Carcinosinum produces phenotypic changes of 4T1 tumor cells *in vitro*

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Considering that there are few published studies that specifically address the exclusive use of *Carcinosinum* in different potencies and, most of them focused on genotypic and clinical effects, the present study was proposed to identify possible phenotypic changes, including viability, expression of HER-2 and metastatic abilities, using 4T1 cells *in vitro* as a model. *Carcinosinum* was tested in different homeopathic potencies (12cH; 30cH; 200cH) mechanically prepared using sterile pure water. The time space between preparing the potencies and using them was 24 hours.The final dilutions were inserted into the culture medium in a volume equal to 10%, at the time of cell seeding. The same succussed vehicle used to prepare the drugs (70% ethanol) diluted 1:100 in sterile pure water was used as control. All treated cells were cultured in 25 mL flasks, with cell density of 5 x 10^5 cells/mL. After 24 hours of treatment, cells were analyzed for apoptosis index using Annexin V kit and the Countess® system. The morphology of 4T1 cells was monitored by staining cell smears with hematoxylin-eosin and Giemsa methods. HER-2 expression was assessed by immunocytochemistry and metalloproteinase activity was assessed by zymography. The determination of the cytokine profile was performed using Cytometric Bead Array (CBA). The samples were evaluated in quadruplicate and the data were analyzed by one-way ANOVA. *Carcinosinum* 30cH presented the highest apoptotic index and reduction of MMP-9-Pro expression; *Carcinosinum* 200cH produced the highest positivity for HER-2 and no specific effect was seen after the treatment with *Carcinosinum* 12cH. No change in cytokine expression was seen among treatments. We conclude that *Carcinosinum* 30cH and 200cH can change phenotypic features important to tumor development *in vitro*. The clinical meaning of these data deserves further investigation.

Keywords: *Carcinosinum*, 4T1 cells, homeopathy, *in vitro* model cancer, breast adenocarcinoma.