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Papain *in vitro* cytotoxicity determination by simultaneous bioassays using murine fibroblasts

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Enzymes are essential to any biochemical industrial process. Papain is a cysteine protease, obtained from papaya latex, which holds among its textile, food, dentistry, pharmaceutical, cosmetic and medical applications, cutaneous absorption enhancement and debridement properties, which are particularly useful for scar and wound treatment, highlighting its relevant potential in such fields and also conferring this biomolecule an ability to be characterized as a model enzyme. Simultaneous *in vitro* cytotoxicity bioassays using vital or non-vital colorants were applied in order to quantify, qualitatively and quantitatively, its bioactivity on murine fibroblast cells (CCL 92). Such tests were performed using papain in several different concentrations (from 0.005 to 0.4 % (w/v)) for 24 or 48 hours of contact at 37°C, 97% humidity and 5% CO_2 in cell culture flasks. The viable cells were measured by the MTS/PMS method, where the active component is a tetrazolium compound and the vital cells reduce it to a colored formazan product that is quantified at 490 nm. After this procedure, the adherent cells were colored using Rhodamine B as a non-vital dye. The qualitative analysis revealed an inhibitory concentration (IC_{50}) of 0.001004 mmol/l after 24 h and 0.001017 mmol/l after 48 h exposition and a lethal dose (LD_{50}) of 0.2092 mmol/kg (24 h) and 0.2104 mmol/kg (48 h), respectively. The qualitative assay indicated a trypsin-like action at the 0.02-0.03% (w/v) concentration range. Despite the difference between the experiments, they seemed to be complementary, revealing the need for a better understanding, standardization and validation of *in vitro* assays for enzymatic compounds.