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by Upik Anderiani Miskad

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SUBMISSION ID	1481397544	CHARACTER COUNT	10995

Selective cytotoxicity Assay in anticancer drug of Melittin Isolated from Bee Venom (*Apis cerana indica*) to several human cell lines: HeLa, WiDr and Vero

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ABSTRACT

Melittin (MEL) is the main toxin constituent of bee venom isolated from *A. cerana*. It is a small basic peptide, consisting of a known amino acid sequence, with powerful anticancer activity. This research aims to determine selective cytotoxicity assay of MEL against several cell lines. The MEL can against HeLa, WiDr and Vero with IC₅₀ 2.54, 2.68 and 3.53 μ g/mL with SI 1.39 and 1.32, respectively. Our results suggest that MEL are promising anticancer agent but also no selective to normal cells.

KEY WORDS: melittin, cytotoxicity, selectivity index.

1. INTRODUCTION

Cancer has been one of the main causes of death in the world. The International Agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer. In 2012, there were 8.2 million people died because of cancer (Globocan, 2012). Nowadays drugs obtained from natural resources play a crucial role in the treatment of cancer, secondary metabolites and their derivatives have been applied to combat cancer (Newman, 2000). Various substances have been developed to cure cancer: alkylating substances, antimetabolite, radiomimetic medicines, hormone and antagonist substance (Nussbaumer, 2011; Wang, 2014). However, none of these substances provides a satisfactory impact and has no harmful side effect (Numico, 2015).

Therefore, there have been many research conducted about the compound found in the arthropods venom that has a big potential as anticancer agent (Ratcliffe, 2014). The research main result is related to active substance from arthropods venom that has anticancer activity (Hendriani and Gorini da Veiga, 2011).

Recent knowledge reveals the potential effect of bee venom and its substance such as melittin (MEL) that cause anti-tumor cytotoxic (Orsolich, 2012), immunomodulatory (Mohammadi, 2015), apoptosis effect to tumor cells differently *in vivo* or *in vitro* (Premratanachai and Chanchao, 2014), etc.

Bee venom is a highly complex blend of at least 18 active components, approximately 50% of its dry weight is MEL (Vogel, 1981). Recent report point to several effect of MEL: anti-proliferative and defined its mechanisms of action in cultured rat aortic vascular smooth muscle cell, effectively inhibited FBS-induced and platelet-derived growth factor BB-induced VSMC proliferation (Wang, 2006); anti-proliferative and pro-apoptotic effects against ovarian cancer (Jo, 2012); anti-cancer effect in prostate cancer cells through activation of caspase pathway via inactivation of NF- κ B (Park et al., 2011). Numerous of studies concerned with the various mechanisms of bee venom cytotoxicity on cancer cells, only very few reports are available on its effects on normal cells (Gajski, 2012).

Hence, this research examined the activity of anticancer compound from MEL that isolated from bee venom to several cell lines. Based on the data from this research, it is expected that this study will provide scientific contribution about the use of MEL as anti-cancer agent.

2. MATERIALS AND METHOD

MEL isolated from bee venom were purchased from Sigma-Aldrich (Sigma-Aldrich Ltd, Singapore Cat. No M2272 Sigma). Human cervical line (HeLa), colon cell line (WiDr) and normal African green monkey kidney epithelial (Vero) cell lines were obtained from Laboratory of Parasitology, Faculty of medicine, Gadjah Mada University, Yogyakarta, Indonesia. HeLa and WiDr cells were cultured in RPMI 1640, while Vero cells were cultured in M199. All media were supplemented with 10% (v/v) fetal bovine serum, 2% streptomycin-penicillin and 5% amphotericin. Cells were maintained in 25 cm flasks with 7 ml of media and were incubated in a 5% CO₂ incubator at 37°C. Cells were harvested using 0.25 trypsin-EDTA when they reach 80% confluence in culture flasks.

Cytotoxic Assay: Cytotoxic effect was determined by a rapid colorimetric assay, using 3-(4, 5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Briefly, 100 μ L cell suspension (10⁴ cell/well) seeded in a 96 well-plate (Iwaki), incubated in a CO₂ incubator at 37°C to allow the cells to adhere. After 24h, cells were treated with various concentration of MEL. MTT consists in the absorption of yellow tetrazolium salts by mitochondrial reductase of

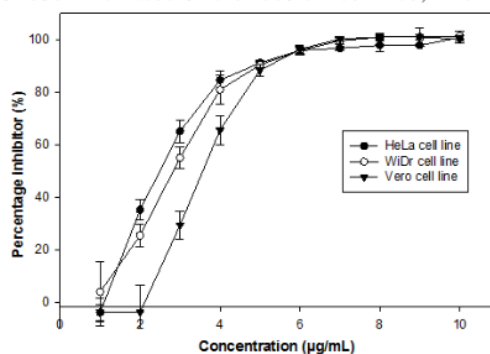
active cells, called formazan and accumulated in intracellular cell, is extracted by adding an organic solvent. SDS 10% was added to each of well to solubilize the MTT formazan. After incubate for 24 h at room temperature, the plates were read with an Elisa Reader (Bio-Rad) at 595 nm (Xu, 2010).

Percentage inhibition: Percentage inhibition (%) was calculated as: $\frac{\text{OD of untreated} - \text{OD of treated}}{\text{OD of untreated}} \times 100\%$

Selectivity index (SI): SI was calculated as follows: $\frac{\text{IC}_{50} \text{ in Vero cell}}{\text{IC}_{50} \text{ in cell lines}}$

3. RESULTS AND DISCUSSION

Cytotoxic effect by *in vitro*: Cytotoxic effect to cell lines is a general basic test for anticancer medicine and chemopreventive substance. The test was conducted using *Inhibitor Concentration* (IC_{50}) parameter through probity analysis to evaluate cytotoxic potency. One method that generally applied to test the cytotoxicity by *in vitro* is MTT assay. The examination principle is by reducing the yellow saline of tetrazolium MTT by reduction enzyme, succinate tetrazolium that entered the respiration chain on the living cell mitochondria and form the purple crystal formazan and did not dissolve in water. Adding stopper reagent could dissolve this colored crystal and its absorbance was measured using ELISA Reader on 595 nm. Based on the absorbance value, viability percentage was determined.



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Figure.1. Cytotoxicity effect of MEL on the growth of HeLa, WiDr and Vero cell lines were examined by MTT assay. Concentration response curves constructed in the range 1.00 – 10.00 µg/mL after 24 h (n= 3)

Figure.1 shows that correlation between MEL concentration and percentage mortality of cell. Applying at higher concentration the effect is increasing and the lower percentage of living cell found as well.

The IC_{50} were determined based on concentration that induced 50% inhibition on the growth of the treated cells as compared to the untreated cells in triplicate after 24 h treated.

Table.1. The IC_{50} of MEL against HeLa, WiDr and Vero cell lines

Cell lines	IC_{50} (µg/mL)	SI
HeLa	2.54	1.39
WiDr	2.68	1.32
Vero	3.53	

MEL has a strong cytotoxic effect and inhibit cell growth on cell lines for both cancer cell (HeLa, WiDr) and normal cell (Vero). The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bio-guided studies if it exerts an IC_{50} value <30 µg/mL (Suffnes and Pezzuto, 1990).

As the SI demonstrates the activity of MEL, the greater the SI value is, the more selective it is. An SI value less than 2 indicates general toxicity of the pure compound (Koch et al., 2005). Based on this, the SI data shown in Table 1 indicate that the MEL was non selective in HeLa and WiDr. This suggests its general toxicity to the cell.

The cytotoxicity effect of MEL has previously been attributed to both necrotic and apoptotic cell death. MEL induction apoptosis has recently been reported in hepatic (Li, 2006), and smooth muscle cells (Son, 2006). This induction of apoptosis was not directly attributed to MEL due to the extensive number of other molecules found in bee venom. MEL was found to induce necrosis of rat thymocytes (Shaposhnikova, 1997). MEL increase by releasing Ca release from endoplasmic reticulum and inducing Ca^{2+} influx. MEL also caused Ca^{2+} -dependent cytotoxicity in a concentration-dependent manner (Gonzalez, 1997).

4. CONCLUSION

MEL has a strong cytotoxic effect and inhibit HeLa and WiDr cell lines and on normal Vero cell, too.

5. ACKNOWLEDGMENTS

Directorate General of Higher Education via Post Graduate Scholarship of Hasanuddin University for the research funding to conduct this research.

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