Review of: "Anti-metastasis After Bee Venom and Melittin by Upregulation of BRMS1 and DRG1 Genes, With Downregulation of WNT7B in Breast Cancer Cells"

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Potential competing interests: No potential competing interests to declare.

- The abstract is generally well-written with clear and coherent sentences.
- Consider revising the sentence: "Apitherapy as an alternative medicine is promised to deal with cancer." to "Apitherapy, as an alternative medicine, has shown promise in cancer treatment."
- In the sentence: "Selective cytotoxicity of bee venom and melittin were higher compared to cisplatin," it should be "was" instead of "were" since "selective cytotoxicity" is singular.
- In the sentence: "Melittin at 0.5 µg/ml was effective at 24h for anti-metastatic function whereas 4 µg/ml was significant in treatments with bee venom or cisplatin," consider rephrasing for clarity. For example, "Melittin at a concentration of 0.5 µg/ml demonstrated effectiveness in anti-metastatic function after 24 hours, whereas a concentration of 4 µg/ml showed significance in treatments with bee venom or cisplatin."
- In the sentence: "Melittin induced overexpression of BRMS1 and DRG1, however bee venom induced DRG1 and KAI1/CD82 expression in breast cancer cells," consider rephrasing for clarity. For example, "Melittin caused overexpression of BRMS1 and DRG1, while bee venom induced expression of DRG1 and KAI1/CD82 in breast cancer cells."
- In the sentence: "WNT7B was downregulated in bee venom-treated breast cancer cells," consider specifying the direction of downregulation (e.g., "WNT7B expression was downregulated").
- It would be helpful to briefly mention the significance of the identified anti-metastatic genes (BRMS1, DRG1, and KAI1/CD82) and the pro-metastatic genes (EGFR and WNT7B) in breast cancer.
- The abstract could benefit from briefly discussing the implications of the findings and their potential application in breast cancer treatment.
- Consider mentioning the sample size and any statistical analyses performed to support the observed gene expression changes.
- It would be useful to provide a brief rationale for comparing the effects of bee venom and melittin to cisplatin, a standard chemotherapy drug, in terms of their cytotoxicity and gene expression profiles.
- The introduction could also provide more background information on the anti-metastatic genes (BRMS1, DRG1, and KAI1/CD82) and the pro-metastatic genes (WNT7B and EGFR) mentioned in the study. This would help readers understand their significance in breast cancer and the rationale behind studying their expression levels.
- Experimental Section:
- In the first paragraph, it should be noted that "This should be noted" can be removed to improve the sentence structure.

- In the second paragraph, there is a repetition of the phrase "were used" in the sentence "Passage 10 and passage 14 were used for MDA-MB-231 and MCF10A cells respectively." It can be revised to "Passage 10 was used for MDA-MB-231 cells, and passage 14 was used for MCF10A cells."
- In the third paragraph, the sentence "Cells were incubated for 6, 24, 30, 48, 54, 72 and 96 hours during wound healing" can be rephrased as "Cells were incubated for 6, 24, 30, 48, 54, 72, and 96 hours for wound healing experiments."
- In the fourth paragraph, the sentence "Counterpart cells were left untreated" can be clarified by specifying which treatment group it refers to, for example, "Corresponding cells in the control group were left untreated."
- In the first paragraph, it would be helpful to provide more information about the origin and characteristics of the MDA-MB-231 and MCF-10A cell lines, such as their phenotype, hormone receptor status, and known genetic alterations.
- In the second paragraph, additional details on the preparation of bee venom and melittin, including the extraction method and any purification steps, would be useful to ensure reproducibility.
- In the MTT cytotoxicity assay paragraph, it would be informative to briefly explain the principle of the MTT assay and how it measures cell viability based on the reduction of MTT dye by metabolically active cells.
- In the wound healing assay paragraph, it would be valuable to provide more information about the criteria used to define a "healed" wound and how the wound area measurements were analyzed statistically.
- Fig,9 should be presented as a Table rather than a figure.
- Results and Discussion:
- In the sentence "Anti-wound healing profiles were defined after bee venom at 4 µg/ml, cisplatin at 4 µg/ml and melittin 0.5 µg/ml (Figures 2 and 3)", it would be clearer to say "The anti-wound healing profiles were defined using concentrations of bee venom at 4 µg/ml, cisplatin at 4 µg/ml, and melittin at 0.5 µg/ml (Figures 2 and 3)."
- In the sentence "Statistical comparisons suggest that incubation up to 24h induced more anti-healing than extended incubations (Figure 3)", it would be more precise to say "Statistical comparisons suggest that incubation for up to 24 hours induced more pronounced anti-healing effects compared to longer incubation times (Figure 3)."
- In the sentence "In MCF1A cells, wounds were still unhealed after bee venom and cisplatin at 4 µg/ml for 24 h, but melittin at 1 µg/ml for 24h incubation was effective for anti-healing (Figure 4 and 5)", it should be "MCF10A" instead of "MCF1A" to match the previous mention of the cell line.
- In the sentence "Detailed statistical comparisons by UNIANOVA are given in Supplementary 2", it would be clearer to say "Detailed statistical comparisons conducted using UNIANOVA are provided in Supplementary 2."
- In the sentence "QPCR were performed after cDNA synthesis using RNA isolates", it should be "QPCR was performed" to match the singular subject.
- The statement "KAI1/CD82 has been shown to function in cancer prevention in particular angiogenesis, therefore considered as an anti-metastatic player [12][13]" is not clear. It would be helpful to provide more specific information about the role of KAI1/CD82 and how it relates to anti-metastatic activity.
- In the sentence "BRMS1 was downregulated after bee venom whereas melittin treatment induced its upregulation (Figure 6A)", it would be useful to explain the significance of BRMS1 and its potential role in cancer.
- The sentence "Dysregulation of DRG1 was found to be related to breast cancer progression in patients [15], however its downregulation after knocking-out resulted in metastasis in MCF7 breast cancer cell line, but no significant effect on

MDA-MB-231 cells [15]" is unclear. It could be rephrased to provide a clearer explanation of the findings.

- In the last paragraph, the statement "Honeybee venom has been known to be effective on cancer cell death and considered as a promising medicine for cancer therapy [16]" should be supported with specific references to studies or research articles.
- The statements about the anti-metastatic function of honeybee venom, melittin, and their involvement in various signaling pathways and cancer types should be supported by specific references to the relevant studies.
- The sentence "Melittin itself induced cancer cell proliferation and invasion in melanoma by inhibiting PI3K/AKT/mTOR [9][23] and MAPK pathways as well as bee venom induced [23]" is not clear. It is unclear how melittin can both induce and inhibit cancer cell proliferation and invasion. The sentence should be clarified or revised for accuracy.
- Overall, it would be beneficial to provide specific references throughout the text to support the scientific claims and findings mentioned.
- It is helpful to provide specific references or studies that support the claims about honeybee venom's effectiveness on cancer cell death and its potential as a cancer therapy.
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