

<https://doi.org/10.15407/exp-oncology.2024.02.174>

**SITI NAZIHASMA HASSAN**<sup>1, 3</sup> **FARIZAN AHMAD**<sup>1,2,3,\*</sup>

<sup>1</sup> Department of Neurosciences, School of Medical Sciences,  
Universiti Sains Malaysia, Kubang Kerian Kelantan, Malaysia

<sup>2</sup> Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia,  
Kubang Kerian Kelantan, Malaysia

<sup>3</sup> Hospital Universiti Sains Malaysia, Kubang Kerian Kelantan, Malaysia

\* Correspondence: Email: farizan@usm.my

## CONSIDERING DIMETHYL SULFOXIDE SOLVENT TOXICITY TO MAMMALIAN CELLS AND ITS BIOLOGICAL EFFECTS

Dimethyl sulfoxide (DMSO) is a common solvent in biological and medical research for dissolving water-insoluble compounds and drugs. However, the impact of DMSO goes beyond its primary function. High-throughput and *in vitro* assays have uncovered various effects of DMSO in mammalian cells. The present article highlights the biological effects of DMSO on normal and cancerous mammalian cells.

**Keywords:** dimethyl sulfoxide, mammalian cells, cytotoxic effects, biological changes, molecular changes, cell behavior.

Dimethyl sulfoxide (DMSO) is an amphipathic organic compound that has been widely used for the dissolution of polar and nonpolar agents. DMSO is a proficient solvent or vehicle with water-mixability, hydrophobic barrier crossing, and enhanced lipid membrane permeability [1, 2]. On top of that, DMSO is commonly used as an intracellular cryoprotectant for mammalian cells.

DMSO is regarded as relatively low-toxic in cell culture applications. It is thought to be non-cytotoxic to both normal and cancerous mammalian cells at concentrations of less than or equal to 0.1%. Exposure levels up to 1.0% are considered acceptable if there is no discernible impact on cell morphology and proliferation patterns relative to the control group. Notably, evidence from the literature

reveals that multiple cell types are susceptible to DMSO-induced cytotoxicity to varying degrees [3–16].

In most cases, cytotoxicity involves mitotic arrest and cell death execution via necrosis (accidental cell death) or apoptosis (programmed cell death). A decrease in the number of viable cells is concentration- and time-dependent. For example, in human embryonic stem cell-derived embryoid bodies (hEBs) and human foreskin-derived mesenchymal stem cells (HF-MSCs), the decrease in cell viability is accompanied by an increase in cell death following exposure to DMSO (0.01%, 0.1%, and 1.0%/24–48 h) [7]. The viability of goat skin fibroblasts was unaffected at 0.01% and 0.1% DMSO for 96 h, but none survived at 5.0%, 7.0%, and 9.0% [8].

**Citation:** Hassan Siti Nazihahasma, Ahmad Farizan. Considering dimethyl sulfoxide solvent toxicity to mammalian cells and its biological effects. *Exp Oncol.* 2024; 46(2): 174-178. <https://doi.org/10.15407/exp-oncology.2024.02.174>

© Publisher PH «Akademperiodyka» of the NAS of Ukraine, 2024. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Conversely, surviving human peripheral blood mononuclear cells (PBMCs) and adult human dermal fibroblasts (HDFa) were observed with 5.0% DMSO over 120 and 168 h, respectively [4, 6]. Besides, DMSO exhibited no toxicity to human PBMCs at concentrations up to 10.0% but only for a short exposure (1 h) [14]. Table 1 shows the cytotoxic effects of DMSO on various mammalian cell types.

Moreover, DMSO impacts mammalian cell physiology in numerous ways. Explicitly, exposure to DMSO (0.05%/2–5 h) induced changes in the

intrinsic excitability of mice-derived neurons, resulting in a decrease in the action potential output [9]. T-cells are functionally affected by DMSO in a concentration- and time-dependent manner [14]. Besides, DMSO (1.0% and 5.0%/24 h) diminished the levels of protein expression for glutamate transporter 1 and glutamate-aspartate transporter in mouse cortical astrocytes [12]. In hEBs, the mRNA expression of markers associated with differentiation toward ectoderm, such as nestin, neuron-specific class III beta-tubulin, neurofilament heavy polypeptide, and keratin 15, was down-

**DMSO-induced cytotoxicity in mammalian cells**

| Type  | Duration (h)    | DMSO (%)                              | Significance  | Reference |
|---|-----------------|---------------------------------------|---|-----------|
| <b>Normal cells</b>   |                 |                                       |   |           |
| Human lymphocytes   | 120             | 0.5, 1.0, and 2.0                     | Cell proliferation  | [4]       |
| 3T3-L1 mouse adipocytes   | 1               | 0.1, 1.0, 10.0, and 100.0             | Cell proliferation, oxidative stress, apoptosis, necrosis | [5]       |
| HDFa  | 24, 48, and 168 | 2.0, 3.0, and 5.0                     | Cell proliferation  | [6]       |
| hEBs and HF-MSCs  | 24–48           | 0.01, 0.1, and 1.0                    | Cell proliferation, differentiation, and death†           | [7]       |
| Goat skin fibroblasts   | 96              | 0.5, 1.0, and 3.0                     | Cell proliferation  | [8]       |
| EA.hy926 human umbilical vein endothelial cells‡  | 120             | 0.2, 0.4, and 0.6                     | Cell proliferation, apoptosis                             | [11]      |
| Mouse cortical astrocytes   | 24              | 1.0 and 5.0                           | Cell proliferation, oxidative stress, apoptosis           | [12]      |
| Human PBMCs   | 168             | 0.2, 0.5, 2.0, 2.5, 3.0, and 5.0      | Cell proliferation and death†                             | [14]      |
| <b>Cancer cells</b>   |                 |                                       |   |           |
| Caco-2/TC7 human colon adenocarcinoma cells   | 2               | 20.0, 50.0, and 100.0                 | Cell proliferation and death†                             | [3]       |
| CL1-5 human lung adenocarcinoma cells   | 48, 72, and 96  | 0.1, 1.0, and 2.0*                    | Cell proliferation  | [10]      |
| Human glioblastoma cells  | 48 and 96       | 0.001, 0.01, 0.1, 1.0, and 10.0       | Cell proliferation  | [13]      |
| Jurkat, Molt-4, U937, and THP1 human leukemic cells   | 24, 48, and 72  | 2.0 and 5.0                           | Cell proliferation  | [15]      |
| <b>Normal/Cancer cells</b>  |                 |                                       |   |           |
| RAW-264.7 mouse macrophage cells, human umbilical vein endothelial cells, and MCF-7 human breast cancer cells | 24              | 0.1, 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 | Cell proliferation  | [16]      |
| Human normal fibroblasts and MDA-MB-231 human breast cancer cells‡  | 24              | 2.0                                   | Cell proliferation  | [17]      |

Note: †The mode of cell death is not specified; ‡G0/G1 cell cycle arrest upon 24 h exposure; \*DMSO (2.0%) also inhibits CL1-5 cell proliferation upon 24 h exposure.

regulated and unchanged in response to DMSO (1.0%/24–48 h) and (0.01% and 0.1%/24–48 h), respectively. On the other hand, DMSO (0.1% and 1.0%/24–48 h) diminished the mRNA levels of the stem cell marker NANOG [7]. Likewise, NANOG mRNA expression in induced human pluripotent stem cells markedly decreased after 24 h of exposure to 1.0% or 2.0% DMSO [17]. Notably, DMSO (0.1%/2–336 h) induced transcriptomic and proteomic changes in human cardiac and hepatic 3D microtissues (MTs) models [18]. In the study, approximately 60.7% and 62.9% of differentially expressed genes (DEGs) were downregulated in cardiac and hepatic MTs, respectively. DMSO (0.05%) also induced DEGs in HDFa, with more than 87.0% of genes upregulated upon 1 h and 24 h of exposure, while 58.0% and 42.0% of genes were up- and downregulated for 48 h, respectively [6].

In the Caco-2/TC7 monolayers, DMSO (2.0%, 4.0%, and 10.0%/2 h) did not induce cytotoxicity [3]. The primary glioblastoma stem-like cells, on the other hand, showed limited sensitivity to DMSO ranging from 0.001% to 1.0%, with no concentration- or time-dependent effects. However, a pronounced sensitivity was observed at 10.0%, with a comparable decrease in cell viability over 48 and 96 h [13]. Noticeably, DMSO inhibited the migration and invasion of CL1-5 lung cancer cells in a concentration- and time-dependent fashion, which was accompanied by an increase in the mRNA and protein expression levels of the tumor-suppressor human liver DnaJ-like protein (HLJ1) [10]. Furthermore, lower concentrations of DMSO ( $\leq 0.1\%$ ) enhanced the HLJ1 protein levels in the human lung cancer cell lines A549 and H1299, while pretreatment of CL1-5 cells with 1.0% and 2.0% DMSO for 24 h enhanced ultraviolet-induced apoptosis.

Notably, DMSO may counteract the effects of dissolved agents. For example, cisplatin in DMSO (10.0 mM) lost cytotoxicity against DLD-1 human colon cancer cells [19]. DMSO aqueous solutions (25.0% and 50.0%, v/v) also abrogated cisplatin cytotoxicity in the MDA-MB-231 breast cancer cells [17]. Rahul et al. [20] delineated that DMSO (0.1% and 0.3%) suppressed cisplatin cytotoxicity in the K562 human chronic myeloid leukemia cells. Furthermore, cisplatin dissolved in

DMSO decreased *in vitro* cytotoxicity and *in vivo* nephrotoxicity due to the formation of cisplatin-DMSO adduct [21]. On the other hand, DMSO (0.5%) potentiated the cytotoxicity of cisplatin in zebrafish sensory hair cells [22].

In a nutshell, the diverse effects of DMSO on mammalian cells challenge the perception of the solvent as biologically and pharmacologically inert. In considering whether it is normal or cancerous, both types of cells are susceptible to transcriptional-level responses. At its core, one possible way by which DMSO affects the cells is through its ability to modulate the membrane structure and function. These effects may contribute to the alterations in the intracellular signaling and pathways, transport processes, and, in turn, impact cellular behaviors, including differentiation, proliferation, and survival. In the context of cell toxicity, its molecular modes of action may be mediated by common mechanisms. For instance, treatment with DMSO induces mitochondrial damage, leading to the accumulation of reactive oxygen species and initiation of pathways to cell death through apoptosis, necrosis, and/or other modes (e.g., autophagy). This ultimately results in a diminution in cell viability. To some extent, the diminished cell viability does not necessarily indicate cell death but rather may result from inhibited cell proliferation and metabolism. All in all, different cell types may exhibit varying degrees of adaptation in the presence of DMSO, depending on the concentration and duration. Henceforth, for greater confidence and precision, one must incorporate the final concentration of DMSO in all assays.

### Declaration of Competing Interests

There are no competing interests to declare.

### Acknowledgments

The authors would like to thank FRGS (203/PPSP/6171203) provided by the Ministry of Higher Education of Malaysia; Postgraduate Funding Grant (311/PPSP/4404811) and Universiti Sains Malaysia Fellowship provided by the Universiti Sains Malaysia.

## REFERENCES

1. Notman R, Noro M, O'Malley B, Anwar J. Molecular basis for dimethylsulfoxide (DMSO) action on lipid membranes. *J Am Chem Soc.* 2006;128(43):13982-13983. <https://doi.org/10.1021/ja063363t>
2. Capriotti K, Capriotti JA. Dimethyl sulfoxide: history, chemistry, and clinical utility in dermatology. *J Clin Aesthet Dermatol.* 2012;5(9):24-26. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3460663>
3. Da Violante G, Zerrouk N, Richard I, et al. Evaluation of the cytotoxicity effect of dimethyl sulfoxide (DMSO) on Caco2/TC7 colon tumor cell cultures. *Biol Pharm Bull.* 2002;25(12):1600-1603. <https://doi.org/10.1248/bpb.25.1600>
4. de Abreu Costa L, Henrique Fernandes Ottoni M, dos Santos M, et al. Dimethyl sulfoxide (DMSO) decreases cell proliferation and TNF- $\alpha$ , IFN- $\gamma$ , and IL-2 cytokines production in cultures of peripheral blood lymphocytes. *Molecules.* 2017;22(11):1789. <https://doi.org/10.3390/molecules22111789>
5. Dłudla PV, Jack B, Viraragavan A, et al. A dose-dependent effect of dimethyl sulfoxide on lipid content, cell viability and oxidative stress in 3T3-L1 adipocytes. *Toxicol Rep.* 2018;5:1014-1020. <https://doi.org/10.1016/j.toxrep.2018.10.002>
6. Moskot M, Jakóbkiewicz-Banecka J, Kloska A, et al. The role of dimethyl sulfoxide (DMSO) in gene expression modulation and glycosaminoglycan metabolism in lysosomal storage disorders on an example of mucopolysaccharidosis. *Int J Mol Sci.* 2019;20(2):304. <https://doi.org/10.3390/ijms20020304>
7. Pal R, Mamidi MK, Das AK, Bhonde R. Diverse effects of dimethyl sulfoxide (DMSO) on the differentiation potential of human embryonic stem cells. *Arch Toxicol.* 2012;86:651-661. <https://doi.org/10.1007/s00204-011-0782-2>
8. Singh M, McKenzie K, Ma X. Effect of dimethyl sulfoxide on in vitro proliferation of skin fibroblast cells. *J Biotech Res.* 2017;8:78-82. <https://www.btsjournals.com/volume-8>
9. Tamagnini F, Scullion S, Brown JT, Randall AD. Low concentrations of the solvent dimethyl sulphoxide alter intrinsic excitability properties of cortical and hippocampal pyramidal cells. *PLoS One.* 2014;9(3):e92557. <https://doi.org/10.1371/journal.pone.0092557>
10. Wang C-C, Lin S-Y, Lai Y-H, et al. Dimethyl sulfoxide promotes the multiple functions of the tumor suppressor HLJ1 through activator protein-1 activation in NSCLC cells. *PLoS One.* 2012;7(4):e33772. <https://doi.org/10.1371/journal.pone.0033772>
11. Yi X, Liu M, Luo Q, et al. Toxic effects of dimethyl sulfoxide on red blood cells, platelets, and vascular endothelial cells in vitro. *FEBS Open Bio.* 2017;7(4):485-494. <https://doi.org/10.1002/2211-5463.12193>
12. Yuan C, Gao J, Guo J, et al. Dimethyl sulfoxide damages mitochondrial integrity and membrane potential in cultured astrocytes. *PLoS One.* 2014;9(9):e107447. <https://doi.org/10.1371/journal.pone.0107447>
13. Herbener VJ, Burster T, Goreth A, et al. Considering the Experimental Use of Temozolomide in Glioblastoma Research. *Biomedicines.* 2020;8(6):151. <https://doi.org/10.3390/biomedicines8060151>
14. Klooverpris H, Fomsgaard A, Handley A, et al. Dimethyl sulfoxide (DMSO) exposure to human peripheral blood mononuclear cells (PBMCs) abolish T cell responses only in high concentrations and following coincubation for more than two hours. *J Immunol Methods.* 2010;356(1-2):70-78. <https://doi.org/10.1016/j.jim.2010.01.014>
15. Hajjhasemi F, Tajic S. Assessment of cytotoxicity of dimethyl sulfoxide in human hematopoietic tumor cell lines. *Iran J Blood Cancer.* 2017;9(2):48-53. <http://ijbc.ir/article-1-719>
16. Jamalzadeh L, Ghafoori H, Sariri R, et al. Cytotoxic effects of some common organic solvents on MCF-7, RAW-264.7 and human umbilical vein endothelial cells. *Avicenna J Med Biochem.* 2016;4(1):e33453. <https://www.sid.ir/paper/343031>
17. Kuroda K, Komori T, Ishibashi K, et al. Non-aqueous, zwitterionic solvent as an alternative for dimethyl sulfoxide in the life sciences. *Commun Chem.* 2020;3:163. <https://doi.org/10.1038/s42004-020-00409-7>
18. Verheijen M, Lienhard M, Schrooders Y, et al. DMSO induces drastic changes in human cellular processes and epigenetic landscape in vitro. *Sci Rep.* 2019;9:4641. <https://doi.org/10.1038/s41598-019-40660-0>
19. Hall MD, Telma KA, Chang K-E, et al. Say no to DMSO: dimethylsulfoxide inactivates cisplatin, carboplatin, and other platinum complexes. *Cancer Res.* 2014;74(14):3913-3922. <https://doi.org/10.1158/0008-5472.CAN-14-0247>
20. Raghavan R, Cheriyaundath S, Madassery J. Dimethyl sulfoxide inactivates the anticancer effect of cisplatin against human myelogenous leukemia cell lines in in vitro assays. *Indian J Pharmacol.* 2015;47(3):322-324. <https://doi.org/10.4103/0253-7613.157132>
21. Fischer SJ, Benson LM, Fauq A, et al. Cisplatin and dimethyl sulfoxide react to form an adducted compound with reduced cytotoxicity and neurotoxicity. *Neurotoxicology.* 2008;29(3):444-452. <https://doi.org/10.1016/j.neuro.2008.02.010>
22. Uribe PM, Mueller MA, Gleichman JS, et al. Dimethyl sulfoxide (DMSO) exacerbates cisplatin-induced sensory hair cell death in zebrafish (*Danio rerio*). *PLoS One.* 2013;8(2):e55359. <https://doi.org/10.1371/journal.pone.0055359>

Submitted: March 20, 2022  
Resubmitted: April 30, 2024

С. Н. Хассан<sup>1,3</sup>, Ф. Ахмад<sup>1,2,3</sup>

<sup>1</sup> Відділ нейробиології, медичний факультет,  
державний університет Малайзії, Кубанг Керіан Келантан, Малайзія

<sup>2</sup> Центр досліджень геному людини, медичний факультет,  
державний університет Малайзії, Кубанг Керіан Келантан, Малайзія

<sup>3</sup> Університетський госпіталь, державний університет Малайзії,  
Кубанг Керіан Келантан, Малайзія

#### ДО ПИТАННЯ ПРО ТОКСИЧНІСТЬ ДИМЕТИЛСУЛЬФОКСИДУ ДЛЯ КЛІТИН ССАВЦІВ ТА РЕАКЦІЇ КЛІТИН НА НЬОГО

Диметилсульфоксид (ДМСО) є загальноприйнятним розчинником для нерозчинних у воді речовин та ліків при проведенні медичних та біологічних досліджень. Однак ДМСО спричинює різноманітний вплив на клітини, що виявлено в багатьох дослідженнях *in vitro*. Біологічні ефекти ДМСО в нормальних та трансформованих клітинах стисло розглянуто в статті.

**Ключові слова:** диметилсульфоксид, клітини ссавців, цитотоксичні ефекти, біологічні зміни, молекулярні зміни, реакції клітин.