Ubiquitous in nature, the phospholipases A₂ (PLA₂) hydrolyze phospholipids at the sn-2 position and exhibit a large variety of biological functions. In the snakes, the PLA₂ are among the most toxic compounds present in the venoms, exhibiting pharmacological effects: myotoxic, neurotoxic, anticoagulant and others. Some PLA₂ possess high inflammatory capacity that can occur by several different paths. The Dominici stain allows the identification of different inflammation involved cells and it can help in the identification of the inflammation pathway induced by the toxin. In the present work, *Bothrops hyophora* venom was fractionated via gel filtration chromatography and a PLA₂ containing pool was submitted to reverse phase HPLC, yielding a new purified acidic PLA₂. Inflammatory activity was monitored by observation of edema formation and cytokines IL-1α, IL-6 and TNF-α concentration in plasma, measured with commercially available kits. Purified toxin was applied in mice gastrocnemius muscle, which was removed and fixed using a 4 % formaldehyde solution in Millonig’s buffer (0.13 M sodium phosphate, 0.1 M NaOH– pH 7.4) for 18 hours at 4º C and washed in water, ethanol dehydrated, diaphanized with xylene and paraffin-embedded. Sections of 7μm were deparaffinized and immersed in water. After that, different sections were stained by hematoxylin-eosin and by a modified Dominici stain: they were submersed for 30 minutes in a mixture of acid fuchsin and orange G (0.5% each in distilled water); after that followed a quickly rinse in 60% ethanol and a counterstain in toluidine blue 0.75% for 20 seconds and another ethanol rinse. The sections were then put in 95% ethanol until the stains differentiate. The images were captured with Zeiss Axio Scope A1 Microscope. Previous experiments showed that this toxin had no myotoxic, anticoagulant or antimicrobial capacities. Nonetheless, it presented moderate edematogenic properties and high inflammatory capacity, elevating several times the concentration of interleukins 1α, 6 and TNF-α. The microscopic observations revealed no muscle damage caused by the toxin, as expected, but it was possible to observe inflammatory infiltrate in the samples. The Dominici stain made possible to identify the cells in the infiltrate as mast cells. These results are coherent with the data obtained by interleukins tests and lead to think that the source of the increase in the cytokines concentration is mast cell degranulation, triggered by the isolated acidic PLA₂ injection.

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Fig. 1: Profile of cytokines IL-1α, IL-6 (pg/ml) and TNF-α (U/ml) plasmatic concentrations along 12 hours after PLA2 injection (filled markers) and phosphate buffer injection (empty markers).

Fig. 2: Sections of the gastrocnemius muscles from mice injected with PLA2 and phosphate buffer, where: A, B, C- section stained with HE of muscle injected with phosphate buffer (A) or PLA2 (B and C). D, E, F- sections stained with Dominici of muscle injected with phosphate buffer (D) and PLA2 (E,F). Bar in A,B,: 40µm. Bar in C,D,E,F: 20 µm.