## 8.07 Establishment of comet assay in haemocytes of *Biomphalaria* glabrata (SAY, 1818).

Grazeffe VS<sup>1</sup>, Tallarico LF<sup>1</sup>, Murasaki NH<sup>1</sup>, Suzuki MF<sup>2</sup>, Okazaki K<sup>2</sup>, Kawano T<sup>1</sup>, Pereira CAB<sup>3</sup>, Nakano E<sup>1</sup>.

<sup>1</sup>Lab. de Parasitologia, Instituto Butantan, SP, Brasil; <sup>2</sup>Centro de Biologia Molecular, IPEN, SP, Brasil; <sup>3</sup>Depto de Estatística, Instituto de Matemática e Estatística, USP, SP, Brasil.

Introduction: The comet assay is a method developed to detect breaks in the DNA. The fragments of the damaged DNA show low molecular weight; on the electrophoresis they migrate first in relation to the weightier ones, acquiring the general aspect of a comet. This is a promising test for studies on genotoxicity, DNA repair, environmental and human monitoring. Objective: To standardize the comet assay in haemocytes of Biomphalaria glabrata. Methodology: Haemolymph of wild-type snails of the species Biomphalaria glabrata exposed to the Co-60 gamma radiation (12,5, 25, 50 e 100Gy) was mixed with lowmelting point agarose and placed on the slide prepared with normal melting point agarose. The cells were lysed overnight, and there after exposed to an alkaline solution (pH>13) for 30 minutes. After the electrophoresis, the slides were neutralized with Tris solution, stained with ethidium bromide and analyzed in fluorescence microscope. Results: The control group did not form comets, but the exposed groups showed comets of several sizes and cells have suffered apoptosis. Discussion: The data has shown that how the bigger the dose of radiation, the greater the damage induced. The doses of 50 and 100Gy have shown citotoxic effect, with a high frequency of apoptotic cells. The obtained results have shown the sensitivity and capacity of this assay to detect the effects caused by Co-60 gamma radiation.