In vitro evaluation of the antimicrobial effect of toxins from the venom of the tarantula *Poecilotheria regalis*.

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Background:The use of antibiotics has a significant impact on human health. However, the emergence of resistant bacteria makes treatment difficult, which has prompted research into new drugs to control bacterial infections. Various organisms, including spiders, have the ability to produce antimicrobial peptides that play a key role in protecting against bacterial agents. However, the proteomic study of tarantula venoms is recent, due to the small amount of venom per specimen and the lack of an adequate extraction protocol.

Objective: Our study aims to evaluate in vitro the antimicrobial effect of the complete venom as well as selected fractions of *P. regalis* venom. For this purpose, we will determine the integrity of the proteins of the total venom of *P. regalis* by electrophoresis. The antimicrobial activity will be determined by plate diffusion. In addition, total venom fractionationing will be performed using RP-HPLC.

Methods: The complete venom of *P. regalis* was obtained by anesthetizing the specimens with concentrated isoflurane, electrically stimulating the venom-producing glands of the chelicerae for 20 seconds with 7V. The venom obtained was quantified using the A230/A280 NANODROP ONE reader. Once the venom was quantified, its complexity was analyzed using the 18% Tricine-SDS-PAGE polyacrylamide gel electrophoresis technique under reducing conditions (120V/90min). Venom fractions were separated in High Efficiency Liquid Chromatography at a concentration of 2 mg, using a reverse phase C18 column, diluting in 0-60% acetonitrile. The fractions were tracked at a wavelength of 280 nm. Each fraction was analyzed by plate diffusion in Gram-negative (*E. coli* ATCC 13706) and Gram-positive (*S. aureus* BAA-1686) bacteria on Müller-Hinton agar culture.

Results: After processing the venom by RP-HLPC, we obtained 50 fractions, of which by plate diffusion analysis, 21 fractions had antimicrobial effect against *E. coli* and 18 fractions had antimicrobial effect against *S. aureus*.

Conclusion: In this study we demonstrated for the first time Gram-positive and Gram-negative antimicrobial activity of a large percentage of the venom fractions. The fractions with antimicrobial activity will be processed to find the minimum inhibitory concentration.

Expositors:

Paul Alexis Bourgade Su, medical student at Universidad Anahuac Mexico. Junior Coordinator of INVESTIGA Health Sciences Research Program. Accepted in a research trainee program in South Florida University. Responsible for a research line that studies the proteomics of tarantula venoms in search of antimicrobial peptides. He's also involved in a project on the pathophysiological explanation of the pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections (PANDAS). Open to collaboration in clinical and basic sciences.

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Dr. Diego Alexander Rojas did his master's program in Health Sciences with a specialization in immunology at ESM-IPN, where he investigated the pathogenesis of the "brain-eating" amoeba (*Naegleria fowleri*) and the immune response to its infection.
In 2018, he started the Health Sciences Phd at ESM-IPN extending the research done in the master's degree. He determined the role of FcγRIII in the nasal mucosa of mice in the model of protection against *N. fowleri* infection, through the interaction between FcγR neutrophils and antigen-antibody complexes.
Currently, He works as a professor at Anahuac University Mexico, where he is also a research associate. He leads a research project on the antimicrobial and amebicidal activity of antimicrobial peptides from *P. regalis* venom, and he collaborates in a study focused on bone marrow regeneration using modified neuronal peptides.

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