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Cytotoxicity evaluation of papain using human keratinocytes

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The value of papain in cleansing tissue wounds has been known for several hundred years. This cysteine protease is extracted from the latex of the immature papaya leaves and fruits. Due to a trypsin-like action, papain cytotoxicity evaluation is difficult. This study intends to show how papain acts in human keratinocytes (HK) using a feeder layer formed by murine fibroblasts (ATCC CCL 92). Papain was tested using several different concentrations (from 0.005 to 0.75% (w/v)) for 24 and 48 h of contact at 37°C, 97% humidity and 5% CO_2 in cell culture flasks. In the attempt to observe a reverse mechanism, the cells were also maintained for 7 days after 24 and 48 h of contact. The viable cells were measured by MTS/PMS, where the active component is a tetrazolium compound and the living cells reduce it to a colored formazan product that is quantified at 490 nm. The different inhibitory concentrations estimated to affect the endpoint in question by 50% were IC_{50} (24h) = 0.000662 mmol/l, IC_{50} (48h) = 0.000397 mmol/l; after 7 days IC_{50} (24h) = 0.000664 mmol/l and IC50 (48h) = 0.000390 mmol/l. Basal cytotoxicity can be used in combination with other information for many purposes in the process of safety or risk evaluation, e.g. to predict starting doses for *in vivo* acute oral LD_{50} values in rodents. HK provides good conditions for analyzing new proteolytic enzyme preparations that may be used to supplement normal digestive activity, or to confer upon an individual a new digestive capability.