THE ROLE OF NATTERINS, MAJORITARY TOXINS OF THE *Thalassophryne nattereri* FISH VENOM IN LOCAL INFLAMMATORY RESPONSE IN MICE

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Natterins, isolated toxins from *Thalassophryne nattereri* (niquim) fish venom, is a family of 5 toxins which present homology amongst themselves and molecular mass around 30-45 kDa. The group of majority toxins Natterin 1, 2 and 3 was used for the evaluation of the inflammatory response in mice. The kinetic of the leukocyte recruitment, cytokine and chemokine levels and matrix metalloproteinases 2 and 9 activity after administration of Natterins in the footpad of Swiss mice was determined. After 6, 24 and 48 hours or 7, 14 and 21 days the animals injected with 10 microgram of Natterins were was sacrificed and the footpad was processed for cellular suspension collection and inflammatory mediators were determined in supernatant of footpad homogenates. The leukocyte cell counts were performed using a hemocytometer using Hema3-stained cytospin preparations. Natterins were not able to induce leukocyte influx into footpad of mice at 6 h after injection, that was characterized by a significant reduction of macrophages and lymphocytes compared with PBS-group, but after 7 days Natterins provoked an intense recruitment of neutrophils, macrophages, and lymphocytes. The footpad levels of PGD2, IL1-beta, IL-6, MCP-1 were augmented after 6 hours and IL1-beta and TNF-alpha after 7 days. High MMP-9 activity was detected in footpad supernatant at 6 h. We can suggest that the impared cellular influx provoked by Natterins was independently of cytokines or chemokines production, and it could be related with the high MMP-9 activity in the footpad.

KEY WORDS: *Thalassophryne nattereri*, venomous fishes, Natterins, inflammatory response.

FINANCIAL SUPPORT: FAPESP, FUNDAP.

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EFFECTS OF THE ALGINATES ISOLATED FROM BROWN SEAWEED *Sargassum vulgare* IN PERFUSED RAT KIDNEY AND MESENTERIC BLOOD VESSELS

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Alginates extracted from seaweed at industrial level are widely used in the food industry, biotechnology area and for medical purpose as microencapsulation of hormone-production, treatment of diabetes mellitus and parathyroid disease. The aim of present work was determining the effects of alginates with high viscous (α-L-mannuronate; +V) and low viscous (β-D gluronate; -V) isolated from seaweed in perfused kidney from Wistar rats, as it was described by Fonteles et al., 1983 (1). The effects of 10µg/mL concentration (n=6, each group) were studied on perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR), sodium (%TNa+), potassium (%TK+) and chloride (%TCl-) tubular transport. All experiments were preceded by a 30 minutes internal control period (C). The data was analyzed by ANOVA and Student t-test (*p<0.05). The -V increased the PP (C = 109.7 ± 0.75 vs -V = 112.15 ± 0.17 mmHg*), RVR (C = 5.76 ± 0.002 vs -V = 0.13 ± 0.004 mmHg.min-1.g-1.min*), UF (C = 0.10 ± 0.01 vs. -V = 0.13 ± 0.004 mL.g-1.min-1*) and GFR (C = 0.761 ± 0.07 vs. -V= 0.422 ± 0.12 mL.g-1.min-1*). However the toxin reduced the %TCl- (C = 79.76 ± 0.56 vs. -V = 59.24 ± 3.15 %*). The +V only changed the PP, RVR and %TCl-. The results of mesenteric blood vessel showed effects only for -V. In conclusion, alginate -V and alginate +V act for different mechanisms. The low viscous alginate probably acts on the endothelial cells and high viscous alginate possibly release of TNFα from mesangial cells in isolated kidney rat.

KEY WORDS: alginates, seaweed, isolated kidney, mesenteric blood vessel.

REFERENCE: (1) FONTELES, M. C. et al. (1983). Am J Physiology 44: 191-197.

FINANCIAL SUPPORT: CNPq.

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BIOCHEMICAL PROFILE AND THE SEARCH OF ANTIMICROBIAL ACTIVITY OF THE MUCUS FROM THE SLUG *Phyllocaulis boraceiense*s (Thomé, 1972)

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Many invertebrates, like mollusks, have been used as source of compounds with antibiotic activity. Biochemical profile of the mucus from the slug *Phyllocaulis boraceiense*s was studied in order to determine if this specie present fraction containing this kind of compound. Assays to quantify proteins, lipids, amino acids, free and bound glucose associated with other substances, mass spectrometry, electrophoretic profile and a high performance liquid chromatography (HPLC) were carried through. Crude samples were collected using a spatula and a saline solution (NaCl-0.06%). This material was stored in freezer at –70ºC, after that an extraction with acetonitrile was performed, and then the material was lyophilized. Finally, the microbiological assays were performed on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* pure cultures. The composition was: protein = 1,15 x 10-4mg/ml; lipids = 6,9 x 10-5mg/ml; amino acid analysis = only few amino acids were detected, probably the material degraded; free glucose = not detected; glucose in association with another compounds = 600mg/ml. The mass spectrometry shows a compound with 17,5kDa molecular weight and probably masses corresponding to monomers, dimmers and trimmers. In the HPLC assay some bands of protein were detected, these results were in accordance to that obtained in the electrophoretic profile. The bactericidal effect was not detected. These preliminarily data suggested that the mucus although being a source of protein does not have any bactericidal and/or bacteriostatic action at least for the bacteria species tested.

KEY WORDS: slugs, *Phyllocaulis boraceiense*s, mollusks, antimicrobial action, biochemical profile.

FINANCIAL SUPPORT: FAPESP and CAPES

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THE FIRST REPORT OF A MICROCYSTIN-PRODUCING CYANOBACTERIA BLOOM IN PARANOÁ LAKE, BRASÍLIA, BRAZIL

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Cyanobacteria blooms occurring all around the world to increase the concern about the water quality used for consumption and recreation. Studies in Brazil reveal cases of cyanotoxins contamination in reservoirs, from the south to the northeast, evidencing the amplitude of occurrence of cyanobacteria. No study of cyanobacteria related to toxicity has already been carried out at Paranoá Lake, a reservoir destined to recreation and power production. Preliminary analyses demonstrated the occurrence of a cyanobacteria bloom , beginning in September and occurring until March of 2005 in the region of the Bananal branch. The toxin was identified by comparison with MCYST-LR, based on retention times, PDA-UV and MS spectral data. Using this approach it was possible to show that the cyanobacterial cells collected from the Paranoá Lake contained the most toxic microcystin-LR.

KEY WORDS: cyanotoxins, microcystin, *Microcystis*, Paranoá Lake.

FINANCIAL SUPPORT: CNPq.

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CHARACTERIZATION OF THE EFFECTS OF CATFISH *Pseudoplatystoma fasciatum* VENOM IN THE MICROCIRCULATION AND MUSCLE FIBERS: AN INTRAVITAL MICROSCOPIC STUDY

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One of the most abundant venomous species of catfish found in the North of Brazil is *Pseudoplatystoma fasciatum* (surubim). The accidents are characterized by local effects as intense pain and edema. The aim of this study was characterize the effects of the venom on the microcirculatory net and on muscle fibers, using intravital microscopy. In addition, eletrophoretical and cromatography profile of the venom was determined. The cremaster muscle of Swiss mice were displayed and different doses of the venom (5, 15, 30, and 60 µg) were topically applyed. Control experiments were performed by applying 30 µl PBS under otherwise identical conditions. Five minutes of observation were recorded before application of the venom to analyze the dynamics in control tissue. Experiments were carried out for up to 30 min. The higher dose of venom (60 µg) induced hypercontraction in muscle fibers, a signicant increase of leukocytes rolling, and a blood clot formation in venules. After application of 30 µg the same alterations were obseverd but in later periods. Using 15 µg it was obseved only hypercontraction in fibers. No alteration were seen after 5 µg application. The electrophoretic profile (SDS-PAGE) showed intense bands at 18, 25, and 66 kDa. Subsequently it was carried out chromatographic profile usig HPLC that showed 3 hidrofilic peaks and 2 hidrofobic peaks. These data suggest that the *P. fasciatum* venom induce an important inflammatory activity characterized for alterations in the microcirculation and muscle fibers, and these modification could be related with toxins present in the venom.

KEY WORDS: Catfish, venom, *Pseudoplatystoma fasciatum*, intravital microscopy.

FINANCIAL SUPPORT: Fapesp and CNPq/PIBIC.

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CYTOTOXICITY EFFECTS OF AMBLYOMIN-X IN CULTURED CANCER CELLS

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One of the most efficient means to control the coagulation is the action of proteinase inhibitors, and several studies have demonstrated that saliva of haematophagous have substances whose interfere in the the blood clotting of their host. Aiming the blood clotting system a recombinant protein which inhibits activated Factor X (FXa) was cloned and expressed. This protein named Amblyomin-X exerts great clinical interest because it could be used as an anticoagulant substance able to inhibit the FXa and don't have any influence in the cell adhesion. The aim of this study is to evaluate the cytotoxic effects of Amblyomin-X on tumor cells, comparing its effect on the normal cells (Human Umbilical Vein Cells - HUVECs). To the experiments confluent cultured cells (HUVECs, B16F10, SK mel-28 and MiaPaca) were treated with different concentrations of Amblyomin-X, the cytological alterations were analyzed by inverse microscopy, and the cell viability analyzed by the MTT method. The obtained data showed that the treatment of the tumor cell lines with the Amblyomin-X induces the cytotoxicity in a dose dependent manner, however it does not affected the normal cells (HUVECs). Due to this result we can suggest that Amblyomin-X can modulate death of tumor cells but not cause alterations in normal cells.

SUPPORTED BY FAPESP and FINEP/COINFAR

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EFFECTS OF AMBLYOMIN-X IN THE CLASSIC COAGULATION ASSAYS

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In the bloodsuckers saliva there are several compounds whose interfere on the physiological systems of their hosts such as coagulation, platelet aggregation and fibrinolysis. Focusing the blood clotting system a recombinant protein named Amblyomin-X was cloned and expressed, this protein is able to inhibit the Factor Xa activity and its effects were compared to the effects caused by Heparin which is a classical anticoagulant “in vivo” and “in vitro”. To perform the “in vivo” experiments normal mice were injected with Amblyomin-X (1 and 2 mg/kg body weight, iv), Heparin (1 and 2 mg/kg body weight, iv) or saline (control), blood was collected 5 and 24 h after the injection and the prothrombin time (PT) and activated partial thromboplastin time (APTT) were assayed. To the “in vitro” experiments a pool of normal mice plasma was obtained and incubated with the anticoagulants (Amblyomin-X 0.02 ug/ul to 1.0 ug/ul and Heparin 0.02 ug/ul to 0.2 ug/ul) or saline as control. The obtained data to the “in vivo” experiment showed that the PT is not affected neither by Amblyomin-X nor by Heparin. On the other hand APTT is prolonged to the both proteins. To the “in vitro” experiments the PT is not affected by Amblyomin-X, however in presence of Heparin a greater PT prolongation is observed. The APTT is prolonged to the both proteins. The anticoagulant action of Heparin was more evident than Amblyomin-X. Heparin catalyzes the antithrombin inhibition and Ambliomin-X acts directly on FXa inhibition.

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STUDIES ON THE ANTINOCICEPTIVE ACTIVITY OF HONEY BEE VENOM, *Apis mellifera*

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Venom from insects, amphibian, arthropods etc, contains toxins with antinociceptive and anti-inflammatory activities. Venom from the honey bee *Apis mellifera* has traditionally been used to treat inflammatory diseases and to relieve pain. Many reports have described the antinociceptive activity of bee venom and some of its components, but no precise mechanism of action has been determined. Whole bee venom and a fraction II obtained from gel permeation chromatography were assayed for antinociceptive activity in some experimental models such as hot plate and formaldehyde injection in the paw of mice. The whole venom and the fraction, injected subcutaneously at the back of the mice, inhibited the nociceptive response induced by formaldehyde in a dose dependent manner. The nociceptive response in the hot plate model was not changed by the venom or the fraction. Neither the venom nor the fraction altered the motor coordination of mice in the rota rod assay. The results suggest a peripheral action probably by inhibition of the production or action of inflammatory mediators.

KEY WORDS: bee venom, pain, nociception, anti-nociception

FINANCIAL SUPPORT: FAPEMIG

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CYANOTOXINS BIOACUMULATION IN THE *Hypophthalmicthys molitrix* (SILVER CARP) OF THE PARANOÁ LAKE – DF- BRAZIL - A SANITARY RISK

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Microcystins are hepatotoxic cyanotoxins, that provoke serious liver internal injuries. Some studies indicate that microcystins can bioaccumulate in aquatic animals, and possibly be transferring through the web food. Paranoá Lake is an artificial reservoir on purposes of offering recreation activities and enhancement of the microclimate in Brazil's Federal Capital. It is the most preferable place for non-professional and professional fisheries and the population that lives around the lake usually consumes the fishes. Three individuals of *Hypophthalmicthys molitrix* (Silver carp), phytoplanktivorous fish, had been collected biweekly, May to June/2006 next to ETE SUL (Treatment Sewer Station of the CAESB). The individuals were dissected in muscle and liver, the tissues were extracted with MeOH (1g/5ml). Then, semipurified with SPE C-18. The analysis were performed in a HPLC system: mobile phase 20 mM ammonium formate in 30% Acetonitrile, corrected pH to 5. The toxin identification was made by comparing the retention time and the spectrogram in 200-300nm range with microcystin-LR standard. In all samples, the microcystin-LR equivalent concentration, were higher than recommended for human consume (0,04 ng of microcystin/g of body weight, Chorus and Bartram, 1999(1)). In this time, liver was between 9,9 to 39,9 ng/g, and muscle 1,5 to 15,9 ng/g. In this study we do not recommend the consume of *H. molitrix*. These preliminaries results are from a 1 year project for monitoring the dynamics of microcystins in aquatic animals of Paranoá Lake.

KEY WORDS: microcystin, cianotoxin, silver carps, *Hypophthalmicthys molitrix*, Paranoá Lake, Microcystis, Cyanobacteria.

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FINANCIAL SUPPORT: CNPq.

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BIOACCUMULATION OF CYANOTOXINS IN BRAZILIAN MOLLUSKS

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Cyanotoxins produced by freshwater cyanobacteria are very common worldwide, and have been reported to cause poisonings and deaths of wild and domestic animals even significant hazards to human health. In this study we examined the presence of Microcystins and Saxitoxins in two mollusks, *Pomacea lineata* (Gastropoda) and *Corbicula fluminae* (Bivalvia). The mollusks were collected in Paranoá Lake, Brasília, Brazil and brought to Laboratório de Toxinologia-UnB, where they were extracted and semipurified for further analysis. For microcystins, the toxins was identified by UV spectrogram in HPLC-PDA/UV system and MS spectral data. And for Saxitoxins a HPLC system accomplished with a fluorescence post-column reaction and MS spectral data. *Pomacea lineata* extracts present 3 types of Microcystins (MC-LR, MC-RR and DleuMC-LR) and a unknown saxitoxin derivative, and *Corbicula fluminae* extract presents GTX1 and GTX5, both saxitoxins derivatives, suggesting that both mollusks are good bioindicators for cyanotoxins. These preliminaries results are from a 1 year project for monitoring the dynamics of microcystins and saxitoxins in aquatic animals of Paranoá Lake.

FINANCIAL SUPPORT: CNPq.

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EVALUATION OF DIFFERENT IMMUNIZING SCHEDULES FOR BOTHROPIC ANTIVENOM PRODUCTION

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The antivenom efficacy resides in providing high titre of specific antibodies. Those antivenoms are prepared using horses gradually immunized with specific whole venoms. The immunization protocol depends on the toxicity and the immunogenicity of the venom, the animal model used and the antigen presentation. The optimum protocol or dose for immunization is generally obtained by trial and error to obtain a sufficient antibody titre. This paper reports the comparative study of the several different immunization schedules and different intervals between cycles using *Bothrops* venoms. The four different groups contain seven animal per group which were submitted to immunizing schedules protocols: G1 was applied with 6 mg of venom, G2 12 mg, G3 18 mg and G4 45 mg. Each cycle spend two or three weeks for immunization and one for bleeding. Therefore, another protocol, were used to evaluated intervals between cycles of immunization composed by 15, 30, 60, 90 and 120 days. The results demonstrated that antivenom obtained from animals of the G2 (12 mg) was more effective in neutralizing the lethal effects of *Bothrops* venom. This study indicated that intervals between cycles of the 90 and 120 days produced high and similar levels of antibodies.

KEY WORDS: immunizing schedules, bothropic antivenom

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TECHNETIUM–99m LABELING OF SCORPION ANTIVENOM FOR BIODISTRIBUTION ANALYSIS

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The species *Tityus serrulatus*, is considered the most venomous of the South America. Serious accidents are observed in children. With the objective to carry through biodistribution analysis of the scorpion antivenom, in 21-22 day-old rats, labeling of the scorpion antivenom (fragment F(ab')2) produced by the Fundação Ezequiel Dias (FUNED) with technetium–99m was effected. The labeling was made using two reducing agents, the stannous chloride and sodium borohydride. In order to calculate the labeling efficiency, was performed thin layer chromatography using silica gel 60 and descending chromatography with Whatman paper n° 1. Preliminary studies to stablish the optimum conditions of labeling were done. The purification was made by filtration using cellulose ester membrane. To verify stability in vitro, after the purification, 99mTc-F(ab')2 samples were incubated with PBS pH 7,4 at 37 °C for 1, 4 and 24 h. Aliquots were then withdrawn for determination % labeling. Protection against venom lethality in mice was checked by incubation, for 30 minutes a 37 °C of 5 DL50 of *Tityus serrulatus* venom with various doses of labeled antivenom. The potency was determined and compared with unlabeled antivenom. *In vitro* the immunoreactivity of 99mTc-F(ab')2 was verified using affinity chromatograph. An yield of labeling of 98,90±0,25% (n=3) after the purification was obtained. The labeling yield of 99mTc-F(ab')2 after incubation remained constant. It was not observed any difference between potencies of the unlabeled (1,21 mg/mL), and labeled (1,12 mg/mL) antivenom using statistical analysis. 99mTc-F(ab')2 presented bound with venom in vitro. This study indicate that technetium–99m labeled scorpion antivenom could be used for biodistribution analysis.

KEY WORDS: scorpion antivenom, technetium–99m, biodistribution

FINANCIAL SUPPORT: FAPEMIG

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PHAGE DISPLAY IN VENOM GLAND IN *Dinoponera australis* (HYMENOPTERA: FORMICIDAE)

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The *Dinoponera* genus presents six species, all with distribution along South America, being that the *Dinoponera australis* is found since Mato Grosso until the Rio Grande do Sul and neighboring countries. These ants are characterized by the presence of a venom gland that is responsible for the production of the toxin, which is composed in its bigger part by proteins. The technology of Phage Display has been used to produce clinical diagnostics, new drugs against several diseases and for interaction protein-protein mapping. This work had as objective to identify specific binding peptides in venom gland of *D. australis* for Phage Display. Two types of libraries presented in the phages´surface had been used. One of linear heptapeptides and other of conformational heptapeptides. After four rounds of election, twelve clones had been chosen for sequencing and the bioinformatic analysis disclosed that they are similar to the sequences expressed in other insects. The analisys of the expression of venom gland binding peptides´protein, showed that some clones are similar to member 9 of the family of the kinesina of *Apis mellifera*, synaptic ras gtpase activating protein, syngap of *Aedes aegypti*, ceramida kinase of *Aedes aegypti*, bacterial periplasmic transport systems, periplasmic binding proteins (PBPs) you transport wide variety of substrates, such, amino acids, peptides, sugars, vitamins and inorganic ions; to vacuolar protein sorting-associated protein. The phage display technique was efficient for the characterization of binding peptides in venom gland of *D. australis*.

KEY WORDS: Phage Display, venom gland, *Dinoponera australis*.

FINANCIAL SUPPORT: CNPq, FAPEMIG, UFU.

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