BIO010

In vitro effects of extracts of sea anemones on the DNA of human lymphocytes.

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Introduction: Sea anemones produce toxins such as hemolysins, neurotoxins and other enzymes that induce various effects such as cardiotoxicity, dermatitis, itching, local swelling, erythema, paralysis, pain and necrosis. Some toxins isolated from *Anemonia*, *Stichodactyla*, *Anthopleura* and *Bunodosoma* sea anemone genera are active on ion channels and receptors, acting on the central nervous system and on immune-mediated responses. Thus, sea anemones are promising sources of molecular models of medical-scientific interest, but the possible action of their toxins on the mammalian genome has not yet been reported. **Objectives:** This study aimed to evaluate the *in vitro* activity of extracts of the sea anemones *Condylactis gigantea*, *Bartholomea annulata* and *Bunodosoma granulifera* 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 cultured for 7 h in 5 mL of RPMI supplemented with FCS, phytohemagglutinin and antibiotics. The cultures were incubated for 3 h with different concentrations of the extracts of sea anemones, the positive control doxorubicin (6 µg/mL) or PBS alone. After incubation, aliquots of these preparations were mixed with 100 µL of LMP agarose and applied to slides previously covered by normal agarose, and then immersed in lysis solution (pH 10) for 2 h to obtain the nucleoids. The slides were maintained for 20 min in alkaline solution and then subjected to electrophoresis (25 V and 300 mA) for 20 min at 4°C. After that, the slides remained for 25 min in a neutralization solution (0.4 M Tris-HCl, pH 7.4) and were dried and fixed with absolute ethanol. Slides were then stained with ethidium bromide and analyzed under an epifluorescence microscope by visual scoring using the classification 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:** The extract of *C. gigantea* presented values of arbitrary units higher than those obtained for the negative control, but was unable to induce level 4 damage. For *Bartholomea annulata* and *Bunodosoma granulifera,* some nucleoid damages were classified as level 3 and 4, but a significant margin of error should be considered when dealing with damage levels close to those on the scores 3 and 4. The extract of *Bartholomea annulata* at a concentration of 50 µg/mL induced approximately twice the damage observed for the negative control, and half that obtained for the positive control. Phospholipase A_2 toxins could be related to the induction of DNA damage in lymphocytes observed in this study, as already described for CB PLA_2 isolated from *Crotalus durissus terrificus* (Marcussi et al., 2011). **Conclusion:** All anemone extracts showed low genotoxic potential at the concentrations tested, and these results should be further investigated by tests that show effects in more than one cell generation. Despite this, sea anemones can be considered as promising sources of natural compounds with broad medical and scientific applications.

Keywords: Comet assay, *Condylactis gigantea*, *Bartholomea annulata*, *Bunodosoma granulifera*, DNA breakage, human lymphocytes.

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