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PP-355 [Apitherapy]

"Anticancer potential of bee venom for head and neck squamous cell cancers – the preliminary results"

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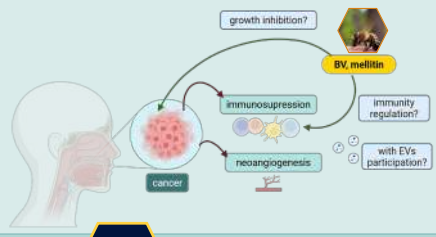
1 Introduction

The **anticancer activity** of **bee venom (BV)** and **melittin** have been shown in various cancers, as well as their beneficial effect on immune system. However, the BV activity in regard to **head and neck squamous cell cancer (HNSCC) remains almost unexplored.**

HNSCC relates to **poor survival outcomes**, unsuccessful treatment in many cases or serious disability. Therefore, new effective therapies are urgently needed. Currently, **immunotherapy** occurs as the novel, promising anticancer strategy. In this aspect a role might play **extracellular vesicles (EVs)** that regulate the activity of cells, including immune cells populations. Cancer induces immunosuppression that favors its growth and metastasis. The solutions that **boost the immune system may improve effective tumor inhibition and drop out in the end.**



2 Aim of the study

The aim of this preliminary study was to evaluate the anticancer potential of BV for pharyngeal carcinoma and healthy human keratinocytes cell lines (HaCat), as well as BV's effect on EVs secretion.

3 Methodology

- **Bee venom** was obtained from honeybees using **low-voltage electrical stimulation**.
- The **melittin** concentration was estimated with **UHPLC-ESI-MS**.
- **Cell lines:** The BV activity was determined in the in vitro studies with **FaDu (squamous cell carcinoma), A549 (lung cancer) and HaCat (keratinocytes) cell lines.**
- After BV treatment:
  - the viability with **MTT assay**,
  - staining with **crystal violet**,
  - **scratch assay**
  - and apoptosis analysis with flow cytometry were performed.
- **EVs** were isolated from cell culture supernatant using pre-clearing with differential centrifugation and **Size Exclusion Chromatography (SEC)** and characterized by **Nanoparticle Tracking Analysis (NTA).**

4 Results

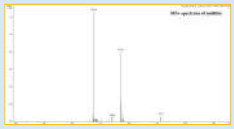


Fig. 1 UHPLC-ESI-MS analysis – the 60,82% of BV was the mellitin.

Fig. 2 Viability of cells (MTT assay) after 24, 48, 72 hours.

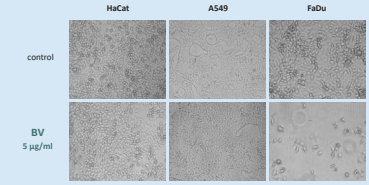


Fig. 3 Cells morphological changes after BV treatment.

Results

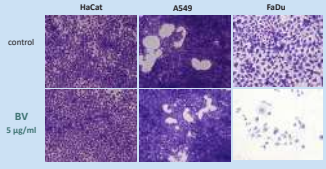


Fig. 4 Cells morphological changes after BV treatment (crystal violet staining)

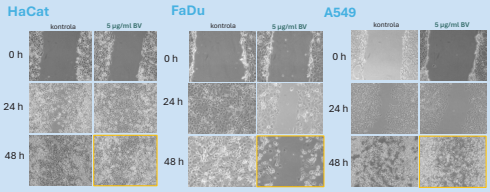


Fig. 5 Scratch assay.

Fig. 6 EVs size and concentration evaluation with NTA measurments.

5 Conclusions

The BV treatment results in case of:

1. **FaDu:** inhibited growth, inhibited wound healing, visible morphological changes, proapoptotic effect and increased level of EVs in the supernatant.
2. **HaCat:** no effect on cell viability, no visible morphological changes and no/ slightly anti-apoptotic effect.

In summary, those preliminary studies revealed no toxic effect of BV for HaCat and potential anticancer activity in case of FaDu in which might take part EVs, however, further investigation with the use of other methodologies is urgently needed.

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Background/PURPOSE:
The anticancer activity of bee venom (BV) and melittin have been shown in various cancers, as well as their beneficial effect on immune system. However, the BV activity in regard to head and neck squamous cell cancer (HNSCC) remains almost unexplored. HNSCC relates to poor survival outcomes, unsuccessful treatment in many cases or serious disability. Therefore, new effective therapies are urgently needed. Currently, immunotherapy occurs as the novel, promising anticancer strategy. In this aspect a role might play extracellular vesicles (EVs) that regulate the activity of cells, including immune cells populations. Cancer induces immunosuppression that favors its growth and metastasis. The solutions that boost the immune system may improve effective tumor inhibition and drop out in the end.

Problem description:
The aim of this preliminary study was to evaluate the anticancer potential of BV for pharyngeal carcinoma and healthy human keratinocytes cell lines (HaCat), as well as BV's effect on EVs secretion.

METHODOLOGY:
Bee venom was obtained from honeybees using low-voltage electrical stimulation. The melittin concentration was estimated with UHPLC-ESI-MS. The BV activity was determined in the in vitro studies with FaDu and HaCat cell lines. After BV treatment the viability with MTT assay, staining with crystal violet, scratch assay and apoptosis analysis with flow cytometry were performed. EVs were isolated from cell culture supernatant using pre-clearing with differential centrifugation and Size Exclusion Chromatography (SEC) and characterized by Nanoparticle Tracking Analysis (NTA).
Results and CONCLUSION: The melittin is 60% of BV composition. The BV treatment results in case of: 1)FaDu: inhibited growth, inhibited wound healing, visible morphological changes, proapoptotic effect and increased level of EVs in the supernatant. 2)HaCat: no effect on cell viability, no visible morphological changes and no/ slightly anti- apoptotic effect. In summary, those preliminary studies revealed no toxic effect of BV for HaCat and potential anticancer activity in case FaDu in which might take part EVs, however, further investigation with the use of other methodologies is urgently needed.