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**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1647241> since 2017-08-31T19:54:42Z

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# EVALUATION OF CYTOTOXIC AND ANTIOXIDANT EFFECTS OF CURCUMIN IN HUMAN AND CANINE MAMMARY CANCER CELLS LINES

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Curcumin is the main component of *Curcuma longa*. It is traditionally used in Ayurvedic medicine and it is also used in Europe, mainly in pharmaceutical preparations, or as food supplement. It is commonly recommended for reducing acute or chronic inflammatory conditions, such as arthritis [1]. In the last decade, Curcumin has been investigated to assess antineoplastic effects, considering a possible correlation between the use of this spice and the decreasing incidence of degenerative and neoplastic diseases [2-3]. The aim of the present study was to evaluate the in vitro cytotoxic effects of Curcumin in cancer cell lines: MCF-7 (derived from metastasis of human mammary adenocarcinoma), CF.41 (primary canine mammary carcinoma), and CHMp (derived from metastasis of canine mammary carcinoma). All cell lines were cultured in Dulbecco Modified Eagle's Medium (DMEM), fetal bovine serum (FBS - 20%), streptomycin, penicillin and amphotericin B solution (2%) and L-glutamine (2%). Cells were incubated at 37°C and 5% CO<sub>2</sub>. At 80% of confluence, cells were detached and seeded in 96-well plates at a concentration of 5\*10<sup>3</sup> cells/well (100 µl) to perform 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay in different conditions: 1) in presence of increasing concentrations of Curcumin (10<sup>-4</sup>-10<sup>-12</sup> M); 2) with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentrations ranging from 2.5 to 200 µM; 3) co-presence of H<sub>2</sub>O<sub>2</sub> (2.5-200 µM) and Curcumin (10<sup>-4</sup>-10<sup>-12</sup> M). For each trial, experimental time points were 2, 24, 48 and 72h. The data obtained were statistically analyzed with GraphPad Prism software, using One-way ANOVA and Bonferroni's post-test (p<0.05). 1) The Inhibitory Concentration(IC)<sub>50</sub> was 10<sup>-4.563</sup> M for MCF-7, 10<sup>-5.386</sup> M for CF.41 and 10<sup>-2.087</sup> M for CHMp cells. At IC<sub>50</sub> it was possible to appreciate a significant reduction of proliferation compared to untreated control; 2) all H<sub>2</sub>O<sub>2</sub> concentrations induced a statistically significant decrease of the cell amount following a time-dependent inhibitory behavior for MCF-7 and CF.41 cells, while only at 2h CHMp cells showed a concentration-dependent inhibitory behavior; 3) Curcumin seemed not to protect cells against oxidative stimuli, except for CHMp and CF.41 cells that demonstrated a low inhibition rate compared to controls after 72h. These results highlighted different behaviors depending on the type of cell origin (human or canine) and suggested a protective activity of Curcumin against oxidative stimuli after a long incubation period.

[1] Gupta S. Anti inflammatory and anti arthritic activity of different milk based formulation of curcumin in rat model. Current Drug Delivery. doi: 10.2174/1567201814666170320142851, 2017. [2] Zubair et al. Cancer Chemoprevention by Phytochemicals: Nature's Healing Touch. Molecules. 22(3). pii: E395. 2017 [3] Steuber et al. Tocotrienol Nanoemulsion Platform of Curcumin Elicited Elevated Apoptosis and Augmentation of Anticancer Efficacy against Breast and Ovarian Carcinomas. International Journal of Molecular Sciences 17(11). pii: E1792, 2016.