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The Lethal Effect of Honey Bee Venom on Human Ovarian Cancer Cisplatin Resistance Cell Line A2780cp

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Abstract. Ovarian cancer seems to be one of the most important causes of death between women in their elderly ages. Because of the vague symptoms most of the time diagnosis at the late stages and treatments become ineffective. Previous studies have shown the inhibitory effect s of Bee venom (BV) on some different cancerous cell lines. Therefore, in the resent investigation, the cytological effects of BV were targeted on ovarian cancerous Cisplatin resistance cell line (A2780cp). A2780cp cell line was incubated in RPMI-1640 with 10% Fetal Bovine Serum (FBS), and sufficient antibiotics for 24 hours for being enough confluent and then were treated with the different concentration of BV to determine IC_{50}. After several MTT assay, concentration of 8 µg/ml BV were considered as IC_{50}. Furthermore , the results of MTT assay showed that BV can inhibits the cell proliferation in low doses and cell death in higher doses, on the other hand results of flow cytometry did not demonstrate the significantly increase in Annexin-V expression at 8 and 16 µg/ml. Therefore, BV maybe induces cell death through necrosis.

Keywords: Bee venom, Ovarian cancer, necrosis, A2780cp cell line

1. Introduction

Ovarian cancer seems to be one of the most important causes of death from gynecological malignancy, with an estimated worldwide prevalence of 192.000 per year [1]. Due to unclear symptoms and signs, the majority of patients diagnose with the advanced stages. Treatment most of the time based on initial debunking surgery which followed by chemotherapy [2]. About 90% of cancers arise originally from ovaries with the unknown reason and the rest with hereditary background and mostly associated with breast and colon cancer [3]. Honey Bee Venom (BV) has been used as a traditional medicine to treat various diseases such as arthritis, rheumatism, backpain, cancerous tumors and skin diseases [7]. BV contains at least 18 active compounds, including Melittin (about 50% of dry weight) which it has the inhibitory effect of calmodulin as a antiproliferation agent of BV and also induces the hyperactivation of PLA2 activity and calcium influx in ras-transformed cells [5] also reported that melittin causes the increase incalpin activity and induces necrosis [6] . PhospholipaseA2 (PLA2, about 10-12% of dry weight), Apamin (the smallest neurotoxin in BV), Adolapin and MCD peptide and BV hyaluronidase.etc are the other compounds in BV [7, 8]. Based on the previous studies on BV, in this investigation, is focused on lethal effect of BV on A2780cp to find out cell death pathway.

2. Materials and Methods

2.1. Cell Culture and MTT Assay

Cell line A2780cp was purchased from NCBI (National Cell Bank of Iran) and cultured with RPMI1640 (Gibco-Invitrogene) with 10% FBS (fetal bovine serum, Gibco-Invitrogene) and antibiotic in 24 wells plate

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for 24 hours , then , were treated with different concentration of BV (BV were collected from Isfahan Province in IRAN) and then the result tested by MTT assay.

2.2. Flow Cytometry

In addition to MTT assay, the cell death in treated cells with BV was also studied using quantative method of Flow cytometry. Briefly, treated and non- treated cells were trypsinized and washed in PBS. After centrifuge (1500 rpm/5min) and aspirate supernatant, after washing, the samples incubated with 100 µl of Annexin-V primary antibody (diluated1:100 in blocking buffer BSA/PBS 3%) overnight at 4°C. The cells were washed with PBS and then centrifuged (2000 rpm/10min). The cells were incubated in the dark with 100 µl fluorescein-labeled goat anti-rabbit secondary antibody (diluted 1:100 in blocking buffer BSA/PBS 3%) for 45 min at 37°C. After washing with PBS and centrifuge (2000 rpm/10min), 500µl of 0.01% formaldehyde was added to each tube and then was measured immediately using a Becton Dickinson FACScan analyzer.

3. Results

3.1. Cell Morphology

The significant morphological difference between control group and treated cells was observed. After 24 hours treatment by BV wild spread cell lysis were seen (Figure 1).

3.2. MTT Assay

Based on MTT assay, the results of IC_{50} value of BV for A2780cp was determined 8µgr/ml.The data has shown in figure 2 indicates the dose depended cell death associated with BV for this cell line.

3.3. Flowcytometry

Using flow cytometry, was found that control group cells exhibited the low expression of Annexin-V (1.23%).However, flow cytometry indicated that the treated cells group did not display the significant increase in Annexin-v expression level(1.64% for 8µgr/ml and 3.92% for 16 µgr/ml).

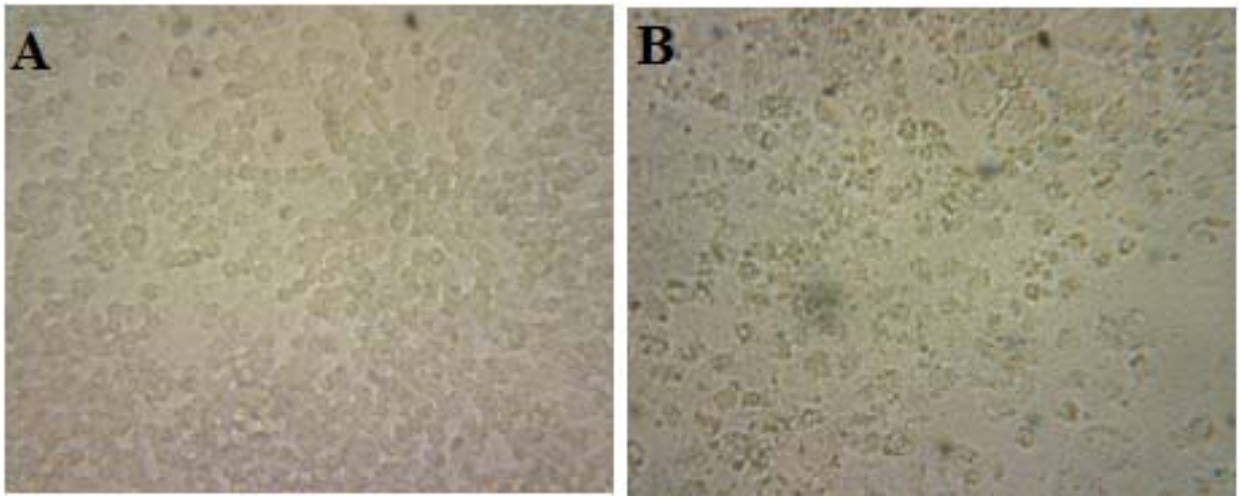

Fig. 1: Light microscope micrograph, Cell death of A2780 treated to 8 µgr/ml of BV (B) comparing with control sample (A) after 24 hours (400X).

4. Discussion

Ovarian cancer is the fifth cause of cancer- related death in women in their elderly ages [8].Honey bee venom possesses different active biological compound [9].The effectiveness of BV on cancerous tumor has been demonstrated in previous studies [10, 11] Melittin, a 26 amino acid, the major compound of BV and hecate-1, a 23 amino acid analog of melittin, which are cationic and amphipathic, might be desirable therapeutic peptides. A novel approach for the treatment of endocrine tumors possessing luteinizing hormone receptors (LHR) was developed. Melittin and a fragment of a melittin-conjugated hormone receptor (e.g., hecate) were shown to have an anti-tumor effect in ovarian and testicular tumors [7].

We hypothesized that BV can inhibit cell proliferation and induce cell death in A2780cp cell line. The present study clearly showed that cell viability is inhibited by BV. Also we found that BV inhibits A2780cp growth on dose-dependent manner. Also our results which are obtained from flowcytometry analyses to indicate that the type of cell death which is induced by BV was not apoptosis and it may be act against cell growth through necrosis. In conclusion, we suggest that BV can reduce the cell viability in Cisplatin resistance A2780cp cells and it might be a regulator of cell death in this kind of cancer.

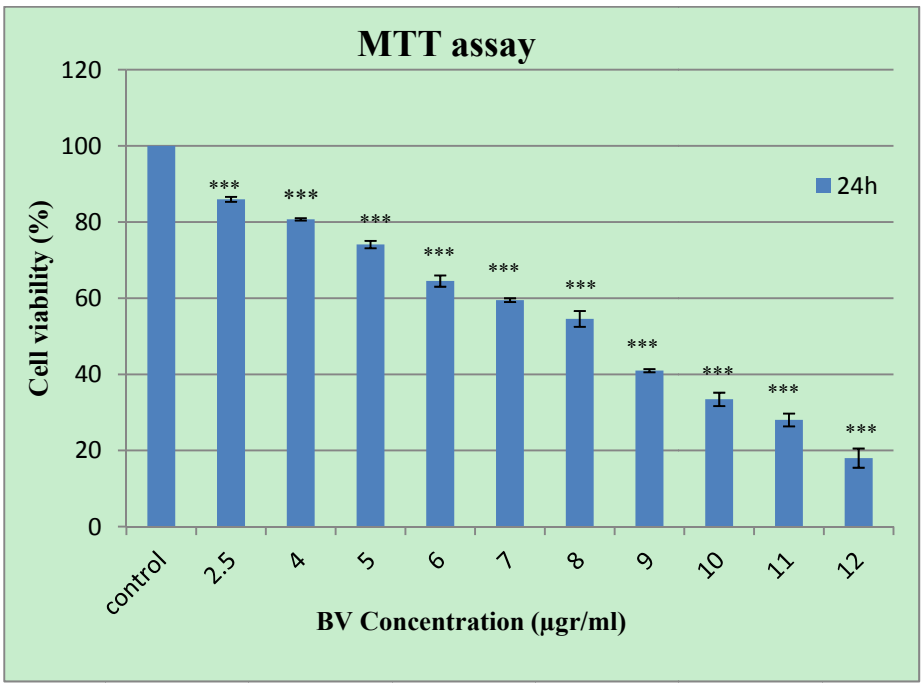

Fig. 2: The effect of BV on viability of A2780cp cell line. The cells were treated with different concentration of BV for 24 hours. The viability was then determined by MTT assay. Data shown are the mean of three independent experiments ±SEM and are statistically significant in comparison to the vehicle control by one-way ANOVA.

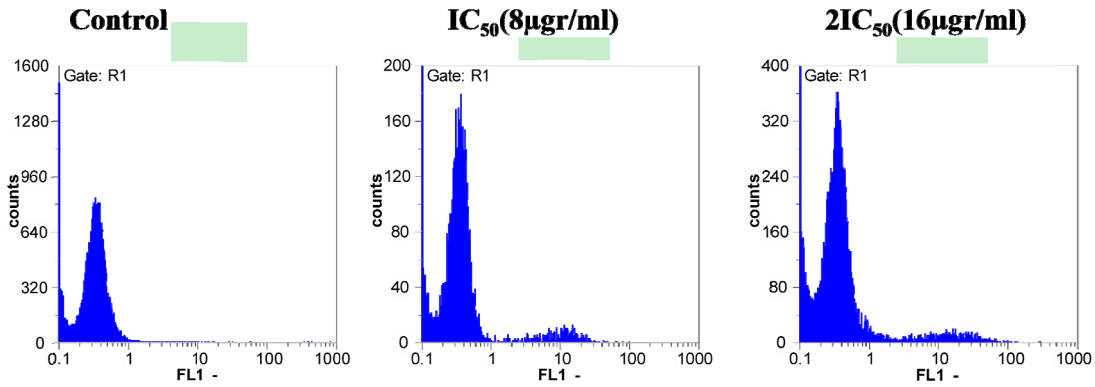

Fig. 3: Effect of BV on Annexin-V expression in A2780cp. After treatment with 8 and 16 µgr/ml of BV for 24 hours. Cell determined by flow cytometric analysis stained with Annexin-V primary antibody followed by FITC conjugated secondary antibody. Results are expressed as relative fluorescent intensity. Control group means the cell are not treated with BV.

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6. References

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