

Melittin-Induced Cell Death Through p53 and 8-OHdG in Breast Cell Cancer MCF-7

Makkasau Plasay^{1,2*}, Rosdiana Natzir³, Muhammad Husni Cangara^{4,5}, Marhaen Hardjo³, Syahrijuita³ and Gita Vita Soraya³

¹Master of Biomedical Study Program, Post Graduate School, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

²Panakkukang College School of Health Sciences, Makassar, South Sulawesi, Indonesia.

³Department of Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

⁴Department of Anatomical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

⁵Installation of Anatomical Pathology, Hasanuddin University Hospital, Makassar, South Sulawesi, Indonesia.

*Corresponding Author E-mail: makkasau_mkes@yahoo.co.id

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Melittin, one of the cytolytic peptides derived from bee venom, is a broad-spectrum efficacy candidate as an antibacterial, antifungal, and antitumor agent. This study demonstrates the cytotoxic effect of melittin isolated from *Apis mellifera* through the induction of p53 and 8-OHdG. The antiproliferative effect was evaluated against breast cell cancer MCF-7 via MTT assay, while the molecular mechanism of melittin on MCF-7 was assayed by p53 and 8-OHdG ELISA. With an IC₅₀ value of 5.86 µg/mL (very toxic), the cytotoxic impact inhibits MCF-7 cell proliferation in a dose-dependent manner. Significant ($p < 0.05$) elevations in the level of p53 and 8-OHdG were evident in the IC₅₀-treated cells compared to control. In conclusion, melittin may have considerable potential as a novel natural product-based for breast cancer.

Keywords: Cytotoxic; Melittin; MCF-7; 8-OHdG; p53.

Cancer is a huge threat to public health across the world. It is the top cause of death globally. Only in the United States (US), in 2019, about 891,480 (100%) cancer cases were recorded, with invasive breast cancer in women accounting for 268,600 (30%) of the cases and 48,100 cases of Ductal carcinoma in situ (DCIS), followed by 41,760 (15%) fatalities^{1,2}. On the Indonesia Health Profile (2018), breast cancer ranks second after cervical cancer³.

Breast cancer is caused by a p53 gene mutation. Various stressors can trigger the p53 response pathway, including anoxia, inappropriate expression of oncogenes (e.g., MYC), and damage to the integrity of the deoxyribonucleic acid (DNA). Tumor-suppressing p53 proteins control cell cycles and play an important role in maintaining DNA integrity^{2,4}. 8-hydroxy-2'-deoxyguanosin (8-OHdG), a biomarker of cancer risk associated with exposure to carcinogen

substances, can be discovered in DNA damaged and cut by Base Excision Repair⁵.

Breast cancer treatment by chemotherapy is an option that many cancer patients in Indonesia choose. However, chemotherapy agents tend to cause cancer cell resistance, which results in treatment failure in most cases⁶. A previous study found that melittin-MIL-2 reduced breast cancer lung metastasis. Combining melittin with a mutant interleukin 2 (IL-2) could be a viable technique for improving immune cell stimulation and anticancer effects. As a result, the melittin-MIL-2 fusion protein is a promising option for cancer immunotherapy⁷.

The goal of this study is to determine the potential of the melittin peptide from bee venom (*Apis mellifera*) in MCF-7 breast cancer cell culture as a chemotherapeutic agent by assessing the levels of p53 and 8-OHdG proteins.

MATERIALS AND METHODS

Material

Melittin was purchased from Sigma-Aldrich (Catalog No. M2272) and was isolated from honeybee venom, with a molecular weight of 2,846.46 g/mol. (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT), amphotericin B (fungizone), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin (Pen-Strep 10,000 U/mL), sodium dodecyl sulphate (SDS), and trypsin-EDTA were bought from Gibco.

Cell culture

MCF7 cells were obtained from Hasanuddin University Medical Research Center (HUMRC). The cells were maintained in DMEM supplemented with 10% FBS, 1% Pen-Strep, and 0.5% amphotericin B at a CO₂ incubator (5% CO₂ - 95% humidified air), 37°C. The medium with supplement was sterilized by aseptic filtration using a sterile polyethylene sulfone filter membrane (d = 0.22 µm) and kept at 4°C.

Cell viability assay

The viability of MCF-7 cells was measured using an MTT assay, as followed by Tanumihardja *et al.* (2020) with a slight modification⁸. In brief, the cells were seeded at 10⁵ cells/mL onto a 96-wellplate (Corning Life Science) and then incubated for 24 h. Un-attached cells were cleaned by washing, and 100 µL of

melittin in various concentrations (1.0–9.0 µg/mL) was added. while control only DMEM medium. MTT (0.5 mg/mL) was added after 24 h of culture, and the cells were incubated for another 4 h. The reaction was stopped by adding 10% SDS. In a microplate reader, the optical density (OD) of each well was measured at 595 nm. The percentage of viable cells was manually determined using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}} \times 100$$

The ten, twenty-five, and half-maximal inhibitory concentrations (IC₁₀, IC₂₅, and IC₅₀) were used to plot x-axis and y-axis and fit the data in a straight line.

Measurement of p53 and 8-OHdG

The cells were seeded at 2×10⁵ cells/mL onto 24-wellplate culture flash (Corning Life Science) and then allowed to stand at 37°C for 24 h. Then, 100 µL of melittin in the concentrations of IC₁₀, IC₂₅, and IC₅₀ were added, and the dishes were allowed to stand at 37°C for 24 h. The culture supernatants were gathered and centrifuged at 10,000 rpm for 3 min at 2-4°C for measurement of p53 and 8-OHdG production. The levels of p53 (Wuhan Fine Biotech, EH4062) and 8-OHdG (Bioassay Technology Laboratory Shanghai, E1436Hu) were measured in accordance with the guidelines given in the protocol kit.

Statistical analysis

The data are shown as means ± SD (standard deviation) for at least 3 independent experiments (n=3). Analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to interpretate the difference between groups, with a significance level of p < 0.05.

RESULTS

The anticancer effect of melittin on MCF-7 was evaluated through culture tetrazolium assay (MTT). Various concentrations of melittin were used, and the inhibitory concentrations were calculated from the dose–response curve. The results of the assay using multiple concentrations of melittin, including 1, 2, 4, 6, 8, and 9 µg/mL, are tabulated in Table 1. The findings of these tests showed that melittin suppressed MCF-7 cells in a dose-dependent way, similar to doxorubicin. As observed, the IC₅₀ value of melittin was 5.86

µg/mL. According to the US NCI, the criteria of cytotoxicity for melittin is very active ($IC_{50} \leq 20$ µg/mL)⁹.

Apoptotic cell death was confirmed and extended to p53 level. During apoptotic cell death,

Table 1. Toxicity effects of melittin against MCF-7 cell after 24 h of incubation

Concentration (µg/mL)	Inhibition (%)	
	Melittin	Doxorubicin
1	23.43 ± 2.09	37.49 ± 1.22
2	30.15 ± 2.31	50.56 ± 2.77
4	66.78 ± 6.62	56.88 ± 2.95
6	89.35 ± 0.64	66.45 ± 3.76
8	98.82 ± 0.34	72.40 ± 2.48
9	98.31 ± 0.50	75.79 ± 1.01
Linearity (R ²)	0.975	0.915
IC ₁₀	2.13	1.33
IC ₂₅	4.52	2.72
IC ₅₀	5.86	3.32

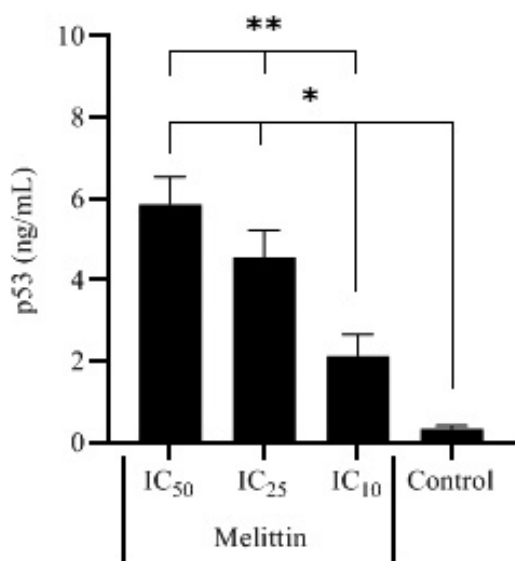


Fig. 1. Effects of melittin on p53 expression in MCF-7 cells. The cells were incubated with melittin in either culture medium at varying concentrations of IC₁₀, IC₂₅, and IC₅₀ or in culture medium alone for 24 h. Data were expressed as the mean ± SD (n=3). The difference between groups was evaluated using ANOVA followed by Dunnett's post hoc test, with a significance level of $p < 0.05$. Superscript (*) signifies the control well, while (**) signifies the IC₅₀

the level of p53 increases. The present study shows that melittin increases the expression of p53 tumor suppressor on MCF-7 cells (Fig 1). The expression of p53 was relatively increased as the concentration of melittin increased. A significant ($p < 0.05$) increase in the level of p53 by 16.25-fold in IC₅₀ compared to control was observed.

Moreover, we compared the level of 8-OHdG after using melittin in three different concentrations. As shown in Fig 2, melittin significantly increased ($P < 0.05$) the level of 8-OHdG in the treated cells by 2.56-fold in IC₅₀. Similarly, the level of p53 and 8-OHdG on MCF-7 cells varied in a dose-dependent manner.

DISCUSSION

The results of the cytotoxic MTT assay on melittin and doxorubicin against MCF-7 cells showed that the inhibitor concentration was below 100 µg/mL. This data is supported by a previous study (10). Melittin has a strong cytotoxic effect and inhibits HeLa and WiDr cell lines, as well as, normal Vero cells. In this study, melittin and doxorubicin showed a dose-dependent action,

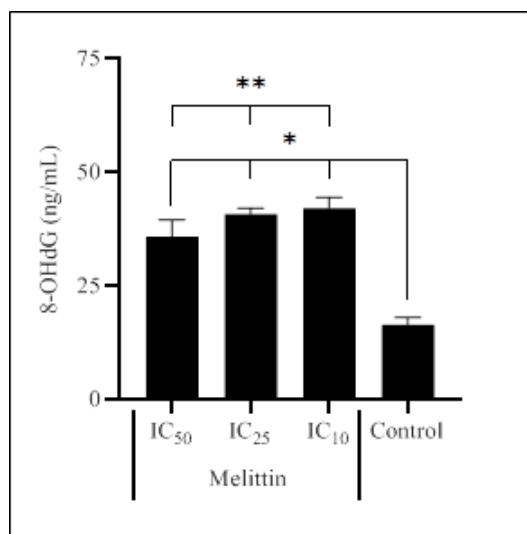


Fig. 2. Effects of melittin on 8-OHdG expressions in MCF-7 cells. The cells were incubated with melittin in either culture medium at varying concentrations of IC₁₀, IC₂₅, and IC₅₀ or culture medium alone for 24 h. Data were expressed as the mean ± SD (n=3). The difference between groups was evaluated using ANOVA followed by Dunnett's post hoc test, with a significance level of $p < 0.05$. Superscript (*) signifies the control well, while (**) signifies the IC₅₀

that is, the higher the concentration, the more the degree of MCF-7 cell apoptosis. This shows that the higher the IC_{50} 24 h value of a compound, the less is the toxicity and more viable cells; moreover, the number of crystals formed also increases. If the absorbance value obtained is very high, then cell death is low. Melittin isolated from *Apis cerana* indica bee venom showed a cytotoxic effect on HeLa, WiDr, and Vero cells after a 24 h-incubation period¹⁰. The tumor suppressor gene p53 is directly engaged in DNA damage-induced apoptosis production. Moreover, the p53 protein regulates the cell cycle and is essential for maintaining DNA integrity¹¹. It also protects the genome by regulating a range of DNA-damage-response (DDR) mechanisms. This protein is presumed to have begun directing the apoptotic death of genomically impaired cells early on during the metazoan evolution. It is a key facilitator of DNA repair, pausing the cell cycle to give the repair machinery enough time to restore genome integrity. Furthermore, p53 fulfills a variety of responsibilities in order to have a direct impact on the activity of numerous DNA-repair mechanisms. As a result, it appears that p53 performs several functions in order to defend against cancer formation by maintaining genome integrity¹².

Previous studies proved that melittin affected p53 expression in HeLa cells after administration of melittin and doxorubicin and observation for 24 h¹⁰. Melittin suppresses cell proliferation and migration of human bladder cancer cell lines T24 and 5637 via the phosphatidylinositol 3-kinase / Protein kinase B (PI3K/Akt) and tumor necrosis factor (TNF) signaling pathways¹³. This shows that both melittin and doxorubicin show a dose-dependent behavior; the higher the concentration administered, the higher the concentration of 8-OHdG in cell MCF-7. According to previous research in human peripheral blood cell, lymphocytes, melittin controls the mRNA expression of genes involved in the response to DNA damage, oxidative stress, and apoptosis¹⁴. The 8-OHdG is the best biomarker to determine DNA damage and is used for evaluating oxidative stress¹⁵.

This suggests that, in addition to causing strong antiproliferation in MCF-7 cells, melittin and doxorubicin also generate reactive oxygen species (ROS) caused by 8-OHdG. In human peripheral

blood, lymphocytes, melittin causes cytogenetic breakdown, oxidative stress, and gene expression changes. Furthermore, after melittin treatment, the production of ROS, loss of glutathione (GSH), and increase in malondialdehyde (MDA) levels, as well as DNA damaging effects and increased phospholipase C (PLC) activity, revealed that melittin acts through LPC and that oxidative stress is implicated in its genotoxic activity. What's more, we discovered that melittin influenced the expression of mRNA from genes involved in DNA damage response, oxidative stress, and apoptosis¹⁴. Future study should concentrate on protein expression and enzyme activity in order to fully comprehend the mechanism of MEL action, keeping in mind that, while melittin may be a promising medicinal agent through by modulating the oxidative stress and DNA damage.

CONCLUSIONS

Our research indicates that melittin is a key peptide ingredient of bee venom, which can enhance the breast tumor-killing ability of chemotherapeutics. These present data suggest that melittin regulates breast cancer by modulating p53 and 8-OHdG.

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Conflicts of interest

The authors declare no conflict of interest.

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