CYTOTOXIC EFFECT OF THE RIBOSOME-INACTIVATING PROTEIN CURCIN FROM *Jatropha curcas* L SEEDS ON CANCER CELL LINES

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Introduction. The fuel-plant *Jatropha curcas* L contains a toxin called curcin with properties like ribosome-inactivating protein (RIP), which is capable to inhibit protein synthesis by its N-glycosidase activity (1). In the present assay the curcin was extracted from fat free cake of *Jatropha* seeds, the purity was checked by SDS-PAGE and N-glycosidase activity was probed on 28s rRNA from *Jatropha* seeds. Curcin extracted has molecular weight of 32.7 kDa, pI of 8.70 and N-glycosidase activity. Besides it has cytotoxic effect against cancer cell lines MDA-MB-231 (IC_{50} of 18.6 $\pm$ 2.4 μg/mL) and SK-BR-3 (IC_{50} of 15.5 $\pm$ 8.3 μg/mL), whereas with healthy cell lines was considerably less toxic, with IC_{50} values of 353.4 $\pm$ 41.9 μg/mL to fibroblasts and 42.12 $\pm$ 8.0 μg/mL to MCF-10. The results suggest that curcin presented higher cytotoxic effect toward breast cancer cells as compared to healthy cell lines. The objective of this study was to determine the cytotoxicity of curcin on normal and cancer cell lines.

Methods. Curcin was extracted from mature seeds from non-toxic cultivar of *J. curcas* L from Puebla, México, and was purified by ion exchange chromatography (2,3). The curcin was analyzed by LC-MS and glycosidase activity was conducted according to Endo et al. (4). Cytotoxic effect was probed on breast cancer cell lines MDA-MB-231 and SR-BR-3, and healthy breast cell line MCF-10 and fibroblasts. In brief, $8x10^4$ cells/mL were incubated during 24 h with different concentrations of curcin, the number of viable cells was determined with MTT reaction. IC_{50} value was calculated using the GraphPad Prism software (5.0) and were analyzed by one-way ANOVA and Tukey means comparisons test (α=0.05)

Results. Curcin was purified to homogeneity by ion exchange chromatography with SP-sepharose and CM-sepharose eluted with gradient of NaCl. According to LC-MS, the molecular mass of curcin was 32.7 kDa and pI of 8.70, which are consisted with those reported to RIP's by Stirpe et.al (5). Figure 1 shows cell viability of MDA-MB-231, SK-BR-3, MCF-10 and fibroblasts cell lines with different concentrations of curcin (μg/mL).

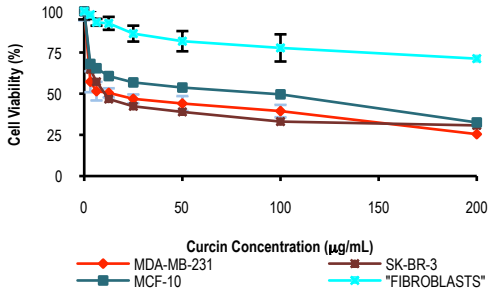

Fig. 1 Cytotoxic activity of curcin on MDA-MB-231, SK-BR-3, MCF-10 and Fibroblasts cell lines. Cells were treated with different concentration of curcin and after 24 h the viability was determined by MTT assay

IC_{50} (Table 1) reflected that MDA-MB-231, SK-BR-3 and MCF-10 were more susceptible to this RIP than fibroblasts.

Table 1. IC_{50} values of curcin in MDA-MB-231, SK-BR-3, MCF-10 and Fibroblasts cell lines.

	MDA-MB-231	SK-BR-3	MCF-10	FIBROBLASTS
IC_{50} (μg/mL)	18.6 $\pm$ 2.4	15.5 $\pm$ 8.3	42.12 $\pm$ 8.0*	353.4 $\pm$ 41.9*

Values were calculated with GraphPad software version 5.0 (α=0.05)

Conclusions. Curcin has higher cytotoxic effect on breast cancer cell lines MDA-MB-231 y SK-BR-3 than on MCF-10 and fibroblasts. Apparently this protein could be an alternative to remove some type of cancer cells.

References.

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