cell proliferation compared to untreated MCF7 as control cells.

**ELISA-based assay using fluorogenic substrates to measure phosphorylated JNK in cells:** The second plate was used and incubated for 24 hours and phosphorylation was detected according instructions. Cells were grown and stimulated with ligands. Following stimulation, cells were fixed and permeabilized in the wells. The target protein...
phosphorylation was measured using a double immune enzymatic labeling procedure. The cells were simultaneously incubated with two primary antibodies: a phospho-specific antibody Phospho-JNK (T183/Y185) and a normalization antibody that recognizes the total protein regardless of phosphorylation status.

The primary antibodies were derived from different species. Two secondary antibodies recognizing the different species were labeled with either horseradish-peroxidase (HRP) or alkaline phosphatase (AP), and two spectrally distinct fluorogenic substrates for either HRP or AP are used for detection. The fluorescence of the phosphorylated protein was normalized to that of the total protein in each well for the correction of well-to-well variations. This two-wavelength assay results in precise analysis of protein phosphorylation with good reproducibility. The plate was read using a fluorescence plate reader (Fluroscan FL detector, Finland, Helfinki) at 600 nm by the total JNK fluorescence at 450 nm in each well.

**ELISA quantitative measurement of IL-6 levels from culture medium:** Interleukin-6 serum level was detected using (Invitrogen Corporation, Camarillo, CA, USA) ELISA kit according instructions.

**TLR4, NF-KB IL-1, IL-8 and VEGF genes expression by qRT PCR**

**Isolation of total mRNA:** Total RNA was extracted from the harvested nine groups of cells in the third plate using SV total RNA isolation system (Promega, Madison, WI), while the medium was collected for IL-6 ELISA assessment.

**cDNA synthesis:** The extracted RNA was reverse transcribed into cDNA using RT PCR kit (Fermentas, USA). M-MuLV reverse transcriptase was used. A total of 3 µL of oligo dT (Roche Applied Science, Indianapolis, IN) to 10 µL of mRNA which was denatured for 2 min at 70° C in the thermal cycler (Biometra, USA). Reaction mixtures contained 10 mM dNTP mix, 40 U RNase inhibitor (Roche), and 50U MMuLV reverse transcriptase in reaction buffer. After 1 h incubation for the last mixture at 37° C in the programmed thermal cycler, reactions were stopped by heating at 95° C, for 10 min.

**Analysis of TLR4, NF-KB IL-1, IL-8 and VEGF genes expression using qRT-qPCR:** For quantitative RT reverse transcriptase (qRT-PCR) analysis, cDNA reaction mixtures were mixed with twice AB solute qRT-PCR SYBR™ green ROX master mix (Abgene, Hamburg, Germany) and the appropriate primers then filled with deionized water to 25 µL. qRT-PCRs
were performed in triplicates in an Applied Biosystems Step One™ RT-PCR system (Applied Biosystems, Foster City, CA, USA) with 2 min as initial stage at 50° C to activate the DNA polymerase, followed by 40 cycles of 95° C for 15 s, 60° C for 1 min and 72° C for 1 min. Statistical analysis was performed on two independent experiments and the ΔΔCt were calculated from:

\[ \Delta\Delta C_t = \Delta C_T \text{ sample assessed gene (CT sample assessed gene–CT reference home gene GAPDH (glycerol aldehyde phosphate dehydrogenase))–CT calibrator (CT control gene–CT reference home gene GAPDH)} \].

Then, \( 2^{\Delta\Delta C_t} \) gives the relative quantification gene expression compared to the control [28]. Standardization was performed by quantification of the GAPDH gene as an endogenous control.

**In vivo evaluation**

Seventy male white Albino mice belonging to local strain weighing between 20-25 gm was obtained from the animal house of faculty of Medicine, Cairo University and included in this study. The animals were housed in wire mesh cages at room temperature with 12:12h light-dark cycles and maintained on standard rat chow and tap water. Veterinary care was provided by animal house Unit of Cairo University. The animals were randomly divided into 7 groups of 10 animals in each;

- 1st group -ve control group (injected phosphate buffer saline (PBS) only).
- 2nd group mice iv injected in tail with 0.5x10^6 hMSCs suspended in 0.5 ml PBS [9].

Then, the rest of the groups were injected iv with 2x10^6 MCF7 suspended in 0.5 ml PBS for induction of breast carcinoma [9]. After three weeks of induction of breast carcinoma the other groups were further divided as follows;

- 3rd group was applied for three injections weekly of 0.5x10^6 hMSCs.
- 4th group was injected 0. 5x10^6 hMSCs+2mg/kg doxorubicin [29].
- 5th group was injected 0.5x10^6 hMSCs + 100 µg LPS [30].
- 6th group was injected 2mg/kg doxorubicin.
- 7th group not injected anything after hMCF7 (+ve control group).

After one week (2) mice were scarified and subjected for pathological examinations to ensure induction of breast carcinoma. The mice were observed for signs of morbidity/death during the experiment period. All the mice were sacrificed after three weeks of stem cells and drug injection by cervical dislocation. Lungs were subjected for pathological examination for detection of metastatic carcinoma and molecular studies.
Histopathological examination: Lung samples were collected and divided into 2 parts; one part fixed overnight in 40 g/L paraformaldehyde in PBS at 4°C. Serial 5-µm sections of the lung were stained with hematoxylin and eosin (HE) stain and all were examined histopathologically [9].

TLR4, NF-κB IL-1, IL-8 and VEGF genes expression using qRT-qPCR: The other part of lung tissue homogenate was further processed for RNA extraction followed by RT (for cDNA synthesis) and qRT PCR as described previously at the in vitro part.

Statistical analysis
Results were disclosed as means ± standard deviations. One-way ANOVA and Tukey’s multiple comparison post hoc tests were performed. $P < 0.05$ was considered significant.

RESULTS
In vitro evaluation
hMSCs was cultured and developed from spindle to fibroblast-like shape. Upon mixing with MCF7 and doxorubicin as coculture, a marked reduction in malignant cells number was shown in figure 1. The shown characterization of hMSCs by flow cytometer was negative for CD34– and was uniformly positive for CD29+CD44+ (Fig. 2).

MCF7 group showed the highest significant increase in cell proliferation when compared to all other groups as illustrated in figure 3A. There was significant proliferation reduction in LPS and doxorubicin groups compared to controls and the maximum significance decrease appeared in hMSCs+MCF7+doxorubicin group compared to all other groups.

Figure 3B showed similar effect of MCF7 and MCF7+LPS groups were the highest phosphorylated JNK in all groups. JNK phosphorylation was reduced in doxorubicin groups compared to controls and the lowest level appeared in hMSCs+MCF7+doxorubicin.

Even if there was significant increase in IL-6 levels in all LPS groups (Fig. 3C), but doxorubicin groups shown an opposite effect and the lowest IL-6 level was in hMSCs+MCF7+doxorubicin group compared to all groups.

Genes’ expressions showed a significant increase in TLR4, VEGF and NF-KB genes for hMSCs+LPS and hMSCs+doxorubicin groups compared to hMSCs group (Fig. 4). Contrarily, genes’ expressions were significantly reduced in TLR4, VEGF, NF-KB, IL-8 and
IL-1 after LPS or doxorubicin exposure for the other groups. MCF7 group mostly showed the highest significant increase in all genes expressions compared to all other groups. hMSCs+MCF7+doxorubicin showed the lowest significant reduction in all genes’ expressions compared to all other groups (Fig. 4).

Figure 1: (A) Spindle shaped hMSCs at one week culture, (B) hMSCs at 2 weeks culture, (C) MCF7 cells, (D) coculture of both MCF7 (red arrow) and hMSCs (black arrow) and (E) co-culture of MCF7 and hMSCs+Doxorubicin with marked decrease in the number of malignant cells.

Figure 2: Flow cytometric characterization analyses of hMSCs.
Figure 3: (A) Cell proliferation by MTT assay, (B) phosphorylated JNK and (C) quantitative measurement of IL-6 in all studied groups. Data are means ± SD and (*) p value < 0.05 comparing with each control group.
Figure 4: *In vitro* quantitative expression for (A) TLR4, (B) VEGF, (C) NF-κB, (D) IL-8 and (E) IL-1 genes in the studied groups. Data are means ± SD and (*) p value < 0.05 comparing with each control group.
Figure 5: (A) Normal lung of thin-walled alveoli separated by a thin layer of connective tissue and numerous capillaries (B) Metastatic carcinoma with heavily infiltration by small, rounded, oval, malignant cells. The cytoplasm is scanty. The nuclei are large and hyper chromatic. (c) Metastatic carcinoma after treatment by hMSCs with decrease in number of malignant cell infiltration (D) Metastatic carcinoma after treatment by hMSCs+Doxorubicin with more decrease in number of malignant cell infiltrations.

**In vivo evaluation**

The induction of breast cancer by MCF7 *in vivo* was established and reached the metastatic phase compared to normal tissue (Fig. 5A and B). Although the treatment of metastatic carcinoma by hMSCs reduced the number of malignant cell infiltration (Fig. 5C), but the treatment by hMSCs+doxorubicin was more effective in the reduction (Fig. 5D).

Confirmed with *in vitro* genes’ expressions results, MCF7 showed an elevation in all genes’ TLR4, VEGF, NF-κB, IL-8 and IL-1 expressions along with a markedly elevation in MCF7+ hMSCs+LPS these genes’ expressions. Even if the treatment doxorubicin alone reduced the genes’ expressions, but the maximum reduction in all genes expressions was shown after treatment of MCF7 with both hMSCs+doxorubicin compared to MCF7 induced groups (Fig. 6).

*In vitro* results supported and confirmed with *in vivo* TLR4, VEGF, NF-κB, IL-8 and IL-1 genes’ expressions in figure 7, when compare each gene expression that reduced to
maximum by the effect of treatment with both hMSCs+doxorubicin compared to MCF7 groups.

Figure 6: In vivo quantitative expression for (A) TLR4, (B) VEGF, (C) NF-kB, (D) IL-8 and (E) IL-1 genes in the studied groups. Data are means ± SD. (*) p value < 0.05 comparing with control groups and (**) p value < 0.05 comparing with MCF7.
Figure 7: Quantitative genes expression in hMSCs+MCF7+doxorubicin group and the MCF7 group either (A) in vitro or (B) in vivo. Data are means ± SD. (∗) p value < 0.05 comparing with MCF7.

Table 1: The primers for the TLR4, NF-KB, VEGF, IL-1, and IL-8

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tbody>
<tr>
<td>TLR4</td>
<td>TGGATACGTTTCCCTTATAAG</td>
<td>GAAATGGAGGCACCCCTTC 18S</td>
</tr>
<tr>
<td>NF-KB</td>
<td>TACCATGCTCTTTTGGTTAC</td>
<td>TCCCTCTTTTCTATTGATGGGA</td>
</tr>
<tr>
<td>VEGF</td>
<td>GCAAGATCGCTAGAACAC</td>
<td>TTATGTCCTTGTCTTCTGATGCTT</td>
</tr>
<tr>
<td>IL1</td>
<td>CCAAAGCCAAGAAAGGGAAG</td>
<td>TTTATTGGGGGGGAAAGTCAG</td>
</tr>
<tr>
<td>IL8</td>
<td>ATCAGGCTGTCATTATGGIGT</td>
<td>AAATTTTCGACCACCTCCAAG</td>
</tr>
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</table>
DISCUSSION

Many cumulative positive impact effects were noticed by the use of hMSCs in combination with doxorubicin that was better than LPS therapy treatment against MCF7 breast carcinoma. Our choice for umblical hMSCs was right according to various advantages including wide spread availability, absence of donor risk, low risk of transmissible infectious diseases, decreased graft-versus-host disease and increased precursors of immune effectors cells [31]. Umblical cord hMSCs was differentiated and reduced the number of breast cancer cells (Fig. 1 and 2) because it possesses tropism to inflammatory process such as various cancer tissues and other inflammatory lesions. Our results have been shown hMSCs to be capable of attenuating the growth of tumor cells both in vitro and in vivo confirmed with many cancer researches [8-10].

LPS and doxorubocin groups showed a significant proliferation reduction compared to controls and exaggerated effects when combined with hMSCs. The maximum effect appeared in hMSCs+MCF7+doxorubicin group (Fig. 3A). LPS proliferation reduction results opposites the reported one that without any change in proliferation or survival of hepatocellular HT-29 after LPS treatment [32]. The cytotoxic effect of doxorubicin coincided many results against MCF7 [27,29] and 4T1 [33] cancer cells, that was more significant decrease in MCF7 number detected in our study after hMSCs addition. Although hMSCs alone effects against breast cancer in this research in contrarily with [34], but it confirmed with activities of hMSCs against breast cancer [9] that elevated by LPS and doxorubicin addition in this study.

JNK phosphorylation and IL-6 levels were significantly reduced in doxorubicin groups specially coculture group (Fig. 3B and C) due to its apoptotic effect against breast cancer [35] and cytokine reduction properties [36], along with cumulative similar effects by hMSCs [37]. As expected on an opposite effects, MCF7 and LPS groups showed an elevation of PJNK because the ability for activation by LPS [38] and elevation of IL-6 level by its effect on cytokines [39]. The elevation in TLR4, VEGF and NF-KB genes’ expressions for hMSCs+LPS and hMSCs+doxorubicin groups may due to irritation of the normal hMSCs by the two substances (Fig. 4). On the other hand, TLR4, VEGF, NF-KB, IL-8 and IL-1 genes’ expressions were significantly reduced after LPS, doxorubicin and/or hMSCs exposure for the other groups because of the cumulative healing effects against breast cancer and the direct response for the genes after treatment [7-22,32,40-43]. Due to the stress state in cancer, MCF7 showed the highest genes expressions. The doxorubicin plus coculture group showed
the lowest significant reduction in all genes’ expressions because of the additional effect for both doxorubicin and hMSCs (Fig. 4).

**Figure 5** *in vivo* histo-pathological images illustrated the effectiveness by hMSCs therapy in the reduction malignancy that was improved when mixed with doxorubicin. Even if all studied genes’ expressions were raised in MCF7+hMSCs+LPS groups caused by the LPS antigenic properties but it stills reduces MCF7 cells proliferation. The effect of doxorubicin to reduce the genes’ expressions was exaggerated by mesenchymal stem cells incorporation therapy on MCF7 induced groups for the previously mentioned reasons (Fig. 6).

Genes’; TLR4, VEGF, NF-KB, IL-8 and IL-1; expressions were reduced to maximum *in vitro and in vivo* by the combination hMSCs+doxorubicin therapy as shown in figure 7.

**CONCLUSION**
Each of the explored breast cancer treatment lines of hMSCs, doxorubicin or LPS was effective alone but appeared more effective when combined together. Umbilical cord hMSCs coculture with human MCF7 combined with doxorubicin therapy achieves the highest benefits for breast cancer treatment. Not only had this combination *in vitro* reduced MCF7 proliferation, PJNK, IL-6 levels and the expression of; TLR4, VEGF, NF-KB, IL-8 and IL-1; genes, but also *in vivo* reduction in cancer cells number and these genes expressions. Even if LPS showed some antigenic properties but it stills reduced MCF7 proliferation.

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**Conflict of interest**
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**REFERENCES**


