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Assessment of the Cytotoxic Effects of Dimethyl Sulfoxide (DMSO) on MCF-7 and HeLa Cell Line

Suharyani[1)], dan Bayu Putra Danan Jaya[2)]

[1]Laboratorium Biokimia, Biologi Molekuler dan Fisiologi, Fakultas Kedokteran Universitas Lampung, Jl. Soemantri Brojonegoro No. 1, Bandar Lampung, 35145
[2] Laboratorium Patologi Anatomi dan Histologi, Fakultas Kedokteran Universitas Lampung, Jl. Soemantri Brojonegoro No. 1, Bandar Lampung, 35145

E-mail: b4yupoetra@gmail.com

ABSTRACT

Cell culture studies on drugs and natural compounds are commonly employed for early compound screening, assessing their potential therapeutic and immunomodulatory effects in vitro. Many of these compounds have limited solubility in water and are typically dissolved in lipophilic solvents. Consequently, it is crucial to employ a biocompatible and non-toxic solvent for cell viability. Dimethyl sulfoxide (DMSO) is a widely utilized solvent in in-vitro testing. This study aims to investigate the effect of DMSO on the viability of HeLa and MCF-7 cell lines. The MTT method was employed to assess cytotoxic effects on the HeLa and MCF-7 cell lines. The results revealed that concentrations of 1% and 2% DMSO significantly reduced the viability of MCF-7 cells ($p<0.05$) compared to the control after 24 hours of incubation. Conversely, the viability of HeLa cell lines exposed to 1% and 2% DMSO concentrations did not significantly differ from the control after 24 hours. Thus, concentrations of 1% and 2% DMSO exhibited cytotoxicity towards MCF-7 cells, while no such effect was observed in HeLa cells.

Keywords: *Cell Viability, Cytotoxic, DMSO, HeLa, MCF-7*

ABSTRAK

Penelitian obat dan bahan alam pada kultur sel, sering dilakukan sebagai penapisan awal senyawa yang berpotensi memiliki efek terapeutik dan imunomodulator secara in vitro. Sebagian besar senyawa tersebut bersifat sulit larut dalam air, dan biasanya larut dalam pelarut yang bersifat lipofilik. Oleh karena itu, diperlukan pelarut yang biokompatibel dan tidak toksik terhadap sel. Salah satu pelarut yang banyak digunakan dalam pengujian secara in vitro adalah DMSO. Penelitian ini bertujuan untuk mengetahui pengaruh DMSO terhadap viabilitas sel line MCF-7 dan sel line HeLa. Pengujian dilakukan dengan cara memaparkan sel MCF-7 dan HeLa dengan DMSO pada kosentrasi 1% dan 2% selama 24 jam untuk mengetahui dosis yang paling aman. Efek sitotoksik pada sel line di uji menggunakan metode MTT. Hasil penelitian menunjukan adanya penurunan viabilitas sel MCF-7 yang signifikan ($p<0,05$) bila dibandingkan dengan kontrol, setelah diinkubasi dalam DMSO 1% dan 2% selama 24 jam. Paparan DMSO 1% dan 2% tidak berpengaruh signifikan terhadap viablitas sel HeLa ($p>0,05$). DMSO pada konsentrasi 1 dan 2% bersifat sitotoksik bagi sel MCF-7, namun tidak bagi sel HeLa.

Kata Kunci: *DMSO, HeLa, MCF-7, sitotoksik, viabilitas sel.*

INTRODUCTION

The identification of new anticancer drugs is crucial due to the severe side effects associated with some existing anticancer medications (Ediriweera et al., 2019). In vitro cell culture studies are commonly employed for the early screening of potential therapeutic compounds (van Tonder et al., 2015) and for evaluating the immunomodulatory effects of pharmaceuticals and natural products (Timm et al., 2013). Conducting cell culture research necessitates the use of a solvent that is compatible with the growth medium and non-toxic to the cells under investigation (Jamalzadeh et al., 2016). DMSO is frequently employed as an organic solvent in most in vitro cell culture studies due to its ability to dissolve and solubilize a wide range of active molecules, including polar and nonpolar compounds (Tuncer et al., 2018; Moskot et al., 2019). However, it is important to note that DMSO has been associated with potential cytotoxicity on cells (Nguyen et al., 2020; Galvao et al., 2014).

DMSO is frequently utilized in cryopreservation due to its high freezing temperature. It exhibits effective interaction with phospholipids, thereby enhancing the delivery of drug molecules across the cell membrane (Galvao et al., 2014). The impact of DMSO on cellular processes has been associated with alterations in critical cellular structures such as proteins and DNA (Sangweni et al., 2021). DMSO is generally regarded as a non-toxic solvent when used below a concentration of 10% (v/v) (Verheijen et al., 2019). Concentrations of DMSO ranging from 1.5% to 5%, and even up to 10% (v/v), are commonly applied to induce differentiation in various cell lines (Moskot et al., 2019), promote the formation of plasma membrane pores, and induce cell death (Galvao et al., 2014). Additionally, DMSO can affect cellular functions such as cell cycle progression and apoptosis (Kita et al., 2015).

A previous study demonstrated that the presence of 2% DMSO in the medium resulted in reduced growth and altered cell morphology (Vesey et al., 1991). In the Hep G2 cancer cell line, a concentration of 5% DMSO significantly decreased cell proliferation (Song et al., 2012). For intestinal permeation assays conducted with the enterocyte-like Caco-2 cell line, 1% DMSO was identified as the optimal concentration (da Violante et al., 2002). However, there is limited information available regarding the non-toxic and optimal concentration of DMSO for use in the MCF-7 and HeLa cell lines, which serve as models

for breast and cervix cancer, respectively. Hence, the objective of this study was to investigate the effects of DMSO on the viability of the HeLa and MCF-7 cell lines.

METHODS

Material

This study used Reagents Roswell Park Memorial Institute (RPMI)-1640 medium, antibiotic (streptomycin and penicillin), fetal bovine serum (FBS), trypan blue (TB), 3-[4, 5-dimethyl thiazol-2, 5-diphenyltetrazoliumbromide] (MTT) kit and dimethyl sulfoxide (DMSO).

Experimental Design

This study employed a randomized block design with two concentrations of DMSO treatment and three replications. The MCF-7 and HeLa cell lines were divided into two groups based on the duration of DMSO exposure: 24 and 48 hours. DMSO concentrations of 0%, 1% and 2% were used in the experiment. Each treatment was replicated three times.

DMSO Preparation

DMSO was diluted in RPMI-1640 medium and stored frozen at -20°C until required. Before use, the frozen DMSO was thawed at room temperature and further diluted to the desired concentration in the RPMI-1640 medium.

Cell Lines Preparation

The MCF-7 and HeLa, human cell lines, used in this study, were obtained from the cell culture and cytogenetics laboratory at the Faculty of Medicine, University of Padjadjaran. The cell lines were cultivated in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 0.2 U/mL bovine insulin, and 100 U/mL of antibiotics (streptomycin and penicillin). Subsequently, the cell lines were incubated at 37°C with 5% CO_2 in a humidified environment.

Cytotoxicity Assay

The cytotoxicity of DMSO on MCF-7 and HeLa cell lines was assessed using an MTT assay. Following two subcultures, cells with a density of 2×10^4 cells/well were seeded in 96-well plates and incubated at 37°C in a 5% CO_2 environment. After 24 hours, the RPMI-1640 medium was aspirated and replaced with a solution containing 1% and 2% DMSO, followed by incubation for 24 hours. Subsequently, 10 µL of MTT solution (5 mg/mL) was added to each well, and the plates were further incubated for 4 hours. Following the incubation period, the supernatant was carefully removed, and 100 µL of DMSO was added to solubilize the formazan crystals. The absorbance was measured at a wavelength of 570 nm using an Elisa Reader. All experiments were performed in triplicate (Jamalzadeh et al., 2016). Cell viability was calculated using the formula: (Absorbance of treated cells - blank / Absorbance of control cells - blank) × 100 (Meiyanto et al., 2008).

Data Analysis

The mean cell viability data at different DMSO concentrations were tested using One Way Anova, followed by the post hoc Least Significant Difference (LSD) test at α=5%.

RESULTS

In the present study, 1% and 2% DMSO concentration were selected to assess and compare the cytotoxic effect on MCF-7 and HeLa cancer cell lines following a 24-hour treatment. The results indicate that the concentration of DMSO influences the viability of MCF-7 and HeLa cell lines. Specifically, at the highest concentration tested, DMSO significantly reduced the viability of the MCF-7 cell line compared to the control cells ($p<0.001$). After a 24-hour incubation, the growth of the MCF-7 cell lines was inhibited by 71.57% in the presence of 2% DMSO compared to the control cell (Table 1).

Table 1 Cytotoxicity of DMSO on HeLa and MCF-7 Cancer Cells Line

Concentration DMSO (%) v/v	% cell viability (mean ± SD)			
	MCF-7 cell line	p-value	HeLa cell line	p-value
0%	100 ± 0.00		100 ± 0.00	
1%	90.21 ± 1.66		100 ± 7.32	
2%	86.87 ± 1.39	<0.05*	100 ± 5.59	>0.05

*significantly differ based on the One Way Anova test at α 5%.

DMSO had different effects on the cervical cancer HeLa cell line. Treatment of HeLa cells with 1% and 2% DMSO did not decrease cell viability. There was no statistically significant difference in mean cell viability between HeLa cells treated with DMSO and control cells (p>0.05).

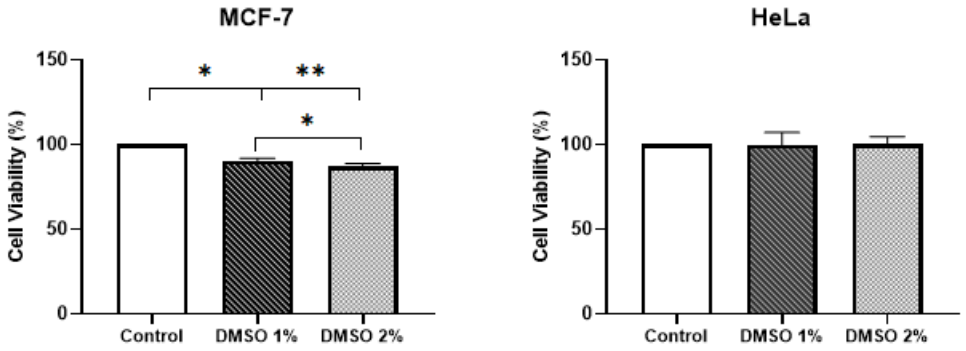

Figure 1. Comparison of MCF-7 and HeLa cell viability exposed to DMSO at 0%, 1% and 2% for 24 h. Data are presented as mean ± SD of the triplicate experiment.

DISCUSSION

This study demonstrates that increasing concentrations of DMSO significantly decrease cell viability in the MCF-7 cell line compared to the control group. These findings align with a previous study Nguyen et al. (2020) study, which observed a decrease in cell viability at DMSO concentrations exceeding 1%. Another study reported a 10% reduction in cell growth with a 1.5% DMSO concentration in cell culture (Tuncer et al., 2018). Moreover, Costa et al. (2017) found that DMSO at 5% and 10% means concentration reduced the relative proliferation index and total lymphocyte count. Yuan et al. (2014)

observed that exposure of astrocyte cultures to 1% DMSO for 24 hours did not significantly affect cell viability, whereas a concentration of 5% significantly inhibited cell viability and induced apoptosis. Furthermore, Nikolau et al. (2016) demonstrated that treating HepG2 cells with 1% DMSO altered their metabolic phenotype, resembling primary human hepatocytes more closely.

In contrast to the MCF-7 cell line, exposure of the HeLa cell line to DMSO concentrations up to 2% for 24 hours did not lead to a significant decrease in cell viability. This finding aligns with a previous study by Forman et al. (1999), which demonstrated that DMSO exposure at concentrations above 2% exerts a cytotoxic effect on the HeLa cell line.

According to the existing literature, the effects of DMSO on cellular structure and membrane properties vary depending on its concentration. Mitochondrial membranes, which possess a unique lipid bilayer structure, are particularly vulnerable to various damaging factors. In the context of DMSO treatment, mitochondrial damage has been identified as a primary contributor to decreased in cell viability (Yuan et al., 2014). Furthermore, DMSO treatment has been associated with the accumulation of cells in the G1 phase, increased chromatin condensation, and decreased total cell count (Sharma et al., 1998).

CONCLUSION

The concentrations of 1% and 2% DMSO exhibited cytotoxicity towards MCF-7 cells, while no such effect was observed in HeLa cells.

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