IJBC 2017; 9(2): 48-53

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir



ORIGINAL ARTICLE

Assessment of Cytotoxicity of Dimethyl Sulfoxide in Human Hematopoietic Tumor Cell Lines

Fatemeh Hajighasemi[1], Shaghayegh Tajik[2]

[1]Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Iran
[2]Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article History:
Received: 05.12.2016
Accepted: 02.02.2017

Keywords:
Dimethyl sulfoxide
Cytotoxicity
Leukemia cell lines
Apoptosis
Proliferative response

**Corresponding author:*
Fatemeh Hajighasemi, PhD
Address: Associate Professor; Department of Immunology, Faculty of Medicine, Shahed University, No. 31, Shahid Abdollahzadeh Street, Keshavarz Blvd., 1415635111, P.O.Box : 14155-7435, Tehran, Iran
Tel: +98 21 88964792
Fax: +98 21 88966310
Email: fatimahajighasemi@gmail.com

ABSTRACT

Background: Dimethyl Sulfoxide (DMSO) is a solvent most broadly used as a cryopreservative agent. Antitumor effects of DMSO is a recently recognized phenomenon. In this study, cytotoxic effects of DMSO on human monocytes and T leukemic cell lines has been investigated in vitro.
Methods: Human leukemic T cells (Molt-4 and Jurkat) and monocytes (U937 and THP1) were cultured in complete RPMI mediums. The cells at different logarithmic growth phases were incubated with different concentrations of DMSO (0.1, 0.2, 0.5, 1, 2 and 5%). Then viability and proliferative response of leukemic cell lines was assessed by trypan blue dye exclusion (TB test) and MTT assays, respectively.
Results: DMSO has a cytotoxic effect on the leukemic cells used in this study; dose and time-dependently. This cytotoxicity for all of these leukemic cells was shown at ≥ 2% concentrations of the DMSO after 24, 48 and 72 hours' incubation time. Moreover, there was not any significant difference between DMSO cytotoxicity in these different leukemic cell lines.
Conclusion: All of the used leukemic cells showed sensitivity to DMSO at ≥2% concentrations time dependently. This sensitivity significantly increased with time. DMSO might be a cytotoxic agent for leukemic cells. It might be a useful candidate in design of chemotherapeutic protocols for leukemia as well as other cancers.

Please cite this article as: Hajighasemi F, Tajik S. Assessment of Cytotoxicity of Dimethyl Sulfoxide in Human Hematopoietic Tumor Cell Lines. IJBC 2017; 9(2): 48-53.

Introduction

Dimethyl sulfoxide (DMSO) is a solvent broadly used as a cryopreservative agent.[1,2] DMSO with various chemical characteristics is an appropriate pharmacological carrier for several drugs and materials.[3,4] Moreover, antioxidant and anti-inflammatory effects of DMSO have been determined.[5,6] The inhibitory effects of DMSO on inflammatory cytokine production in autoimmune arthritis have been proposed.[7] Meanwhile, antitumor effects of DMSO have been shown in different studies.[8-10] There are also some roles for DMSO in management of pain in cancers.[11] There are numerous reports of induction of antitumor immunotherapy by DMSO-treatment.[12]

On the other hand, pretreatment by DMSO has been reported to potentiate toxic effect of cisplatin on sensory hair cells.[13] Furthermore, antitumor effects of two ruthenium (II)-DMSO-chalcone complexes has been described.[10] Anticancer activities of two DMSO-complexes have also been demonstrated.[14] Tumor suppressor gene activation, induction of apoptosis and inhibition of the several cancer cells' proliferation by DMSO have also been shown.[8,15,16] Treatment of mouse hepatocellular carcinoma cell line with 2% solution of DMSO depressed proliferation and induced cell cycle arrest with no notable apoptosis or reduced viability.[12] It is clear that enhancement of anti-inflammatory, antioxidant and antineoplastic effects of several drugs and medicinal plants is dependent on their delivery vehicles. DMSO as

[Downloaded from ijbc.ir on 2024-03-29]

a polar solvent can dissolve numerous nonpolar and polar tiny ingredients, enhances cell membrane permeability, avoids free radical development and increases the penetration of pharmaceutical mediators in antitumor drugs through the cells.[17]

Moreover, epigenetic modifications by DMSO have also been reported,[18,19] and the role of epigenetic variations in cancer progress have been discovered.[20,21] In the above mentioned studies, different mechanisms of anti-cancer properties of DMSO such as induction of leukemic cell differentiation, tumor suppressor genes activation, apoptosis induction, inhibition of the various neoplasms proliferation and increase in penetration of pharmaceutical agents have been explored.[15-17] Considering the modulatory epigenetic anti-tumor properties of DMSO and its complexes, in the current study the cytotoxic effects of DMSO on human monocytic and T leukemic cells has been investigated in vitro to find out if anti-cancer effects of DMSO might be somewhat due to its direct cell cytotoxicity.

Materials and Methods

Reagents

RPMI-1640 medium, penicillin, streptomycin, dimethyl sulfoxide (DMSO) and trypan blue (TB) were purchased from Sigma (USA). Fetal calf serum (FCS) was obtained from Gibco (USA) and MTT (3-[4, 5-dimethyl thiazol-2, 5-diphenyltetrazoliumbromide]) kit was purchased from Invitrogen (USA). Microtiter plates, flasks and tubes were purchased from Nunc (Falcon, USA).

Preparation of DMSO

DMSO was dissolved in RPMI-1640 medium and stored at -20°C until use. DMSO was diluted in culture medium to prepare appropriate concentrations before use.

Cell Lines

Human leukemic T cells [Molt-4 (NCBI C149) and Jurkat (NCBI C121)] and monocytes [U937 (NCBI C130)] and THP1 (NCBI C563) were obtained from NCBI (National Cell Bank of Iran, Pasteur Inst. of Iran, Tehran). The cells were maintained in RPMI-1640 medium supplemented with 10% FCS in 5% CO2 at 37°C.

Cell Culture and Treatment

Human leukemic and mouse fibrosarcoma cells were cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C in 5% CO2. The cells were seeded at a density of 2×10^{4} cell/well and then incubated with different concentrations of DMSO (0.1, 0.2, 0.5, 1, 2 and 5%) for 24, 48 and 72 hours. All experiments were done in triplicate.

Cell Proliferation Assay

To evaluate the effect of different concentrations of DMSO on viability of leukemic cell lines, we used trypan blue dye exclusion (TB test)[22] and MTT assay.[23]

Trypan Blue Dye Exclusion Test

Principle of trypan blue dye exclusion test is exclusion of dye by viable cells and taking it up by dead cells. Viability is evaluated by direct counting of viable and dead cells. Percentage of the number of viable cells to the total number of cells is considered as viability percentage.

MTT Assay

In MTT test, the conversion of yellow water soluble MTT to a blue-insoluble formazon was assessed according to the method developed by Mosmann.[23] At the end of incubation time, the medium was replaced with 100 µl of fresh medium. The amount of 10 µl of MTT solution (5 mg/ ml in PBS) was then added to each well and incubated at 37°C for 4 hours. Then, 100 µl of the SDS-HCl solution (100 mg SDS was dissolved in 1 ml HCl) was added to each well and incubated at 37°C for 4 hours. The insoluble formazon derivative was dissolved and absorbance at 570 nm was measured using a microplate reader (Awarness Technology INC). The results were expressed as cell numbers per control.

Statistical Analysis

Effect of the DMSO on each cell line was performed in three independent experiments (n=3) and the results were expressed as mean±SD. Statistical comparisons between groups were made by analysis of variance (ANOVA). $P<0.05$ was considered significant. Test of multiple comparison of Tukey was applied (5%) for statistically significant differences. For statistical analysis and graph making the software SPSS-16.0 and Excel 2003 were used respectively.

Results

Cytotoxic effect of DMSO on human leukemic THP1, U937, Jurkat and Molt-4 cells and mouse fibrosarcoma Wehi 164 cells in different concentrations and time intervals are illustrated in figures 1 to 4. In every figure, "A and B" indicate the results of trypan blue dye exclusion and MTT tests, respectively.

Cytotoxic Effect of DMSO on Human Leukemic THP1 Cells

DMSO significantly decreased proliferative response of human leukemic THP1 cells in both staining techniques in all time intervals dose-dependently ($P<0.05$, figures 1A and B). As shown, DMSO significantly diminished the proliferation of THP-1 cells at ≥2% after 24 hours incubation compared with untreated control cells ($P<0.05$). DMSO cytotoxicity at ≥2 % concentration, significantly increased with time in this order: 72h >48>24 h in THP-1 cells ($P<0.05$, figure 1).

Cytotoxic Effect of DMSO on Human Leukemic U937 Cells

According to the results depicted in figure 2A and 2B, DMSO significantly reduced proliferation of human leukemic U937 cells in both staining methods in all time intervals in a dose dependent manner ($P<0.05$). The results displayed in figure 2(A and B) showed that DMSO significantly decreased the proliferation of U937 cells at ≥2 % after 24 hours' incubation in comparison

[Downloaded from ijbc.ir on 2024-03-29]

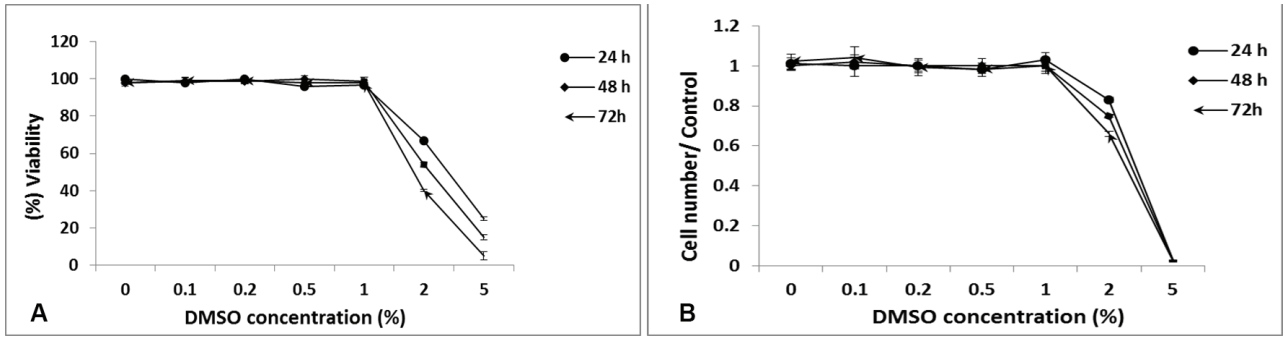


Figure 1: Effect of DMSO on proliferation of human leukemic THP1 cells (A) and (B). The THP1 cells (2×10^4 cell/well) were treated with different concentrations of DMSO (0.1, 0.2, 0.5, 1, 2 and 5%) for 24, 48 and 72 hours. The results are presented as % of viability demonstrated by trypan blue dye exclusion (TB) test (A) and cell number/control demonstrated by MTT assay (B). Data are mean±SEM of three independent experiments. *P<0.05 was considered significant

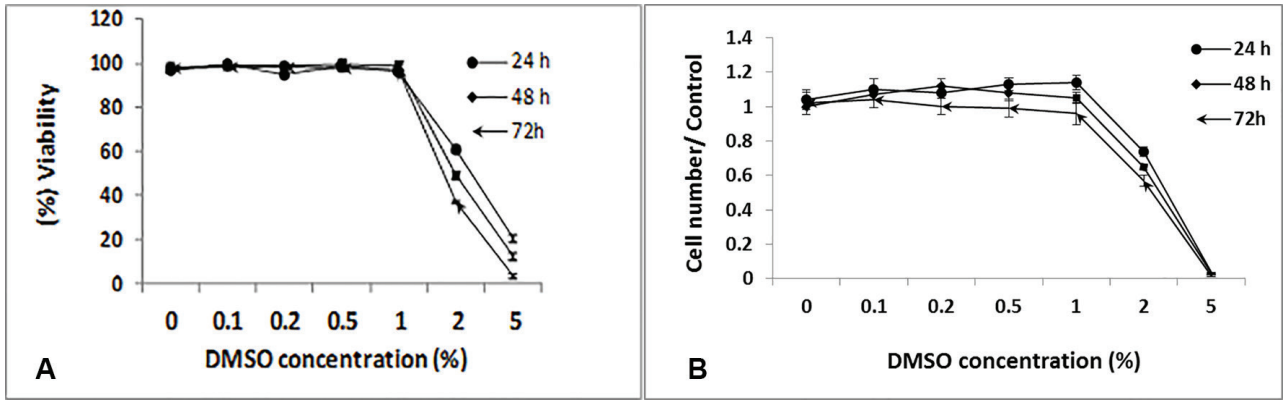


Figure 2: Effect of DMSO on proliferation of human leukemic U937 cells (A) and (B). The U937 cells (2×10^4 cell/well) were treated with different concentrations of DMSO (0.1, 0.2, 0.5, 1, 2 and 5%) for 24, 48 and 72 hours. The results are presented as % of viability demonstrated by trypan blue dye exclusion (TB) test (A) and cell number/control demonstrated by MTT assay (B). Data are mean±SEM of three independent experiments. *P<0.05 was considered significant

with untreated control cells (P<0.05).

DMSO cytotoxic effect at ≥2 % concentration, significantly augmented with time in this order: 72h>48h>24 h in U937 cells (P<0.05, figure 2).

Cytotoxic Effect of DMSO on Human Leukemic Jurkat Cells

DMSO significantly decreased proliferation of human leukemic Jurkat cells in every staining method in all time intervals dose-dependently (P<0.05, figure 3A and 3B). As shown, DMSO considerably diminished the proliferation of Jurkat cells at ≥2% after 24 hours incubation compared with untreated control cells (P<0.05). DMSO cell cytotoxicity at ≥2% concentration, significantly increased with time in this arrangement: 72h>48h>24 h in Jurkat cells (P<0.05, figure 3).

Cytotoxic Effect of DMSO on Human Leukemic Molt-4 Cells

DMSO significantly diminished proliferative response of human leukemic Molt-4 cells by both staining methods in all time intervals in a concentration-dependent manner (P<0.05, figure 4A and 4B). The results exhibited that DMSO significantly decreased the proliferation of Molt-4 cells at ≥2% after 24 hours' incubation compared with untreated control cells (P<0.05). DMSO cytotoxic effect at ≥2% concentration, significantly increased with time in this order: 72h>48h>24 h in Molt-4 cells (P<0.05, figure 4).

Discussion

In the present study, the effects of DMSO on proliferation of four human leukemic cell lines were evaluated. The results of this study showed that DMSO has a cytotoxic effect on the leukemic cells used in this study; dose and time-dependently. This cytotoxicity for all of these leukemic cells was shown at ≥2% concentrations of the DMSO after 24, 48 and 72 hours' incubation time. Moreover, there was not any significant difference between DMSO cytotoxicity in these different leukemic cell lines.

Justo and colleagues reported that DMSO had not any significant effect on proliferation of mouse peritoneal macrophages at ≥ 0.1%.[17] Similarly; in our study, DMSO did not show any cytotoxicity at 0.1% concentration on proliferation of leukemic cells. We also demonstrated that DMSO has no significant cytotoxicity on human leukemic cell lines at <2% concentration; however, Justo and co-workers had not studied the DMSO effect at >0.1% concentrations.[17] Furthermore, the decrease of clonogenic ability in some normal and leukemic cells by DMSO at >2% concentration has been shown in vitro.[24] Elisia and co-workers showed that DMSO significantly reduced monocyte viability at 2% concentration.[7] However, Elisia and colleagues used normal cells, but we assessed DMSO cytotoxicity effect on leukemic cell lines. DMSO cytotoxicity at less than 10% concentration has also been reported in vivo.[25]

[Downloaded from ijbc.ir on 2024-03-29]

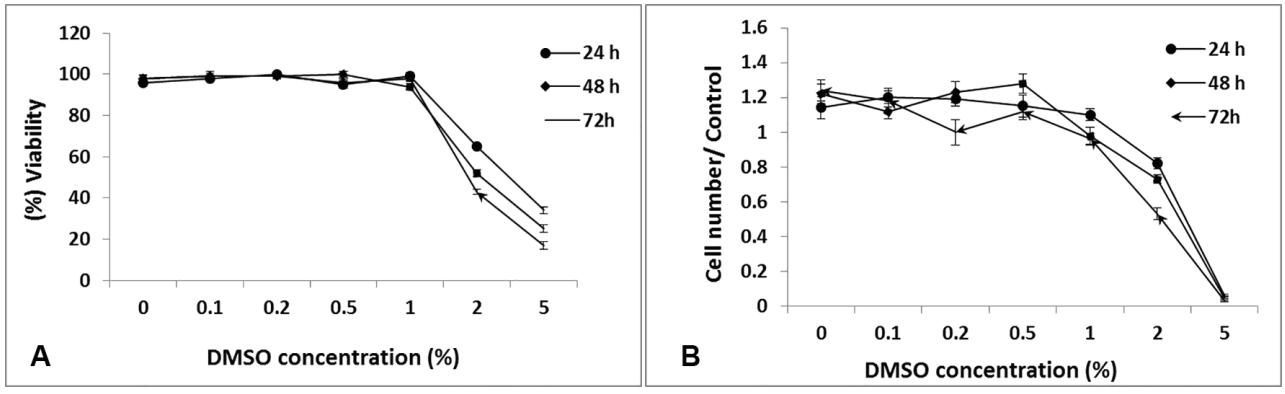


Figure 3: Effect of DMSO on proliferation of human leukemic Jurkat cells (A) and (B). The Jurkat cells (2×10^4 cell/well) were treated with different concentrations of DMSO (0.1, 0.2, 0.5, 1, 2 and 5%) for 24, 48 and 72 hours. The results are presented as % of viability demonstrated by trypan blue dye exclusion (TB) test (A) and cell number/control demonstrated by MTT assay (B). Data are presented as mean±SEM of three independent experiments. $P<0.05$ was considered significant

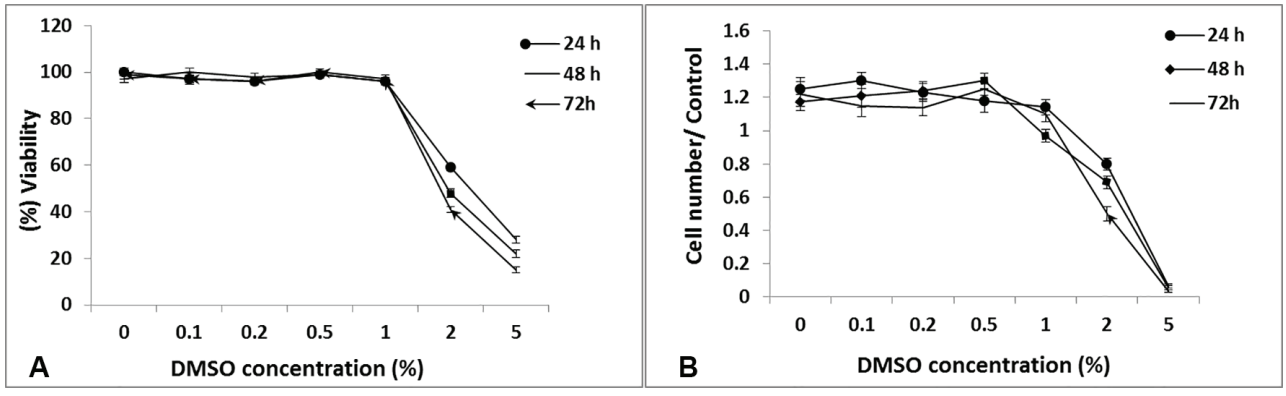


Figure 4: Effect of DMSO on proliferation of human leukemic Molt-4 cells (A) and (B). The Molt-4 cells (2×10^4 cell/well) were treated with different concentrations of DMSO (0.1, 0.2, 0.5, 1, 2 and 5%) for 24, 48 and 72 hours. The results are presented as % of viability demonstrated by trypan blue dye exclusion (TB) test (A) and cell number/control demonstrated by MTT assay (B). Data are presented as mean±SEM of three independent experiments. $P<0.05$ was considered significant

Antioxidant and anti-inflammatory properties of DMSO and inhibitory effects of DMSO on inflammatory cytokine secretion in autoimmune arthritis have also been shown.[5-7] As the monocytes and T-cells play a key role in inflammation,[26] the anti-inflammatory effects of DMSO may be partly due to its cytotoxic effects on these cells as was shown in our study. Additionally, there are numerous reports of antitumor effects of DMSO and induction of antitumor immunotherapy by DMSO.[8-10,12] The antitumor effects of DMSO might be in part owing to its direct cytotoxic effects on neoplastic cells as we showed in present study.

On the other hand, pretreatment by DMSO potentiated the toxic effect of cisplatin on sensory hair cells.[13] Furthermore, antitumor effects of two ruthenium (II)-DMSO-chalcone complexes has been described.[10,14] The augmentation of antitumor effects of some anticancer drugs by DMSO may probably result form its cytotoxic effects which acts synergistically in cancer therapy.

Consistent to our results, treatment of mouse hepatocellular carcinoma cell line with 2% DMSO, depressed cell proliferation.[12] However, in contrast to our study, there was no reduced cell viability.[12] The difference between our results and the mentioned study may be due to the type and number of the cells and the methods used for the assessment of cell viability. Jiang and colleagues used 1×10^5, 1×10^4 and 1×10^3 mouse hepatocellular carcinoma cell/well and used CCK-8 reagent to screen cell viability, while we used 2×10^4 cell/well of human leukemic cells and used trypan blue dye exclusion test for assessment of cell viability.

In our study, all of the used leukemic cell lines showed time-dependent sensitivity to DMSO at ≥2% concentrations. Taken together our findings suggest that DMSO might be a cytotoxic agent for leukemic cells. According to the results of the present study, DMSO might be a useful candidate in design of chemotherapeutic protocols for leukemia as well as other cancers.

However, additional studies such as evaluation of DMSO toxicity on normal and other tumor cells are required for further conclusions. As DMSO is commonly used as a solvent, it is noteworthy to investigate its toxicity on different cells at lower concentrations especially between 1% and 2% in various time intervals in-vitro to prevent inaccuracy in evaluating the characteristics (such as cytotoxicity) of other drugs/ agents which have been dissolved in DMSO.

Conclusion

DMSO shows cytotoxic effects on leukemic cells and might be a useful candidate in design of chemotherapeutic approaches for leukemia as well as other cancers.

[Downloaded from ijbc.ir on 2024-03-29]

Conflict of Interest: None declared.

References

1. Mi HY, Jing X, Salick MR, Cordie TM, Turng LS. Carbon nanotube (CNT) and nanofibrillated cellulose (NFC) reinforcement effect on thermoplastic polyurethane (TPU) scaffolds fabricated via phase separation using dimethyl sulfoxide (DMSO) as solvent. J Mech Behav Biomed Mater. 2016; 62:417-27. doi: 10.1016/j.jmbbm.2016.05.026.
2. Svalgaard JD, Haastrup EK, Reckzeh K, Holst B, Glovinski PV, Gørløv JS, et al. Low-molecular-weight carbohydrate Pentaisomaltose may replace dimethyl sulfoxide as a safer cryoprotectant for cryopreservation of peripheral blood stem cells. Transfusion. 2016; 56(5):1088-95. doi: 10.1111/trf.13543. PubMed PMID: 26991781.
3. Cheng CY, Song J, Pas J, Meijer LH, Han S. DMSO induces dehydration near lipid membrane surfaces. Biophys J. 2015; 109(2):330-9. doi: 10.1016/j.bpj.2015.06.011. . PubMed PMID: 26200868. PubMed Central PMCID: PMC4621616.
4. Dando R, Pereira E, Kurian M, Barro-Soria R, Chaudhari N, Roper SD. A permeability barrier surrounds taste buds in lingual epithelia. Am J Physiol Cell Physiol. 2015; 308(1):C21-32. doi: 10.1152/ajpcell.00157.2014. PubMed PMID: 25209263. PubMed Central PMCID: PMC4281669.
5. Liang C, Xue Z, Cang J, Wang H, Li P. Dimethyl sulfoxide induces heme oxygenase-1 expression via JNKs and Nrf2 pathways in human umbilical vein endothelial cells. Mol Cell Biochem. 2011; 355(1-2):109-15. doi: 10.1007/s11010-011-0844-z. PubMed PMID: 21533649.
6. Li YM, Wang HB, Zheng JG, Bai XD, Zhao ZK, Li JY, et al. Dimethyl sulfoxide inhibits zymosan-induced intestinal inflammation and barrier dysfunction. World J Gastroenterol. 2015; 21(38):10853-65. doi: 10.3748/wjg.v21.i38.10853. PubMed PMID: 26478676. PubMed Central PMCID: PMC4600586.
7. Elisia I, Nakamura H, Lam V, Hofs E, Cederberg R, Cait J, et al. DMSO Represses Inflammatory Cytokine Production from Human Blood Cells and Reduces Autoimmune Arthritis. PLoS One. 2016; 11(3): e0152538. doi: 10.1371/journal.pone.0152538. PubMed PMID: 27031833. PubMed Central PMCID: PMC4816398.
8. Koiri RK, Trigun SK. Dimethyl sulfoxide activates tumor necrosis factorα-p53 mediated apoptosis and down regulates D-fructose- 6-phosphate-2-kinase and lactate dehydrogenase-5 in Dalton's lympho- ma in vivo. Leuk Res. 2011; 35:950–6. doi: 10.1016/j.leukres.2010.12.029. PubMed PMID: 21269693.
9. Tan C, Hu S, Liu J, Ji L. Synthesis, characterization, antiproliferative and anti-metastatic properties of two ruthenium-DMSO complexes containing 2,2'-biimidazole. Eur J Med Chem. 2011; 46(5):1555-63. doi: 10.1016/j.ejmech.2011.01.074. PubMed PMID: 21354673.
10. Jovanovic KK, Gligorijevic N, Gaur R, Mishra L, Radulovic S. Anticancer activity of two ruthenium (II)-DMSO-chalcone complexes: Comparison of cytotoxic, pro-apoptotic and antimetastatic potential. J BUON. 2016; 21(2):482-90.
11. Hoang BX, Levine SA, Shaw DG, Tran DM, Tran HQ, Nguyen PM, et al. Dimethyl sulfoxide as an excitatory modulator and its possible role in cancer pain management. Inflamm Allergy Drug Targets. 2010; 9 (4):306-12. PubMed PMID: 20887267.
12. Jiang Z, Zhang H, Wang Y, Yu B, Wang C, Liu C, et al. Altered Hepa1-6 cells by dimethyl sulfoxide (DMSO)-treatment induce anti-tumor immunity in vivo. Oncotarget. 2016; 7(8):9340-52. doi: 10.18632/oncotarget.7009. PubMed PMID: 26824185. PubMed Central PMCID: PMC4891044.
13. Osman AM, Alqahtani AA, Damanhouri ZA, Al-Harthy SE, ElShal MF, Ramadan WS, et al. Dimethylsulfoxide excerbates cisplatin-induced cytotoxicity in Ehrlich ascites carcinoma cells. Cancer Cell Int. 2015; 15:104. doi: 10.1186/s12935-015-0258-1. PubMed Central PMCID: PMC4625967.
14. Chen ZF, Qin QP, Qin JL, Liu YC, Huang KB, Li YL, et al. Stabilization of G-quadruplex DNA, inhibition of telomerase activity, and tumor cell apoptosis by organoplatinum(II) complexes with oxoisoaporphine. J Med Chem. 2015; 58(5):2159-79. doi: 10.1021/jm5012484. PubMed PMID: 25650792.
15. Breitman TR, He RY. Combinations of retinoic acid with either sodium butyrate, dimethyl sulfoxide, or hexamethylene bisacetamide synergistically induce differentiation of the human myeloid leukemia cell line HL60. Cancer Res. 1990; 50(19):6268-73. PubMed PMID: 2400989.
16. Wang J, Lin D, Peng H, Huang Y, Huang J, Gu J. Cancer-derived immunoglobulin G promotes tumor cell growth and proliferation through inducing production of reactive oxygen species. Cell Death Dis. 2013; 4(12): e945. doi: 10.1038/cddis.2013.474. PubMed Central PMCID: PMC3877547.
17. Justo OR, Simioni PU, Gabriel DL, Tamashiro WM, Rosa Pde T, Moraes ÂM. Evaluation of in vitro anti-inflammatory effects of crude ginger and rosemary extracts obtained through supercritical CO2 extraction on macrophage and tumor cell line: the influence of vehicle type. BMC Complement Altern Med. 2015; 15:390. doi: 10.1186/s12906-015-0896-9. PubMed PMID: 26511466. PubMed Central PMCID: PMC4625945.
18. Iwatani M, Ikegami K, Kremenska Y, Hattori N, Tanaka S, Yagi S, et al. Dimethyl sulfoxide has an impact on epigenetic profile in mouse embryoid body. Stem Cells. 2006; 24(11):2549-56. doi: 10.1634/stemcells.2005-0427. PubMed PMID: 16840553.
19. Thaler R, Spitzer S, Karlic H, Klaushofer K, Varga F. DMSO is a strong inducer of DNA hydroxymethylation in pre-osteoblastic MC3T3-E1 cells. Epigenetics. 2012; 7(6):635-51. doi: 10.4161/epi.20163. PubMed PMID: 22507896. PubMed Central PMCID: PMC3398991.
20. Reis AH, Vargas FR, Lemos B. Biomarkers of

[Downloaded from ijbc.ir on 2024-03-29]

genome instability and cancer epigenetics. Tumour Biol. 2016; 37(10):13029-13038. doi: 10.1007/s13277-016-5278-5. PubMed PMID: 27468720.
21. Xu Y, Li X, Wang H, Xie P, Yan X, Bai Y, et al. Hypermethylation of CDH13, DKK3 and FOXL2 promoters and the expression of EZH2 in ovary granulosa cell tumors. Mol Med Rep. 2016; 14(3):2739-45. doi: 10.3892/mmr.2016.5521. PubMed PMID: 27431680.
22. Moldeus P, Hogberg J, Orrenius S. Isolation and use of liver cells. Methods Enzymol. 1978; 52:60-71. PubMed PMID: 672656.
23. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65(1-2):55-63. PubMed PMID: 6606682.
24. Su C, Allum AJ, Aizawa Y, Kato TA. Novel glyceryl glucoside is a low toxic alternative for cryopreservation agent. Biochem Biophys Res Commun. 2016; 476(4):359-64. doi: 10.1016/j.bbrc.2016.05.127. PubMed PMID: 27235553.
25. Galvao J, Davis B, Tilley M, Normando E, Duchen MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. FASEB J. 2014; 28(3):1317-30. doi: 10.1096/fj.13-235440. PubMed PMID: 24327606.
26. Taleb S. Inflammation in atherosclerosis. Arch Cardiovasc Dis. 2016; 109(12): 708–715. doi: 10.1016/j.acvd.2016.04.002. PubMed PMID: 27595467.

[Downloaded from ijbc.ir on 2024-03-29]