EXPERIMENTAL STUDY

Inhibitory effects of mouse bone marrow mesenchymal stem cell soup on staurospurine-induced cell death in MCF-7 and AGS

Zhaleh H, Azadbakht M, Bidmeshki Pour A

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran.
a.bidmeshkipour@gamil.com

ABSTRACT

BACKGROUND: Staurospurine induces apoptosis in cell line. Bone Marrow Mesenchymal stem cells Soup is a promising tool for cell proliferation *via* a variety of secreted factors. In this study, we examined the effects of BMSCs Soup on Staurospurine induced-cell death in MCF-7 and AGS cells.
METHODS: There were three Groups: Group I: no incubation with BM Soup; Group II: incubated with 24 h BM Soup; Group III: incubation with 48 h BM Soup. There were two treatments in each group. The treatments were 1µM Staurospurine (Treatment 1) and 0.0 µM Staurospurine (Treatment 2). The cells were cultured in culture medium containing 0.2 % BSA. We obtained the cell viability, cell death and NO concentration.
RESULTS: Our results showed that BM soup administration for 48 hours protectsed against 1µM staurosporine concentration induced cell death and reduced cell toxicity in MCF-7 and AGS cells. Cell viability and cell toxicity assay showed that BM soup in time dependent manner increased cell viability (p < 0.05) and cell death assay showed that cell death in time dependent manner was decreased(p < 0.05). Our data showed that BM soup with increasing NO concentration reduced staurospurine induced cell death and cell cytotoxicity (p < 0.05).
CONCLUSION: It's concluded that BMSCs soup suppressed staurospurine-induced cytotoxicity activity process in MCF-7 and AGS cells *(Fig. 9, Ref. 79)*. Text in PDF *www.elis.sk.*
KEY WORDS: BM-MSC soup, cell viability, cell death, NO.

Introduction

Many reports proposed that adult bone marrow mesenchymal stem cells (BM-MSCs) can differentiate into osteoblasts, chondroblasts, adipocytes, and hematopoiesis supporting stroma (1–3). Besides the mesenchymal tissue, many studies demonstrated that MSCs could differentiate into various non-mesenchymal tissue lineages under appropriate experimental conditions in vitro and in vivo, such as: hepatocytes (4, 5), cardiomyocytes (6, 7), lung alveolar epithelium (8), even neuron and glia (9–13). Recently, the amazing potential of MSCs attracted intense interest in the possible applications of MSCs in cell and gene therapy for disease, because mesenchymal Stem Cells can be obtained from bone marrow easily and expanded rapidly in vitro (14, 15). It has been showed that MSCs could easily induce differentiation in vitro by chemical inducers (9), cytokines (4, 16, 17), co-culture with neurons (18, 19), chemical inducers plus cytokines (10, 20) and transfect plus cytokines (21), etc.

Many studies had reported that MSCs transplantation improved cellular and functional recovery, increased endogenous cell proliferation, decreased apoptosis, promoted reduced lesion size and angiogenesis of neuronal cells (22) in CNS injuries, such as stroke and spinal cord injury in animal models. It showed that transplanted MSCs might exert beneficial effects in injury including their ability to produce growth factors (22–24). However, MSCs can secrete a variety of molecules including trophic factors and antiapoptotic molecules, which may provide the main mechanism responsible for their therapeutic effect (25). In addition, MSCs can inhibit the release of proinflammatory cytokines and promote the survival of damaged cells (26). For example, the therapeutic benefit of MSC cytokines has been observed in acute lung injury (27, 28), myocardial infarction (29), acute renal failure (30), cerebral ischemia (31, 32) and Alzheimer's disease (33).

On the other hand, the most recent mechanism of action is that BMSC cells provide a local paracrine effect (34–36) such as tissue regeneration and improvements. This have been proposed as paracrine effect of stem cell action (35–37). However, after closer investigation, we could not fully explain organ improvement.

Meanwhile, several studies have shown that stem cells soup include soluble factors that actin a paracrine fashion, contribute to organ repair and regeneration. These factors are cytokines; growth and other factors inducing cytoprotection, neovascularization, and medium endogenous tissue regeneration via activation of resident tissue stem cells. In addition, tissue re-modelling and organ function is affected by these paracrine factors (35).

Recent study showed that BMSC soup paracrine mechanism is the main cause behind the reported improvement of salivary organ

Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran.

Address for correspondence: A. Bidmeshki Pour, Department of Biology, Faculty of Science, Razi University, Postal Code: 6714967346, Kermanshah, Iran.
Fax: +98 08334274545 Phone: +98 08334274545

function (38). Many studies showed that once MSCs have promoted the development of fibroblasts, endothelial cells and tissue progenitor cells, which carried out tissue regeneration and repair by secretion of paracrine factors, including cytokines such as TNF-a, IL-1, IFN-g, toxins of infectious agents and hypoxia can stimulate the release of many growth factors by MSCs, including EGF, FGF, PDGF, TGF-b, VEGF, HGF, IGF-1, keratinocyte growth factor (KGF) and stromal cell derived factor-1 (SDF-1) (39–42).

There has been no evidence about any effect of mBMSC Soup on improving any form of the staurosporine induced apoptosis or cell death in breast and gastric cell line. The aim of this experimental study was to investigate the efficacy of mBMSC Soup on this proposal. In this context, we aimed to test the efficiency of mBMSC Soup in suppressing the staurospurine induced cell death in MCF-7 and AGS cell lines. The hypothesis is that mBMSC Soup protects cells and increases cell viability.

Material and methods

Preparation of mouse bone marrow cells

Isolation and culture of mBMSCs were carried out as previously described (43). Bone marrow was obtained from 6–8-week-old NMRI mice. For isolation of MSCs, tibias and femurs were dissected and the ends of the bones were cut. The marrow was extruded with flushing the shaft with DMEM culture medium (Gibco) completed with 10 % FBS (Gibco), 1 % L-Glutamine (Sigma), 1 % Non-essential amino acids (Sigma) and 100 IU/ml penicillin (Sigma), 100 µg/ml streptomycin (Sigma) using a 27-gauge syringe. Cells were disaggregated by gentle pipetting several times on 25 cm^2 plastic flask. The cell pellet was re-suspended in DMEM for each 10^8 cells. The cell suspension was incubated at 37 °C in humidified atmosphere containing 5 % CO_2 for 72 hour. After that, the nonadherent cells were removed by replacing the medium. Culture medium was replaced every 2 or 3 days about 2 weeks. When cell cultures reached to 80 % confluency, they were harvested with trypsin-EDTA 0.25 % (Sigma) for 5 minutes, again cultured to next confluence and harvested. Expanded cells from passages five-eight were used for further testing.

The surface marker expression of mBMSC cells was assessed by flow cytometry. mBMSC were suspended (1×10^6 cells/ml) and stained with FITC- or R-phycoerythrin (PE)-conjugated monoclonal antibodies, such as: CD14 (eBioscience), CD105 (eBioscience), CD73 (eBioscience), CD29 (Biolegend), CD34 (Santa Cruz), CD45 (eBioscience), CD90 (Biolegend).

Before being used in inducing neuroectodermal differentiation, the mBMSC cells were subculture at a density of 10^5 cells/well in 24-well culture plates.

mBMSC characterization

As quality controls, the capacity of isolated cells to form colonies (CFU-f) and their ability to undergo differentiation into chondrocytes, osteocytes, and adipocytes were evaluated. To evaluate the clonogenic capacity of cultured mBMSCs, after 14 days in culture and before colony coalescence, cells in the low-density plates were washed with PBS, fixed with 4 % paraformaldehyde,

washed, and stained with methylene blue for 2 hours to identify all colonies. To evaluate the in vitro differentiation ability of cultured mBMSCs, cells were trypsinized, harvested, and plated at 5×10^4 cells per well in 24-well plates in standard medium. Medium was replaced 24 hours after plating. Osteogenic differentiation was induced in mBMSC cultures by adding ascorbic acid (50 µg/mL), sodium b-glycerophosphate (10 mM), and dexamethasone (10^{-8} M) in standard medium. After 2 weeks, plates were washed with PBS, fixed with 4 % paraformaldehyde, and stained with 1 % alizarin red. Adipogenic differentiation was induced in another aliquot of cells of the initial culture by the addition of dexamethasone (10^{-7} M) and insulin (6 ng/mL), in DMEM medium supplemented with 1 % foetal calf serum, 1 % glutamine, and 1 % penicillin-streptomycin to the cell culture.

After 3 weeks, plates were washed with PBS, fixed with 4 % paraformaldehyde.

For von Kossa staining, cells were incubated in 5 % silver nitrate in the dark for 30 minutes, and then they were exposed to ultraviolet light for 1 h. The secretion of calcified ECM was observed as black nodules with von Kossa staining.

mBM soup preparation

After confirming and harvesting the mBMSC cells, mBMSC condition medium as mBM Soup was prepared. Briefly, mBMSCs cells were cultured in DMEM culture medium, culture medium was replaced every 2 or 3 days about 2 weeks. When cell reached to 80 % confluency, the medium was changed by fresh DMEM free serum and 0.2 % BSA culture medium. The cells were maintained for 24 h or 48 h. Then, the condition mediums were harvested as mBM soup (mBM soup 24 and mBM soup 48 h, respectively). Protein concentration of mBM Soup was 0.41 ug/ul (from 10^7 cells). mBM Soup was kept on –70 °C until further uses.

Cell line

MCF-7 and AGS cells were grown in a 25-cm2 tissue culture flask in DMEM culture medium (Gibco), supplemented with 10 % foetal bovine serum (FBS, Gibco; UK), 1 % NEAA (Sigma), 100 u/ml of penicillin (Sigma) and 100 mg/ml streptomycin (Sigma). The cells were maintained at 37 °C in a humidified, 5 % CO_2 environment.

Cell treatment

One day after plating the MCF-7 and AGS cells, the cells were washed with PBS, pH 7.4. There were six treatments. Treatment 1: 1 µM Staurospurine, Treatment 2: control cells, Treatment 3: mBM Soup 24 h, Treatment 4: mBM Soup 24 h together with 1 µM Staurospurine, Treatment 5: mBM Soup 48 h and Treatment 6: mBM Soup 48 h together with 1 µM Staurospurine.

Then, the cells were placed in the incubator at 37 °C with 5% CO_2. The cells were cultured in DMEM culture medium containing 0.2 % BSA.

Cell viability measurement

Trypan blue is a vital stain that leaves nonviable cells with a distinctive blue colour when observed under a microscope, while

viable cells appear unstained. Viable cells have intact cell membranes and hence do not take in dye from the surrounding medium. On the other hand, nonviable cells do not have an intact and functional membrane and hence do take up dye from their surroundings. This results in the ability to easily distinguish between viable and nonviable cells, since the former are unstained, small, and round, while the latter are stained and swollen. The method does not differentiate between apoptotic and necrotic cells.

The traditional method of performing trypan blue (0.4 g/100 ml in PBS) cell viability analysis involves manual staining and use of a hemocytometer for counting (44).

MTTassay

To perform the test, 15×10^3 MCF-7 and AGS cells were loaded into a 96-well plate and 200 µL of DMEM medium containing 0.2 % BSA was added. After 24-hour incubation, 200 µL of treatments medium as described was added to the wells. The cells were separately incubated with different treatments medium for 24 and 48 hours.

After incubation, the MTT test was performed in which the supernatant from each well was removed and 50 µL of MTT solution (5 mg/mL) was added to each well and incubated for 3 hours. The supernatant from each well was then removed and 100 µL of dimethyl sulfoxide was added to dissolve the formazan crystals at room temperature for 30 minutes. The optical density of each well was measured using a microplate reader (EL800; USA) at 570 and 630 nm.

The viability of the cells for each concentration was calculated using the following formula (45):

Cell viability (%)=(A570, 630(sample)/A (control))×100

NO assay

NO was measured using the Griess staining method. For each sample, 400 µL of thawed supernatant was deproteinized by adding 6 mg of zinc sulfate. The samples were centrifuged at 4 °C and 12,000 g for 12 minutes. Separately, 100 µL of 0, 6.25, 12.5, 25, 50, 100, and 200 µM sodium nitrite was poured into standard wells. We then added 100 µL of the deproteinized samples to each well, and 100 µL of vanadium chloride, 50 µL of sulfanilamide, and 50 µL of N-(1-naphthyl) ethylenediamine dihydrochloride were added to all wells (standard and samples). All wells were incubated for 15 minutes and were assessed using a microplate reader (EL800; USA) at wavelengths of 570 and 630 nm (46).

Quantification of cell death incidence

Hoechst/PI nuclear staining was carried out as previously described (47). Briefly, cells were plated in 24 well -cultured plates with 5×10^4 cells/well density overnight. Then, cells were treated with different treatment mediums for a range of times in differentiation medium (6, 12, 24 and 48 h). Then cells were incubated for 30 min at 37 °C with Hoechst 33342 dye (10 ng/ml in PBS), washed twice in PBS. PI (50 ng/ml in PBS) was added just before microscopy. Cells were visualized using an inverted florescence microscope (Olympus IX-71, Japan). Nuclear morphology was scored as follows: 1, viable cells had blue stained nuclei with smooth appearance; 2, viable apoptotic cells had blue-stained nuclei with multiple bright specks of condensed chromatin; 3, non-viable apoptotic cells had red-stained nuclei with either multiple bright specks of fragmented chromatin or one or more spheres of condensed chromatin (significantly more compact than normal nuclei); 4, non-viable necrotic cells had red-stained, smooth
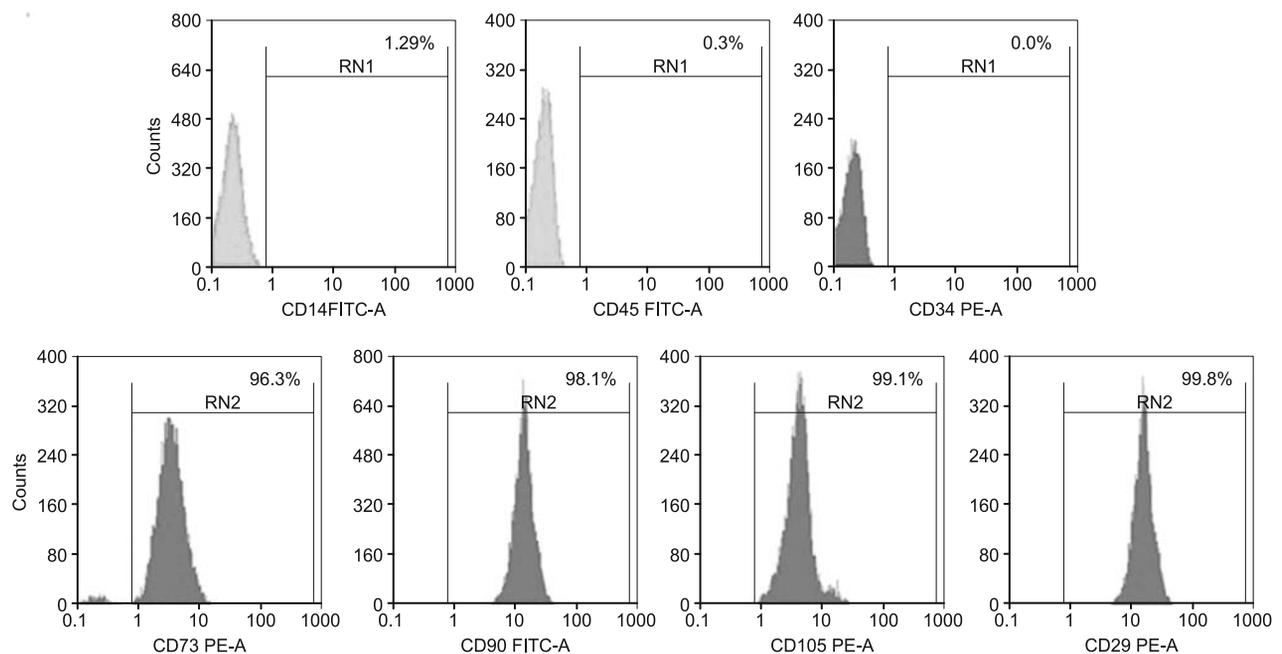
Fig. 1. The characterization of mouse BMSCs by flow cytometry. BMSCs, expressed CD73 but not CD45, CD34, or CD14. F) M-SMSCs detected by flow cytometry. Of the M-SMSCs, 96 % expressed CD73, CD90, CD105 and CD29 but not CD45, CD34, or CD14.

and homogeneous nuclei that were about the same size as normal (control) nuclei. The cell death index was calculated by the fraction of numbers of apoptotic cells on the total cell count in 100 (300 cells), respectively.

Data analysis

Data are reported as the mean ± SEM. Differences among treatment groups were tested using one-way ANOVA followed by Tukey's test. $p < 0.05$ was considered statistically significant.

Results

Characteristics of BMSCs from NMRI mice

The cells as mBMSCs were derived from female and male NMRI mice. The 5th passage of cells had similar morphology. Most of the mBMSCs cells were spindle-shaped similar to the fibroblast-like cells. The cells exhibited a rapid growth with cell clustering. We used Flow cytometry to detect the phenotype of the 5th passage cells. The results showed that about 98 % cells of mBMSCs are CD90-positive and lack the expression of CD14, CD45 and CD34. These results showed that mouse Bone marrow cells had the characteristics of mesenchymal stem cells (Fig. 1).

Multipotency of bone marrow mesenchymal stem cells were tested and specified by differentiation assay. Differentiation assays showed that isolated mBMSC cells could be induced into osteo-blast and adipocytes, as evinced by Alizarin red, Von Kossa for
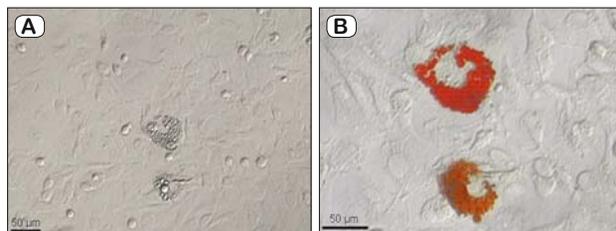
Fig. 2. adipogenic differentiation of BMSCs. Before (A), after (B) Sudan III staining.
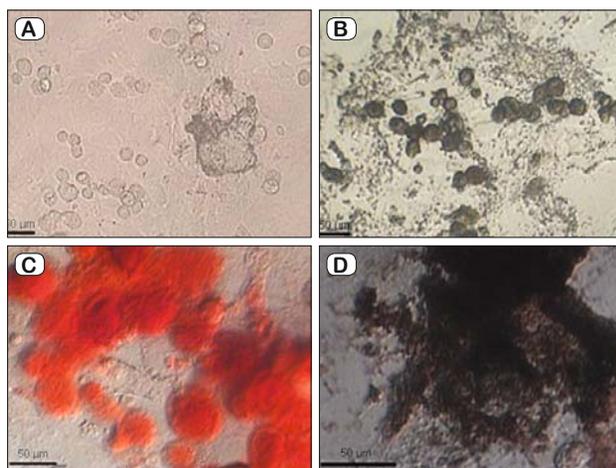
Fig. 3. Osteogenic differentiation of BMSCs. 12 day (A), 21 days (B), 21 days, alizarin red staining (C), and 21 days, von Kossa staining.
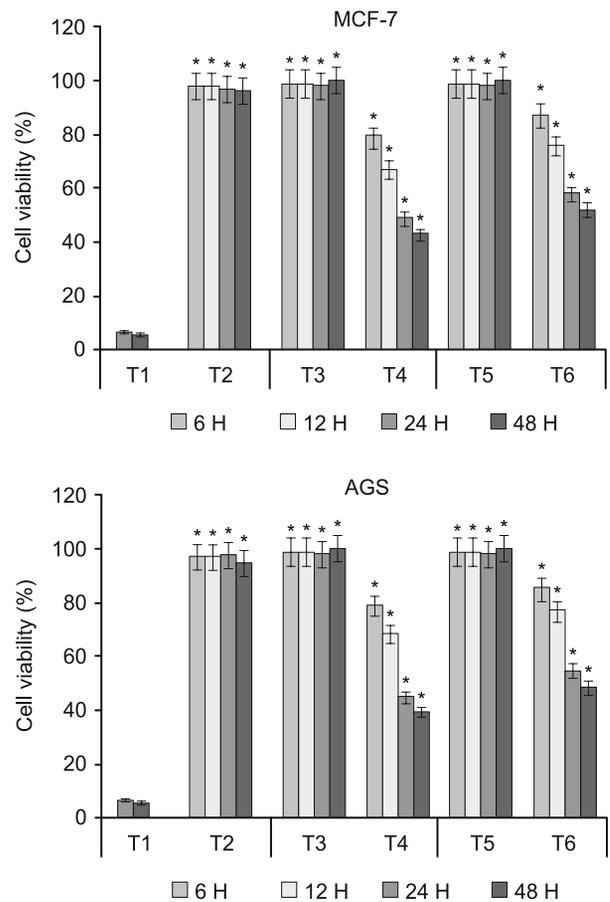
Fig. 4. MCF-7 cells and AGS cells, cell line viability (%) assessed by trypan blue in different treatment mediums and different culture periods. * $p < 0.05$; significant difference between the experiments and control were seen.

detection of osteogenic differentiation and Sudan III staining for adipogenic differentiation. Adipogenic differentiation in mBMSC resulted in distinct, round lipid vacuoles (Fig. 2) visualized with Sudan III. In contrast, osteogenic differentiation of mBMSC led to a considerable increase of extracellular matrix (ECM) deposits consisting of hydroxyapatite, calcium and magnesium salts stained dark red with Alizarin Red (AR) (Fig. 3). A detailed analysis of the osteogenic differentiation was accomplished by von Kossa staining.

Von Kossa staining revealed a distinct positivity for mBMSC, which was confirmed by AR-staining. Both stainings demonstrated the presence of calcite crystals as a sign of matrix ossification during osteogenic differentiation.

Cell viability

Comparison of the mean MCF-7 and AGS cell viabilities analysed using the trypan blue method after 6, 12, 24 and 48 hours indicated a significant difference between the treatments ($p < 0.05$).

After 6, 12, 24 and 48 hours, the percentage of cell viability in treatments 1, 4 and 6 were decreased compared to the treatment 2 (97 %) ($p < 0.05$). After 6, 12, 24 and 48 hours the percentage of

cell viability were not different in the treatments 3 and 5 compared to the treatment 2. The percentage of cell viability was increased in the treatment 3 compared to the treatment 4 p ($p < 0.001$) (Fig. 4). The percentage of cell viability was increased in the treatments 4 and 6 compared to the treatment 1 ($p < 0.05$).

Comparison of the mean cell viability analysed using the MTT assay after 24 and 48 hours revealed a significant increase between the treatments compared to the treatment 1, respectively ($p < 0.05$). Cell viability was again significantly decreased by increasing the time in the treatment 1 compared to the treatment 2 ($p < 0.05$). The percentages of cell viability were increased in the treatments 4 and 6 compared to the treatment 1 ($p < 0.05$). The percentage of cell viability was lowest in the treatment 1 and was highest in the treatments 3 and 5 ($p < 0.05$) (Fig. 5).

NO levels

NO concentration was evaluated using the Griess method. The effect of different concentrations of staurospurine on MCF-7 and AGS cells after 24 and 48 hours indicated a time-dependent decrease in NO secretion compared to the control treatment, respectively ($p < 0.05$).
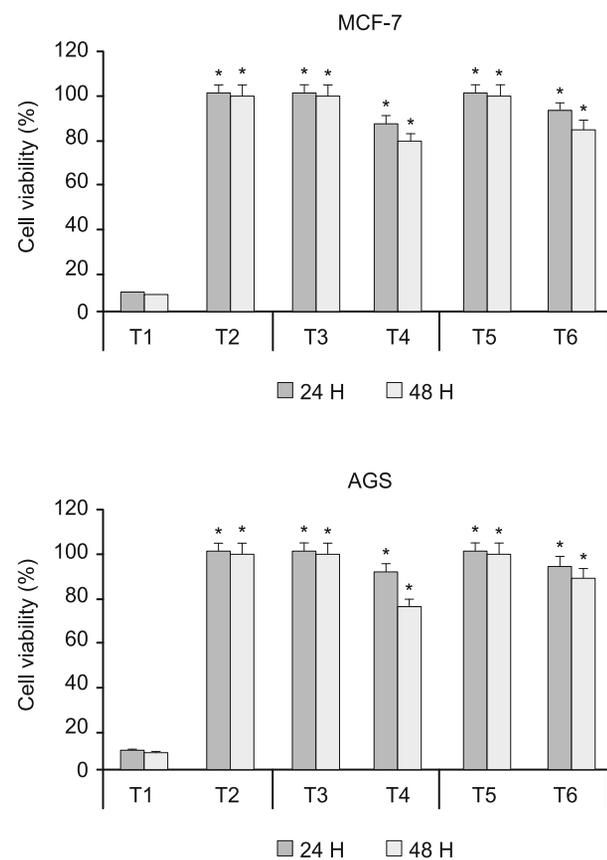
Fig. 5. MCF-7 cells AGS cell line viability (%) assessed by MTT in different treatment mediums and different culture periods. *p < 0.05; significant difference between the experiments and control were seen.
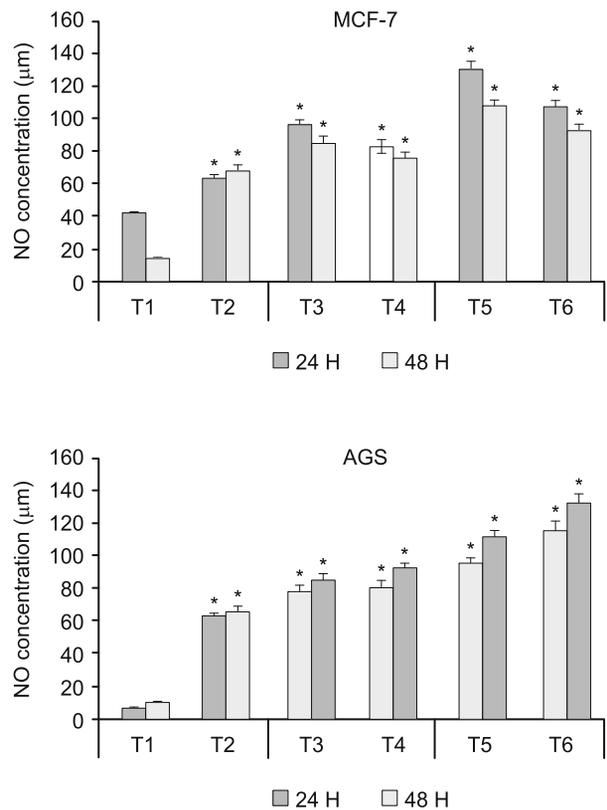

Fig. 6. Nitric oxide (NO) levels in different groups and different culture periods. * p < 0.05; significant difference between the experiments and control were seen.

Figure 6 shows NO concentration amounts (μM) in the culture medium of MCF-7 and AGS cells that contained staurospurine (1 μM) plus different mBM soup for 24 and 48 incubation times.

After 24 and 48 h incubation, in the treatment 1, the NO concentration was decreased in the medium compared to the control sample (treatment 2), respectively ($p < 0.05$). After the 24 and 48 h incubation, NO concentration significantly increased when cells were treated with treatments 2–6 compared to the treatment 1 ($p < 0.05$). Data showsedthat the highest NO concentration was in the treatment 6 and the lowest concentration was in the treatment 1 in culture medium for 24 and 48h, respectively ($p < 0.05$) (Fig. 6).

Cell death indexes

The cell death index of MCF-7 and AGS cells treated with different treatments showed an increase from the treatment 1 to the other treatments in time order, respectively ($p < 0.05$). After 6, 12, 24 and 48h, the cell death index was highest in the treatment 1 and lowest in the treatments 3 and 5, respectively ($p < 0.05$). The cell death index was increased in the treatment 1 compared to the treatment 2, respectively ($p < 0.05$). The cell death index of MCF-7 and AGS cells in the treatment 4 was increased compared to the treatment 3 ($p < 0.05$). The cell death index of cells in the treatment 6 was increased compared to the treatment 5 ($p < 0.05$).
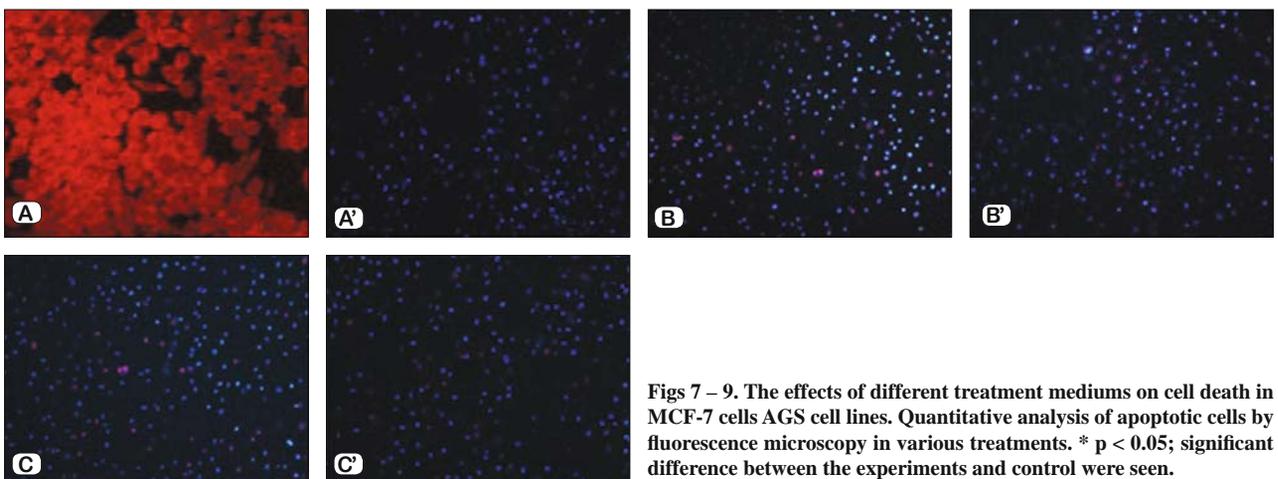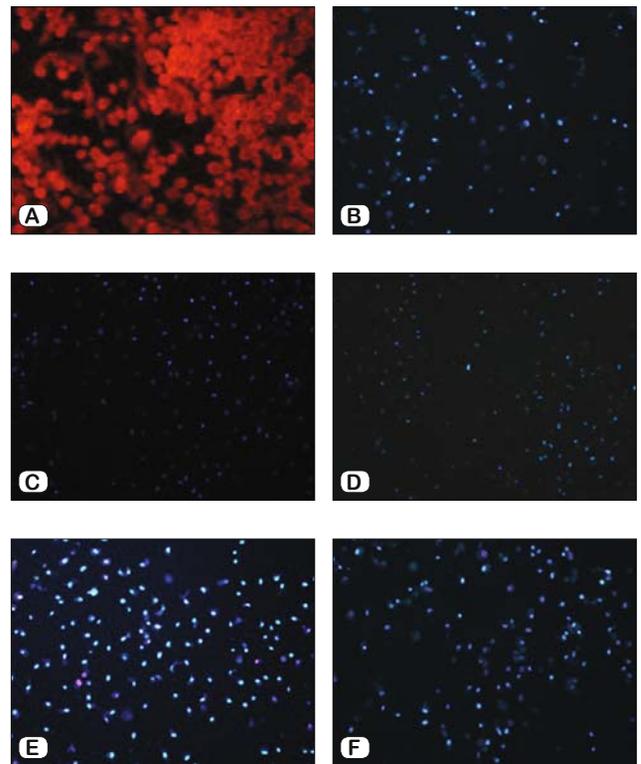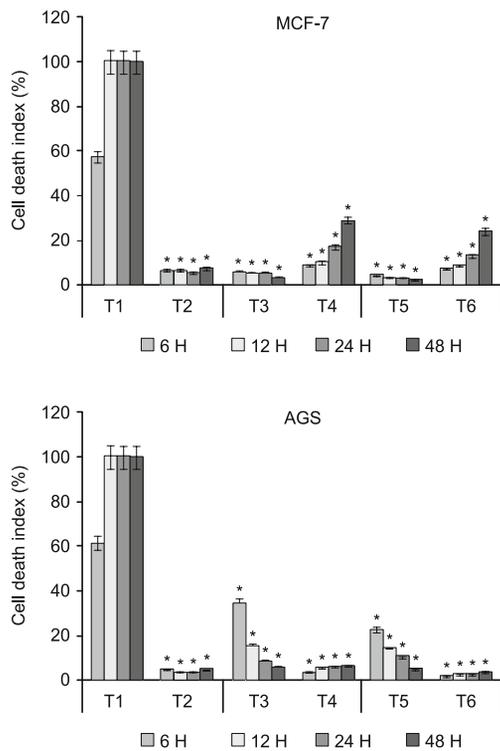
Figs 7 – 9. The effects of different treatment mediums on cell death in MCF-7 cells AGS cell lines. Quantitative analysis of apoptotic cells by fluorescence microscopy in various treatments. * $p < 0.05$; significant difference between the experiments and control were seen.

The cell death index of MCF-7 and AGS cells was decreased in the treatments 2, 3 and 5 compared to the treatment 1 after 6-48h incubation, respectively ($p < 0.05$) (Figs 7–9).

Discussion

In the present study, we showed that administered mBM Soup was capable multiple effectively: (1) to enhance the cell viability, (2) to enhance the NO concentration in culture medium, and (3) to suppress cell death or cell death index in cells lines.

Here, for the first time, we showed that administrations of mBM Soup were effective for prevention of Staurospurine-induced cell death in MCF-7 and AGS cells. Mesenchymal Stem Cells might exhibit extremely different functions in distinct tumour microenvironments because various signalising pathways can be activated in these cells. Highly controversial results have been reported in prior studies that addressed the role of MSCs in tumour development. For example, MSCs can inhibit tumour cell proliferation when co-cultured with tumour cells in vitro. Ramasamy et al has showed that MSCs could inhibit the proliferation of leukaemia cell lines and solid tumour cell lines in vitro

(48). The results showed that MSCs could also induce apoptosis in tumour cells (49) by upregulation of caspase 3, an apoptosis-related protease (50). It has been showed that MSCs indirectly suppressed tumour growth by inhibiting angiogenesis in vascular endothelial cells (51, 52) or by directly inhibiting vascular network formation (53).

However, opposing results had been documented. For example, in PC3 cells (an animal model of prostate cancer) MSCs promoted fibroblast growth factor 2 (FGF2) secretions. It has been showed that MSCs promoting tumour angiogenesis by enhance the expression of endothelin-1 in colon cancer cell lines (54, 55). It has been showed that co-culturing gastric cancer cell lines with MSCs increased the proportion of CD133-positive gastric cancer cells (56, 57). Therefore, in this study, we tried to examine the effect of BM-MSC Soup (cell free system) on a cancer cell line (MCF7 and AGS). It is very important to examine how mBM Soup in different states affects tumour cell lines and it can also show the growth factor supporting role for the cells against the apoptosis inducers.

In the present study, the enhancing and supporting effect mBM Soup on a breast cancer cell line and gastric cell line was associated with NO increase. Yin et al has showed that the inhibitory effect on MCF7 cells was due to decreasing NO secretion (58), although decreasing cyclooxygenase 2 and cytokine expression are additional factors for consideration.

Staurosporine, a broad -spectrum protein kinase inhibitor (59), has been used to induce cell death in a wide range of cell types at 1μM concentration (60, 61). The exact mechanism responsible for staurosporine-induced cell death is not known. Several authors showed that the exact mechanism responsible for staurosporine-induced cell death in treated cells depends on calcium ions, but several controversial reports exist about the effects of staurosporine on extra or intracellular calcium sources on different treated cells. For example, it has been showed that staurosporine induces cell death by increasing cytoplasmic Ca^{2+} concentration, which is responsible for releasing mitochondrial cytochrome C, caspase-3 activation (62), intracellular ROS accumulation (63), and increasing (Ca^{2+}) (64) and cell death in various cell types (eg. PC12 cells). Recently, we showed that that endogenous ROS generation, extra-cellular Ca^{2+}, and calcium homeostasis regulation were related by mechanisms playing an important role in regulation of STS-induced cell death (65, 66). Recently, we reported that inhibition of IP3R and PLCγ led to attenuating of the rise in intracellular calcium triggered by staurosporine, thus strongly suggesting that ROS generation could potentiate the observed intracellular calcium overload (67). Our data showed that saturospurine at 1μM concentration induced cell death and reduced cell viability by increasing the NO secretion. Maybe, that saturospurine upregulates NOS biosynthesis and intracellular calcium overload, but it needs to be investigated for these effects. This paper highlights the importance of the paracrine effects of BMSC soup on tumour cell lines, and that intact BM cells may not be necessary. Previous studies showed that paracrine cross-talking between salivary gland (SG) cells and other cell populations (such as MSC or amniotic epithelial cells) was demonstrated by using a culture system that physically separated the cell populations (68, 69). These findings on the paracrine effects of MSC showed that organ repair was due to the secretion of cytokines, chemokines, and growth factors (70). It had been showed that injecting conditioned medium from MSC cultures exerted cardiomyocyte protection and improved cardiac function in mouse infarcted hearts (71, 72). Administrating mBM Soup does not require the injection of cells, which carry the risk of differentiating into unwanted/tumorigenic cell types in organs and is not patient-specific. Study showed that BMSC Soup included all cell types of the whole BM and consequently numerous proteins, cytokines and paracrine factors (73, 74). It has been showed that TGF-β expression in MSCs promotes tumour growth of prostate cancer cell lines in mice. This tumour growth-promoting effect of MSCs can be blocked by siRNAs against TGF-β (75). Cell survival factor inhibits cell apoptosis through activating specific signalising pathway(s), including the PI3K/Akt pathway. It has been showed that, transfection of constitutively active Akt prevents cell apoptosis while a dominant negative Akt induces cell apoptosis (76). In the other hand, recent studies showed that inhibitors of the PI3K/Akt pathway can sensitize cells to apoptotic stimuli (77, 78). For example, Osaki et al, showed that inhibition of PI3K caused inhibition of cell proliferation without induction of cell apoptosis and that inhibition of the PI3K-Akt signalising pathway significantly increased the sensitivity of cell apoptosis (79).

It has been showed, that BMSC Condition medium enhanced Erk1/2 and Akt phosphorylation in neurons, and the specific MEK1 inhibitor PD98059 or the phosphoinositide-3 kinase (PI3-K) inhibitor Ly294002 abolished the neuroprotective effect from staurospurine induced cell death. They showed that these findings demonstrated that BMSC trigger endogenous survival signalising pathways in neurons that mediate protection against apoptotic insults (79).

Moreover, the interaction between breast and gastric cells and mBM Soup were not clear. Our results provided evidence that mBM soup was capable to suppress cell death and induced cell viability MCF-7 and AGS cells. Studying the paracrine factors that are differentially produced in BM- MSC like NO, TGFβ and activation of PI3K/Akt, could induce the effects, but it is not really clear.

Conclusion

According to our results in the present study, application of mBMSC Soup enhance the cell viability, NO concentration and decrease cell death.

In conclusion, mBMSCs Soup suppressed the staurospurine induced cell death in breast cancer cell line and gastric cell line in a time-dependent manner. mBMSCs Soup also enhanced the proliferation of cells. It seems that the Soup exerts its effect through an induction of NO production and induction of proliferation. However, more factors need to be investigated in these effects.

References

1. **Prockop DJ.** Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997; 7 (4): 276–285.

2. **Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR.** Multilineage potential of adult human mesenchymal stem cells. Science 1999; 14 (7): 284–293.

3. **Bianco P, Riminucci M, Gronthos S, Robey PG.** Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 2001; 19 (3): 180–192.

4. **Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM.** Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418 (3): 41–49.

5. **Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund, T, Lenvik T, Johnson S, Hu WS, Verfaillie CM.** Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 2002; 109 (4): 1291–1302.

6. **Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S.** Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 1999; 103 (11): 697–705.

7. **Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD.** Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult Murine heart. Circulation 2002; 105 (2): 93–98.

8. **Kotton DN, Ma BY, Cardoso WV, Sanderson EA, Summer RS, Williams MC, Fine A.** Bone marrow-derived cells as progenitors of lung alveolar epithelium. Development 2001; 128 (12): 5181–5188.

9. **Woodbury D, Schwarz EJ, Prockop DJ, Black IB.** Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000; 61 (7): 364–370.

10. **Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR.** Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 2000; 164 (4): 247–256.

11. **Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ.** Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats e similarities to astrocyte grafts. Proc Natl Acad Sci USA 1998; 95 (8): 3908–3913.

12. **Kopen GC, Prockop DJ, Phinney DG.** Bone Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA 1999; 96 (9): 10711–10716.

13. **Mu~noz-Elias G, Marcus AJ, Coyne TM, Woodbury D, Black IB.** Adult bone marrow stromal cells in the embryonic brain: engraftment, migration, differentiation, and long-term survival. J Neurosci 2004; 24 (3): 4585–4595.

14. **Colter DC, Class R, DiGirolamo CM, Prockop DJ.** Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. Proc Natl Acad Sci USA 2000; 97 (5): 3213–3218.

15. **Javazon EH, Colter DC, Schwarz EJ, Prockop DJ.** Rat marrow stromal cells are more sensitive to plating density and expand more rapidly from single-cellderived colonies than human marrow stromal cells. Stem Cells 2001; 19 (3): 219–225.

16. **Jiang Y, Henderson D, Blackstad M, Chen A, Miller RF, Verfaillie CM.** Neuroectodermal differentiation from mouse multipotent adult progenitor cells. Proc Natl Acad Sci USA 2003; 100 (12): 11854–11860.

17. **Hermann A, Gastl R, Liebau S, Popa MO, Fiedler J, Boehm BO, Maisel M, Lerche H, Schwarz J, Brenner R, Storch A.** Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 2004; 117 (7): 4411–4422.

18. **Wislet-Gendebien S, Leprince P, Moonen G, Rogister B.** Regulation of neural markers nestin and GFAP expression by cultivated bone marrow stromal cells. J Cell Sci 2003; 116 (14): 3295–3302.

19. **Wislet-Gendebien S, Hans G, Leprince P, Rigo JM, Moonen G, Rogister B.** Plasticity of cultured mesenchymal stem cells: switch from nestin-positive to excitable neuron-like phenotype. Stem Cells 2005; 23 (7): 392–402.

20. **Kohyama J, Abe H, Shimazaki T, Koizumi A, Nakashima K, Gojo S.** Brain from bone: efficient "meta-differentiation" of marrow stroma-derived mature osteoblasts to neurons with Noggin or a demethylating agent. Differentiation 2001; 68 (2): 235–244.

21. **Dezawa M, Kanno H, Hoshino M, Cho M, Matsumoto N, Itokazu Y, Tajima N, Yamada H, Sawada H, Ishikawa H, Mimura T, Kitada M, Suzuki Y, Ide C.** Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest 2004; 113 (11): 1701–1710.

22. **Parr AM, Tator CH, Keating A.** Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 2007; 40 (3): 609–619.

23. **Chopp M, Li Y.** Treatment of neural injury with marrow stromal cells. Lancet Neurol 2002; 1 (2): 92–100.

24. **Si YL, Zhao YL, Hao HJ, Fu XB, Han WD.** MSCs: Biological characteristics, clinical applications and their outstanding concerns. Ageing Res Rev 2011; 10 (9): 93–103.

25. **Meirelles Lda S, Fontes AM, Covas DT, Caplan AI.** Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009; 20 (7): 419–427.

26. **Uccelli A, Moretta L, Pistoia V.** Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008; 8 (3): 726–736

27. **Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG.** Interleukin 1 receptor antagonist mediumtes the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci USA 2007; 104 (11): 11002–11007.

28. **Gupta N, Su X, Popov B, Lee JW, Serikov V, Matthay MA.** Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J Immunol 2007; 179 (22): 1855–1863.

29. **Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ.** Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 2009; 5 (3): 54–63.

30. **Togel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C.** Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. Am J Physiol Renal Physiol 2005; 289 (11): 31–42.

31. **Sheikh AM, Nagai A, Wakabayashi K, Narantuya D, Kobayashi S, Yamaguchi S, Kim SU.** Mesenchymal stem cell transplantation modulates

neuroinflammation in focal cerebral ischemia: contribution of fractalkine and IL-5. Neurobiol Dis 2011; 41 (5): 717–724.

32. Lee JK, Jin HK, Endo S, Schuchman EH, Carter JE, Bae JS. Intracerebral transplantation of bone marrow-derived mesenchymal stem cells reduces amyloid-beta deposition and rescues memory deficits in Alzheimer's disease mice by modulation of immune responses. Stem Cells 2010; 28 (11): 329–343.

33. Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. Stem Cells Dev 2005; 14 (3): 595–604.

34. Tyndall A, Gratwohl A. Adult stem cell transplantation in autoimmune disease. Curr Opin Hematol 2009; 16 (9): 285–291.

35. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008; 103 (10): 1204–1219.

36. Prockop DJ. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther 2009; 17 (8): 939–946.

37. Phinney DG, Prockop DJ. Concise review: mesenchymal stem/ multipotent stromal cells: the state of transdifferentiation and modes of tissue repair–current views. Stem Cells 2007; 25 (12): 2896–2902.

38. Tran SD, Liu Y, Xia D, Maria OM, Khalili S. Paracrine Effects of Bone Marrow Soup Restore Organ Function, Regeneration, and Repair in Salivary Glands Damaged by Irradiation. PLoS ONE 2013; 8 (4): 61632–61639.

39. Shi Y, Su J, Roberts AI, Shou P, Rabson AB, Ren G. How mesenchymal stem cells interact with tissue immune responses. Trends Immunol 2012; 33 (7): 136–143.

40. Ma XL, Liu KD, Li FC, Jiang XM, Jiang L, Li HL. Human mesenchymal stem cell increases expression of a-tubulin and angiopoietin 1 and 2 in focal cerebral ischemia and reperfusion. Curr Neurovasc Res 2013; 10 (1): 103–111.

41. Aguilar S, Scotton CJ, McNulty K, Nye E, Stamp G, Laurent G. Bone marrow stem cells expressing keratinocyte growth factor via an inducible lentivirus protects against bleomycin-induced pulmonary fibrosis. PLoS One 2009; 4 (2): 8013–8021.

42. Hung SP, Yang MH, Tseng KF, Lee OK. Hypoxia-induced secretion of TGF-beta 1 in mesenchymal stem cell promotes breast cancer cell progression. Cell Transplant 2012; 22 (3): 1869–1882.

43. Nadri S, Soleimani M, Hosseni RH, Massumi M, Atashi A, Izadpanah R. An efficient method for isolation of murine bone marrow mesenchymal stem cells. Int J Dev Biol 2007; 51 (2): 723–729.

44. Patel S, Gheewala N, Suthar A, Shah A. In vitro cytotoxicity activity of solanum nigrum extract against Hela cell line and vero cell line. Pharmacy and Pharmaceutical Science 2009; 1 (3): 176–198.

45. Ghosh K, Chandra K, Ojha AK, Sarkar S, Islam SS. Structural identification and cytotoxic activity of a polysaccharide from the fruits of Lagenaria siceraria (Lau). Carbohydr Res 2009; 344 (11): 693–698.

46. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5 (2): 62–71.

47. Yuan W, Guo J, Li X, Zou Z, Chen G, Sun J, Wang T, Lu D. Hydrogen peroxide induces the activation of the phospholipase C-g1 survival pathway in PC12 cells: protective role in apoptosis. Acta Biochem Biophys Sin (Shanghai) 2009; 41 (8): 625–630.

48. Ramasamy **R, Lam EW, Soeiro I, Tisato V, Bonnet D, Dazzi F**. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on in vivo tumor growth. Leukemia 2007; 21 (2): 304–310.

49. Sun B, Roh KH, Park JR, Lee SR, Park SB, Jung JW. Therapeutic potential of mesenchymal stromal cells in a mouse breast cancer metastasis model. Cytotherapy 2009; 11 (3): 289–298.

50. Lu YR, Yuan Y, Wang XJ, Wei LL, Chen YN, Cong C. The growth inhibitory effect of mesenchymal stem cells on tumor cells in vitro and in vivo. Cancer Biol Ther 2008; 7 (2): 245–251.

51. Secchiero P, Zorzet S, Tripodo C, Corallini F, Melloni E, Caruso L. Human bone marrow mesenchymal stem cells display anti-cancer activity in SCID mice bearing disseminated non-Hodgkin's lymphoma xenografts. PLoS One 2010; 5 (6): 11140–11149.

52. Otsu K, Das S, Houser SD, Quadri SK, Bhattacharya S, Bhattacharya J. Concentration-dependent inhibition of angiogenesis by mesenchymal stem cells. Blood 2009; 113 (18): 4197–4205.

53. Ho IA, Toh HC, Ng WH, Teo YL, Guo CM, Hui KM. Human bone marrow-derived mesenchymal stem cells suppress human glioma growth through inhibition of angiogenesis. Stem Cells 2013; 31 (1): 146–155.

54. Huang WH, Chang MC, Tsai KS, Hung MC, Chen HL, Hung SC. Mesenchymal stem cells promote growth and angiogenesis of tumors in mice. Oncogene 2013; 32 (37): 4343–4354.

55. Lin G, Yang R, Banie L, Wang G, Ning H, Li LC. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. Prostate 2010; 70 (10): 1066–1073.

56. Li Z. CD133: a stem cell biomarker and beyond. Exp Hematol Oncol 2013; 2 (1): 17–24.

57. Nishimura K, Semba S, Aoyagi K, Sasaki H, Yokozaki H. Mesenchymal stem cells provide an advantageous tumor microenvironment for the restoration of cancer stem cells. Pathobiology 2012; 79 (6): 290–306.

58. Yin MB, Li ZR, Tóth K, Cao S, Durrani FA, Hapke G. Potentiation of irinotecan sensitivity by Se-methylselenocysteine in an in vivo tumor model is associated with downregulation of cyclooxygenase-2, inducible nitric oxide synthase, and hypoxia-inducible factor 1alpha expression, resulting in reduced angiogenesis. Oncogene 2006;25 (11): 2509–2019.

59. Cai J, Jones DP. Superoxide in apoptosis. J Biol Chem 1998; 273 (6): 11401–11404

60. Kruman I, Guo Q, Mattson MP. Calcium and reactive oxygen species mediumte staurosporine-induced mitochondrial dysfunction and apoptosis in PC12 cells. J Neurosci Res 1998; 51 (7): 293–308

61. Abe S, Ohnishi H, Tsuchiya K, Ishizawa K, Torii M, Kanematsu Y, Kawazoe K, Minakuchi K, Yoshizumi M, Tamaki T. Calcium and Reactive Oxygen Species Mediumted Zn2+ Induced Apoptosis in PC12 Cells. J Pharmacol Sci 2006; 102 (5): 103–111.

62. Kirkland RA, Franklin JL. Evidence for redox regulation of cytochrome c release during programmed neuronal death: antioxidant effects of protein synthesis and caspase inhibition. J Neurosci 2001; 21 (3): 1949–1963

63. Prehn JH, Jordan J, Ghadge GD. Ca2+ and reactive oxygen species in staurosporine-induced neuronal apoptosis. J Neurochem 1997; 68 (4): 1679–85

64. Dawson VL, Dawson TM. Free radicals and neuronal cell death. Cell Death Differ 1996; 3 (2): 71–78

65. Zhaleh H, Zhaleh M. ROS Mediumted the Effects of Extracellular Calcium on Staurosporine-induced Cell Death in PC12 Cells. EJBS 2012; 5 (1): 112–119.

66. Zhaleh H, Azadbakht M, Bidmeshki Pour A. Effects of extracellular calcium concentration on neurite outgrowth in PC12 cells by staurosporine. Neuroscience Lett 2011; 498 (9): 1–5.

67. Zhaleh H, Azadbakht M, Bidmeshki Pour A. Phosphoinositid signal pathway mediumte neurite outgrowth in PC12 cells by staurosporine. Bratisl Lek Listy 2014; 115 (4): 112–121.

68. Maria OM, Tran SD. Human mesenchymal stem cells cultured with salivary gland biopsies adopt an epithelial phenotype. Stem Cells Dev 2011; 20 (3): 959–967.

69. Huang GL, Zhang NN, Wang JS, Yao L, Zhao YJ. Transdifferentiation of human amniotic epithelial cells into acinar cells using a double-chamber system. Cell Reprogram 2012; 14 (9): 377–383.

70. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediumtors. J Cell Biochem 2006; 98 (6): 1076–1084.

71. Takahashi M, Li TS, Suzuki R, Kobayashi T, Ito H. Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury. Am J Physiol Heart Circ Physiol 2006; 291 (3): 886–893.

72. Gnecchi M, He H, Liang OD, Melo LG, Morello F. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005; 11 (7): 367–368.

73. Yeghiazarians Y, Zhang Y, Prasad M, Shih H, Saini SA. Injection of bone marrow cell extract into infarcted hearts results in functional improvement comparable to intact cell therapy. Mol Ther 2009; 17: 1250–1256.

74. Angeli FS, Zhang Y, Sievers R, Jun K, Yim S. Injection of human bone marrow and mononuclear cell extract into infarcted mouse hearts results in functional improvement. Open Cardiovasc Med J 2012; 6 (1): 38–43.

75. Cheng J, Li L, Liu Y, Wang Z, Zhu X, Bai X. Interleukin-1alpha induces immunosuppression by mesenchymal stem cells promoting the growth of prostate cancer cells. Mol Med Rep 2012; 6 (5): 955–960.

76. Suzuki S, Chuang LF, Doi RH, Chuang RY. Morphine suppresses lymphocyte apoptosis by blocking p53-mediumted death signaling. Biochem Biophys Res Commun 2003; 308 (10): 802–808.

77. Fahy BN, Schlieman M, Virudachalam S, Bold RJ. AKT inhibition is associated with chemosensitisation in the pancreatic cancer cell line MIA-PaCa-2. British Journal of Cancer 2003; 89 (7): 391–397.

78. Osaki M, Kase S, Adachi K, Takeda A, Hashimoto K, Ito H. Inhibition of the PI3K-Akt signaling pathway enhances the sensitivity of Fas-mediumted apoptosis in human gastric carcinoma cell line, MKN-45. J Cancer Res Clin Oncol 2004; 130 (1): 8–14.

79. Nicola BI, Hea SL, Landshamer S, Straube A, Padovan CS, Plesnila N, Culmsee C. Bone marrow stromal cells mediumte protection through stimulation of PI3-K/Akt and MAPK signaling in neurons. Neuint 2006; 8 (9): 213–221.