EVALUATING THE EFFECT OF PRODUCTS EXTRACTED FROM PANAX BIPINNATIFIDUS SEEM ON MULTIPLE MYELOMA CELLS

Vu Thi Thu[1*], Pham Thi Bich[1], Pham Trong Kha[1], Ngo Thi Hai Yen[1]

[1]VNU University of Science

* Corresponding author,

E-mail address: vtthu2015@gmail.com

ABSTRACT

Panax bipinnatifidus Seem (PB) is a precious medicinal plant in Vietnam. Although known for its many biological activities, the impact of this herb on multiple myeloma has not been elucidated. ***Objective:*** *The study was conducted to evaluate the effects of total saponin extract from PB on the viability, reactive oxygen species (ROS), and mitochondrial cardiolipin content of multiple myeloma cells.* ***Methods:*** *Human myeloma cell line (KMS-20) cells were divided into 2 groups: control group and experimental group. In the control group, cells were grown in medium containing DMSO 0.05%. In the experimental group, cells were grown in medium containing total saponin extract (PB-TS) at different concentration ranges. Taxol is an anti-cancer drug used as a positive control in the study. Cell viability, ROS, and cardiolipin content were determined using suitable kits.* ***Results:*** *IC50 values of PB-TS for KMS-20 cells were 4.43±0.78 (µg/ml), higher than the IC50 value of Taxol (2.88±0.51 ng/ml). Besides, the CM-H_2DCFDA fluorescence density of the cell group treated with Taxol and PB-TS increased to 121.52 ± 5.16% and 134.27 ± 3.59% compared to the control group ($p<0.05$). Moreover, the NAO fluorescence density of the cell group treated with Taxol and PB-TS also decreased to 62.73±6.87% and 41.56± 8.52%, respectively ($p<0.05$ compared with the control).* ***Conclusion:*** *The results of the study showed that total saponin from PB exhibited strong toxicity to KMS-20 cells through stimulation of ROS production and reduction of mitochondrial cardiolipin content.*

Keywords: *KMS-20 cells, Panax bipinnatifidus Seem, cardiolipin, ROS.*

1. INTRODUCTION

Multiple myeloma is an incurable cancer of mature B lymphocytes [8] and accounts for about 10% of all blood cancers [6]. The disease also accounts for about 1% of cancer deaths and nearly 20% of deaths from hematological malignancies [2]. Although the understanding of the disease and the discovery of novel therapies have resulted in a significant increase in overall survival time, almost all patients relapse and eventually succumb to their condition [4]. In fact, many studies have shown that mitochondria play an important role in the development and progression of multiple myeloma [6, 9]. Therefore, the search for mitochondrial-targeting drugs or/and natural compounds to inhibit the growth of cancer cells has paid attention to scientists.

In Vietnam, Panax bipinnatifidus Seem (PB, Vu Diep ginseng) is a precious herb with identified biological activities such as anti-aging, neurological disorders, enhancing immunity, and improving cardiovascular system activity [14]. However, in multiple myeloma subjects, the role of Vu Diep ginseng as well as products extracted from Vu Diep ginseng have not yet been studied. Therefore, in this study, we evaluated the effects of total saponin extract from Panax Bipinnatifidus Seem on the human multiple myeloma cell line (KMS-20) by evaluating the rate of viable cells, the changes in mitochondrial inner membrane cardiolipin content and the production of ROS free radicals.

2. METHODS

2.1. Object

The KMS-20 cell line (ATCC® - USA) was donated by the Center for

Cardiovascular Metabolic Disease Research, Inje University, Korea. Ethanol extract and SVD total saponin extract were provided by Phenikaa University, Vietnam.

2.2. Materials

RPMI 1640 (RPMI, Gibco, USA); Fetal bovine serum (FBS, Gibco, USA); Penicillin-Streptomycin (PS, Gibco, USA); Phosphate buffered saline (PBS, Gibco, USA); MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide; Sigma, USA); Cell Counting Kit-8 (CCK-8, Dojindo, Japan); Dimethyl sulfoxide (DMSO, Sigma, USA); 10-Nnonyl acridine orange (NAO, excitation/emission: 495/519 nm, Invitrogen, USA); 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA, 495/5169 nm, Invitrogen, USA); Culture dishes 90x20 mm (SPL, Korea); 96-well black, glass bottom plates (SPL, Korea); CO_2 Incubator (Shellab, USA); and Microplate reader (Tristar, USA). The study was carried out at the Animal Cell Biotechnology Laboratory, Life Science Research Center, Faculty of Biology, VNU University.

2.3. Methods

2.2.1. Cell cultured and treatments

KMS-20 cells were grown in RPMI medium containing 10% FBS, and 1% of PS at 37°C with 5% CO_2. The culture medium was changed every 2-3 days. For drug treatment, KMS-20 cells were further transferred to 96-well at the density of 2.10^4 cells per well and three cells per group at 37°C, 5% CO_2. After 24 h, the cells were further divided into three groups:

+ The control group (RPMI, control): KMS-20 cells were continuously cultured in RPMI medium plus DMSO 0.05% for 48 h;

+ The positive control drug: KMS-20 cells were continuously grown for 48 h in new media containing Taxol at the range of concentration from 0.1 to 30 ng/ml;

+ The PB-TS group: KMS-20 cells were continuously grown for 48 h in new media containing PB-TS at the range of concentration from 0.1 to 50 µg/ml.

2.2.2. Measurement of cell viability

Cell viability was determined using CCK-8. KMS-20 cells were seeded at a density of 2.10^4 cells per well in 96-well plates. After different treatments, CCk-8 was added to each well with a ratio of 10% v/v at 37°C for 1.5 h as our previous study [12]. The optical density (OD) was determined at 450 nm using a microplate reader (Spectramax plus 384, Molecular Devices, USA). The live cell number in each well (3 wells per group) was expressed as a value relative to the normal control well. Experiments were repeated three times.

2.2.3. Measurement of reactive oxygen species

Reactive oxygen species (ROS) were measured by CM-H_2CMDCFDA fluorescence kits following the previous reports [13]. KMS-20 cells were seeded at a density of 2.10^4 cells per well in 96-well black, glass bottom plates. After treatments, the cells were stained with 5 µM NAO (ex/em: 495/516 nm) at room temperature for 30 min. After treatments, the cells were stained with either 0.1 µM NAO (ex/em: 495/519 nm) at room temperature for 30 min. The cells were washed twice with PBS before measuring fluorescence intensity [10]. The CM-H_2CMDCFDA intensity in each well was expressed as a percentage value relative to the normal control. There were three wells per group for each repeat. Experiments were repeated three times.

2.2.4. Measurement of mitochondrial cardiolipin

The mitochondrial cardiolipin content was measured by NAO fluorescence kits following the previous reports [13]. KMS-20 cells were seeded at a density of 2.10^4 cells per well in 96-well black, glass bottom plates. After treatments, the cells were stained with either 0.1 µM NAO (ex/em: 495/519 nm) at room temperature for 30 min. The cells were washed twice with PBS before measuring fluorescence intensity [10]. The NAO intensity in each well was expressed as a percentage value relative to the normal control. There were three wells per group for each repeat. Experiments were repeated three times.

2.2.5. Statistical Analysis

Data are presented as means ± Standard deviation (SD) by using Excel 2016, Origin 8.5. Statistical significance was evaluated by one-way analysis of variance followed by T-test. P<0.05 was considered to be a statistically significant difference.

3. RESULTS AND DISCUSSION

3.1. The toxicity of PB-TS on KMS-20 cells

The effect of PB-TS at different concentrations on KMS-20 cells was evaluated through the change in cell survival by CCK-8 kit. The results are illustrated in Figure 1, and Table 1.

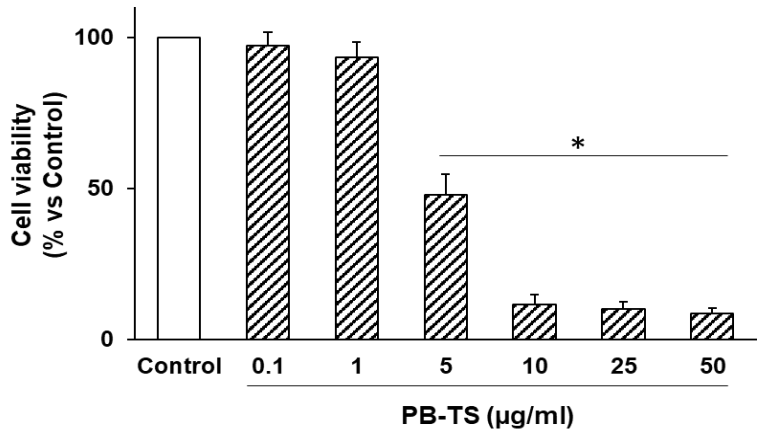

Figure 1. The cell viability of KMS-20 cells in medium supplemented with PB-TS

*Control: KMS-20 cells cultured in normal condition; PB-TS (tested compound): KMS-20 cells cultured in medium containing PB-TS at range of concentration from 0.1 to 50 µg/ml. *p<0.05 of Control*

Results from Fig.1 show the survival rate of cell groups supplemented with PB-TS at concentrations of 0.1 µg/ml (97.43±4.28%) and 1 µg/ml (93.31±5.27%) was not different from the Control group (p>0.05). However, this ratio in the group treated with PB-TS at concentration from 5 to 50 µg/ml decreased sharply to 46.21±7.04% at 5 µg/ml, and lowest at 8.06±1.63% at the dose of 50 µg/ml. The difference was statistically significant compared to the control group (p<0.05). This suggests that PB-TS exhibits strong cytotoxicity against KMS-20 cells. Based on this data, the IC50 value of PB-TS for KMS-20 cells was also determined and compared with the Taxol positive control. The results are shown in Table 1.

Table 1. IC50 value of PB-TS for KMS-20 cells

Compounds	IC50 value			
	1st	2nd	3rd	Mean ± SD
PB-TS (µg/ml)	4.80	3.53	4,97	4.43±0.78
Taxol (ng/ml)	2.95	2.34	3.26	2.88±0.51

The results from Table 1 show that the mean IC50 value of PB-TS for KMS-20 cells is 4.43±0.78 (µg/ml). This value is higher than the IC50 of Taxol (2.88±0.51 ng/ml), proving that PB-TS extract exhibits weaker toxicity to KMS-20 cells than Taxol. The results from Table 1 show that the mean IC50 value of PB-TS for KMS-20 cells is 149.49±1.98 (µg/ml). This value is higher than the IC50 of Taxol (0.89±0.14 µg/ml), proving that PB-TS extract exhibits weaker toxicity to KMS-20 cells than Taxol. However, this IC50 value shows that PB-TS has great potential in the treatment of multiple myeloma.

3.2. PB stimulates ROS production in KMS-20 cells

One of the pathways by which substances exhibit anticancer effects is by activating the apoptotic process in cancer cells, leading to cell death [7]. Many studies have shown that several signaling pathways promote cancer cell apoptosis by activating or inhibiting ROS production [5]. In this study, the impact of PB-TS and Taxol at IC50 value on ROS production was determined through the change in the CM-H_2DCFDA fluorescence signal. The results are presented in Fig 2.

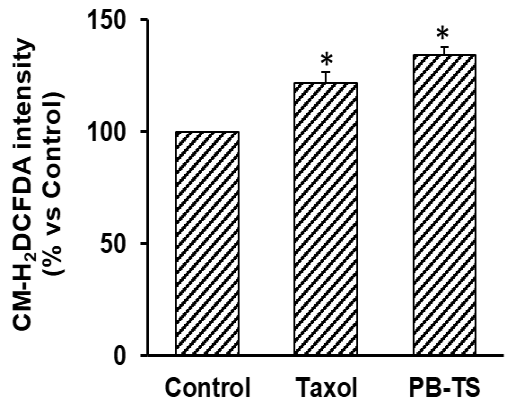

Figure 2. The CM-H_2DCFDA fluorescence intensity of KMS-20 cells under different conditions

*Control: KMS-20 cells cultured in normal condition; Taxol: KMS-20 cells cultured in medium containing Taxol 3 ng/ml; PB-TS: KMS-20 cells cultured in medium containing PB-TS 5 µg/ml; *p<0.05 of Control.*

Figure 2 shows that the CM-H_2DCFDA fluorescence density of reflecting ROS production of Taxol and PB-TS treated cells increased to 121.52 ± 5.17% and 134.27±3.59% compared with the Control group ($p<0.05$). This shows that, similar to Taxol, PB-TS stimulates ROS production in KMS-20 multiple myeloma cells. This result is consistent with the results on the percentage of living cells

above. Overproduction of ROS is responsible for the oxidation of macromolecules, which in turn activates a series of reactions that lead to cancer cell death [1].

3.3. PB-TS reduces the mitochondrial cardiolipin content of KMS-20 Cells

Cardiolipin is a phospholipid specific to the inner mitochondrial membrane, accounting for 13–15% of the total mitochondrial phospholipids. Depletion of cardiolipin content as well as structural changes lead to mitochondrial dysfunction. Cardiolipin is closely related to the opening of the inner mitochondrial membrane pore and leads to the release of cytochrome C, thereby stimulating apoptosis. Therefore, cardiolipin is the target of many studies to inhibit the growth of cancer cells [3]. In this study, the effects of PB-TS and Taxol on the mitochondrial cardiolipin content of KMS-20 cells were evaluated through changes in NAO fluorescence density. The results are shown in Figure 3.

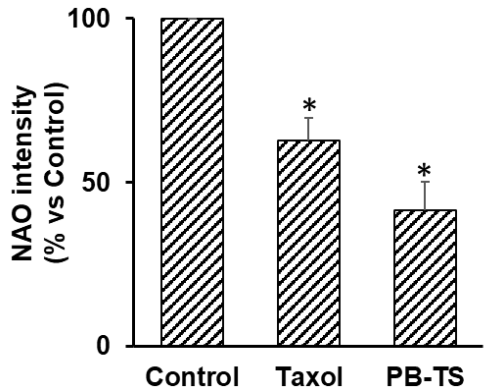

Figure 3. The NAO fluorescence intensity of KMS-20 cells under different conditions *Control: KMS-20 cells cultured in normal condition; Taxol: KMS-20 cells cultured in medium containing Taxol 3 ng/ml; P-TS: KMS-20 cells cultured in medium containing PB-TS 5 µg/ml; *p<0.05 of Control.*

The results in Figure 3 show that the KMS-20 cell group treated with Taxol and PB-TS had a fluorescence density of NAO reflecting a sharp decrease in the mitochondrial membrane cardiolipin content to 62.73±6.87 % and 41.56±8.52% ($p<0.05$ compared with Control). This result is consistent with the ROS content results above because stimulation of ROS production can cause increased cardiolipin oxidation, thereby reducing phospholipid content. A decrease in cardiolipin levels can lead to mitochondrial damage and cell death [15]. The effect of PB-TS is quite similar to the effect of Curcurmin and Naringin in the privious studies of Vu Thi Thu et al [11, 12]. In particular, Curcumin extracted from turmeric and Naringin extracted from citrus fruit peels have the ability to reduce mitochondrial membrane cardilipin, thereby limiting the growth ability of KMS-20 cells.

4. CONCLUSSION

The current findings indicate that PB-TS is potential to inhibit the growth of multiple myeloma cell by stimulating ROS production, and reducing cardiolipin levels. This might be the basis for further studies on the mechanism of action of PB-TS on multiple myeloma.

AKNOWLEDGEMENTS

We thank Dao Thi Thu Yen, Associate Professor Nguyen Huu Tung, Associate Professor K. H. Kyu and Professor H. Jin for their helps and support.

REFERENCES

1. **Liou, G.Y. and Storz P. (2010)**, "Reactive oxygen species in cancer". *Free Radic Res*. **44**(5): p. 479-96.
2. **Aisen, Y., Gatt M.E., Hertz R., et al. (2021)**, "Suppression of multiple myeloma by mitochondrial targeting". *Scientific Reports*. **11**(1): p. 5862.
3. **Dudek, J. (2017)**, "Role of Cardiolipin in Mitochondrial Signaling Pathways". *Frontiers in Cell and Developmental Biology*. **5**(90).
4. **Jöhrer, K. and Çiçek S.S. (2021)**, "Multiple Myeloma Inhibitory Activity of Plant Natural Products". *Cancers*. **13**(11): p. 2678-43.
5. **Nakamura, H. and Takada K. (2021)**, "Reactive oxygen species in cancer: Current findings and future directions". *Cancer Sci*. **112**(10): p. 3945-3952.
6. **Ortiz-Ruiz, A., Ruiz-Heredia Y., Morales M.L., et al. (2021)**, "Myc-Related Mitochondrial Activity as a Novel Target for Multiple Myeloma". *Cancers*. **13**(7): p. 1662-17.
7. **Ouyang, L., Shi Z., Zhao S., et al. (2012)**, "Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis". *Cell Prolif*. **45**(6): p. 487-98.
8. **Palumbo, A. and Anderson K. (2011)**, "Multiple Myeloma". **364**(11): p. 1046-1060.
9. **Song, I.S., Kim H.K., Lee S.R., et al. (2013),** "Mitochondrial Modulation Decreases the Bortezomib-resistance in Multiple Myeloma Cells". *Int J Cancer*. **133**(6): p. 1357-67.
10. **Thi Thu, V. and Yen N.T.H. (2021),** "Naringin Effectively Protects Cardiomyocytes Against Hypoxia/Reoxygenation Injury". **37**(3).
11. **Thu V.T, K.H.K., Long L.T, Thuy T.T, B. Nyamaa, P.T. Bich, N. Kim and J. Han (2016)**, "Curcuminoids suppress the development of KMS-20 cells via alternating mitochondrial function". *Vietnam Journal of Physiology*. **Vol. 20, No. 2**: p. 1-9.
12. **Thu, V.T. and Yen N.T.H. (2022)**, "Naringin Inhibits Multiple Myeloma Cells Proliferation by Altering Mitochondrial Function". **38**(2).
13. **Thu, V.T., Yen N.T.H., Tung N.H., et al. (2021)**, "Majonoside-R2 extracted from Vietnamese ginseng protects H9C2 cells against hypoxia/reoxygenation injury via modulating mitochondrial function and biogenesis". *Bioorganic & Medicinal Chemistry Letters*. **36**: p. 127814.
14. **Zhang, Y., Shi C., Liu C., et al. (2016)**, "Saponins from Panax bipinnatifidus Seem.: New strategy of extraction, isolation, and evaluation of tyrosinase inhibitory activity based on mathematical calculations". *J Chromatogr B Analyt Technol Biomed Life Sci*. **1039**: p. 79-87.
15. **Fiorucci, L., Erba F., Santucci R., et al. (2022)**, "Cytochrome c Interaction with Cardiolipin Plays a Key Role in Cell Apoptosis: Implications for Human Diseases". **14**(4): p. 767.