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2ND INTERNATIONAL ELECTRONIC CONFERENCE ON TOXINS
14-28 July 2023
IECT 2023

Natural Peptides and Plant Extracts protect Skin Cells in culture against venom-induced cytotoxicity

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Introduction

Snake envenomation still remains one of the most significant factors of morbidity and mortality worldwide mainly affecting low-income countries. In many cases, the tissue is damaged locally around the site of the bite leading to severe disabilities. Limited therapeutic pharetra includes antivenoms administration as well as analgesics, haemodialysis and antibiotics. Currently, novel therapeutic alternatives are under investigation exploring additional treatments.

Objective

Evaluation of the venom toxicity of the Cypriot venomous snake *Macrovipera Lebetina Lebetina* on human keratinocytes (HaCat cells).
Antivenom exploration of natural compounds (synthetic peptides and endemic plant extracts).

Methods

- Two different cell lines were used for testing the venom toxicity, human keratinocytes (HaCaT) and human cancer cell line (A549).
- A549 cells were primarily used for the evaluation of venom's cytotoxicity, while the HaCaT cell line was used for testing both the cytotoxicity and the protective properties of the natural products
- Peptides (humanin, 1 mg/ml) and plant extracts (origanum vulgare hirtum, 2.5 mg/ml) were tested for their antivenom potency by treating cells at 3 and 6 hours after adding venom in 4 concentrations (10, 5, 2.5, 1.25 mg/ml)

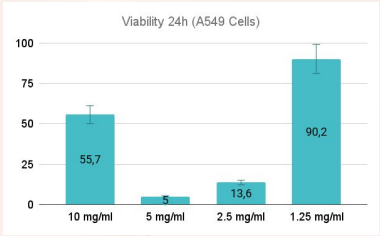
Key points

i) In the first experiment, cells were treated with humanin and origanum 3 hours after the venom was added

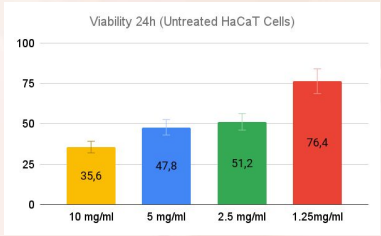
ii) In the second experiment cells were treated with humanin and origanum 3 + 3 hours after the venom was added

iii) The levels of cytoxicity were measured using the MTT assay protocol

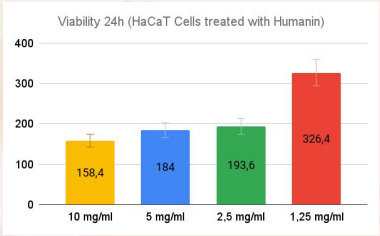
Results



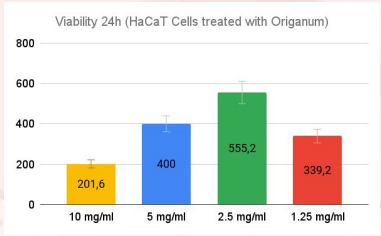
A549 cells' viability after the add of MLL venom
X axis: Venom concentration, Y axis: Cell viability



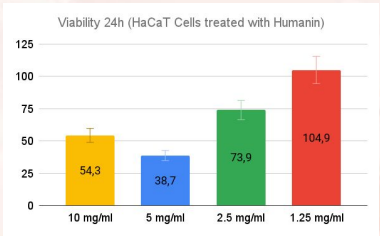
HaCaT cells' viability after the add of MLL venom
X axis: Venom concentration, Y axis: Cell viability



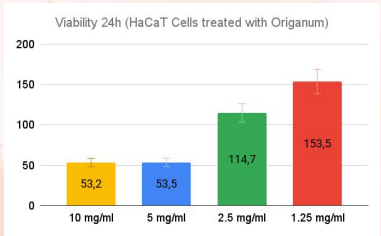
HaCaT cells' viability after the add of MLL venom and treatment with Humanin 3 hours later
X axis: Venom concentration, Y axis: Cell viability



HaCaT cells' viability after the add of MLL venom and treatment with Origanum 3 hours later
X axis: Venom concentration, Y axis: Cell viability



HaCaT cells' viability after the add of MLL venom and treatment with Humanin 6 hours later
X axis: Venom concentration, Y axis: Cell viability



HaCaT cells' viability after the add of MLL venom and treatment with Origanum 6 hours later
X axis: Venom concentration, Y axis: Cell viability

Discussion

i) The MLL venom shows remarkable levels of cytotoxicity on both cell lines

ii) Both humanin and origanum vulgare hirtum seem to reverse the venom's effect.

3 hours: Both our natural compounds show a great contribution to cells' viability
6 hours: Cells' viability is definitely lowered (compared to 3 hours), but the numbers are still higher compared to untreated cells.
Therefore, we can conclude to the fact that both humanin and origanum vulgare hirtum possess anti-venom properties

iii)Comparing the percentage of viability between the two experiments it is indicated that:
i) The MLL venom exhibits its cytotoxicity after the first 3 hours.
ii) Origanum vulgare hirtum is more efficient in protecting the cells than humanin

References

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iii) "World Health Organization (WHO)." *World Health Organization*, www.who.int/. Accessed 31 May 2023.

Acknowledgements

i) We acknowledge MedVenom Ltd, a Cyprus start-up company, for gifting the venom.
ii) The origanum vulgare hirtum was bought from the Agricultural Research Institute, Nicosia, Cyprus.
iii) Both of the cell lines were given by Aglaia Pappa's laboratory of Molecular Physiology - Department of Molecular Biology and Genetics