7.20 Genotoxicity of textile dye Remazol Black B after vinylization in snail *Biomphalaria glabrata* (Say 1818) determined by the alkaline comet assay

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Introduction: Synthetic dyes represent an important group of xenobiotic chemicals. Of textile dyes, approximately 10-15 % of world production is lost to the environment during synthesis, processing and application. The presence of these compounds in aqueous ecosystems may cause negative impacts deteriorating water quality. Furthermore, these dyes and/or their degradation products may be toxic and mutagenic. Single cell gel electrophoresis, or comet assay, has been the major tool in the evaluation of genotoxic effects in genetic toxicology. **Objectives**: The aim of this work was to evaluate the genotoxic effects of the synthetic textile dye Remazol Black B after vinylization in hemocytes of *B. glabrata* by the alkaline single cell gel electrophoresis assay. **Methods**: The chemical structure of the textile dye Remazol Black B was modified by increasing pH (pH 12) and temperature (40 °C) for 30 min in a process called vinylization. Snails were exposed for 7 days at three different dye concentrations (100 mg/L, 500 mg/L and 1 g/L) and the solutions were renewed every 48 h. To perform the comet assay, about 100 µL of hemolymph containing hemocytes of each animal were collected by pedal stimulus, and then added to 500 µL of LMP agarose 0.5% (w/v), mixed, and placed on two microscope slides pre-coated with NMP agarose 1.5% (w/v). The slides were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 20% DMSO, pH 10.0), kept frozen (4 °C) and protected from the light for at least 12 h. They were subsequently incubated in freshly prepared alkaline buffer (300 mM NaOH and 200 mM EDTA, pH>13) for 10 min for DNA unwinding. Electrophoresis (30 min at 300 mA and 23 V (0.74 V/cm) was performed in the same buffer. After electrophoresis, the slides were neutralized in 400 mM Tris (pH 7.5) and fixed for 10 min in alcohol. Prior to their examination, the slides were stained with 20 µg/ml ethidium bromide and 100 cells per slides (200 per each animal) were analyzed using a Zeiss Axiopian epifluorescence microscope. The extent of the DNA damage was determined by visual analysis. **Results and discussion**: DNA damage was measured as the percent number of comets (classes 1, 2 and 3) and normal cells (class 0). The quantitative damage was calculated and the results showed a significant increase in DNA damage after exposure to textile dye at doses of 500 mg/L and 1 g/L. This study confirmed that the comet assay applied to *Biomphalaria glabrata* hemocytes may be a useful tool in determining the potential genotoxicity of water pollutants.

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