BIO008

Evaluation of DNA damage in human lymphocytes induced by the venom of *Lachesis muta*.

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Introduction: The genus *Lachesis* along with the genera *Bothrops*, *Crotalus* and *Micrurus* are the main responsible for cases of snakebites in Brazil. There are few studies with *Lachesis* snake venoms, and no reference to their action on human DNA, though the number and severity of accidents involving these snakes present great medical and scientific importance. **Objectives**: This study aimed to evaluate the genotoxic potential of the crude venom of *Lachesis muta* on the DNA of human lymphocytes using the Comet assay. **Methods**: To perform the comet assay, 500 µL of peripheral blood (collected from healthy donors aged between 18 and 35 years after prior consent and approval by the Research Ethics Committee), were diluted in 500 µL of PBS and incubated for 3 h with different venom concentrations (10, 20, 30 and 50 µg/mL). After incubation, aliquots of these preparations were mixed with 100 µL of low melting point (LMP) agarose and applied to slides previously coated with a thin layer of normal agarose and immersed in lyses solution (pH 10) for 2 h in order to obtain the nucleoids. The slides were maintained for 20 min in a solution of electrophoresis (pH 13) for the exposure of the alkali-labile sites of the DNA molecules, and then subjected to electrophoretic run for 30 min at 300 mA and 25 V. At the end of the electrophoresis, the slides remained for 25 min in a neutralization solution (0.4 M Tris-HCl, pH 7.4) and were then dried at room temperature and fixed with absolute ethanol. For the observation of nucleoids, slides were stained with propidium iodide and analyzed on an epifluorescence microscope. All steps of the Comet test were performed at low temperatures and in the dark. The results were analyzed by visual scoring as described by Tice et al. (2000), where level 0 represents damage > 5%, level 1, 5-20% damage, level 2, 20-40% damage, level 3, 40-85% damage and level 4, damage > 85%. **Results**: Damages in DNA molecules were observed for all tested concentrations and, in general, were slightly higher than those observed for the positive control (Doxorubicin, an antitumor mutagenic drug at a concentration of 12 µg/mL), considering the values of arbitrary units. Marcussi et al. (2011) reported higher percentages of DNA damage for *Crotalus durissus terrificus* venom and its isolated toxins than those observed in this study for *L. muta* venom. The different composition of these venoms, with *C.d.t.* presenting different neurotoxic peptides while *L. muta* possesses several proteases with main local action, could explain the differences in their genotoxic potential, considering the uniformity of the methodology used for both analyses. Interestingly, the concentration of 50 µg/mL induced similar levels of DNA damage to the concentration of 10 µg/mL, which could indicate a saturation of the blood solution with venom components, thus hampering the access of potentially genotoxic molecules. The concentration of 30 µg/mL resulted in values of arbitrary units three times greater than those obtained for the negative control. **Conclusion**: The crude venom of *L. muta* showed low genotoxic potential. These results provide important information on the effects of snake venoms on the human DNA and also supplement the functional characterization of this particular venom.

Keywords: Comet assay, *Lachesis muta*, DNA breakage, human lymphocytes.

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