

## EVALUATION OF AN EXPERIMENTAL BOTROPIC ANTIVENOM PRODUCED WITH VENOM BLOCKED WITH Na<sub>2</sub>-EDTA

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Snake envenoming is a serious medical problem in tropical developing countries and antivenoms are the only effective therapy. Antisera are produced by immunizing horses with snake venom and adjuvant, usually Freund's adjuvant. Botropic venoms contain metalloproteinases (SVMPs), responsible for the local effects of envenoming, such as hemorrhage, edema and myotoxicity as well as systemic bleeding. SVMPs represent around 34,2 % of the protein composition from *B. diporus* venom and causing lesions during the immunization of animals. In search of new processes, focused on animal welfare for the production immunobiologicals, and taking into account that the toxic action of SVMPs is inhibited by Na<sub>2</sub>-EDTA, in the present work, the immune response of animals inoculated with *B. diporus* venom blocked with Na<sub>2</sub>-EDTA was evaluated. For this proposal, the *B. diporus* venom (1,9 mg/mL) was blocked by Na<sub>2</sub>-EDTA (200 mM, B.dV/ Na<sub>2</sub>-EDTA) and used as antigen. Previously to the inoculation, the excess of chelate was removed by molecular exclusion chromatography (Sephadex G-25). Likewise, the venom without inhibitor (B.dV) received the same treatment and in both cases, the effective neutralization of SVMPs using azocasein as substrate was determined. Group of 5 CF-1 mice were immunized subcutaneously on days 0, 15 and 30 with B.dV or B.dV/ Na<sub>2</sub>-EDTA (7-30µg or 14-60 µg) emulsified with complete Freund's Adjuvant and incomplete (booster). On days 14, 29, 37 blood samples were collected from the tip of the animals tail and day 45 the final bleeding and the separation of the different sera were performed. These were destined for immunoassays and neutralization assays for proteolytic, indirect hemolytic, and coagulant activity. The results of the enzyme-linked immunosorbent assay showed that both anti-B.dV and anti-B.dV/EDTA serum had high antibody titers (1/74.850 – 1/186.150) at the end of the immunization protocol. Regarding the Western Blot, the anti-B.dV/EDTA serum recognized the main bands, corresponding to the venom proteins, in a similar way as the anti-B.dV. Additionally, the experimental sera produced showed neutralizing capacity of the main toxic activities tested *in vitro*. This result shows that Na<sub>2</sub>-EDTA does not affect the immunogenicity of proteins since animals immunized with B.dV/ Na<sub>2</sub>-EDTA respond to *B. diporus* venom in a similar way to animals immunized with venom without inhibitor.