EFFECT OF DECATROPIS BICOLOR AGAINST BREAST CANCER CELLS

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Introduction. Breast Cancer is an important health problem. In Mexico, it represents the second cause of women death (1). Nowadays, there are different methods for the treatment of breast cancer such as chemotherapy. It uses antineoplastic drugs to weaken and destroy cancer cells but also they can produce resistance and side effects. Because of that, new alternatives are being used such as traditional medicine. It uses natural sources as plants that can be more effective and can reduce collateral effects (2). By this reason, *D. bicolor* is a medicinal plant used for the treatment of different illness and also for breast cancer (3).
So, the main objective of the present work was to evaluate the effect of *Decatropis bicolor* against a breast cancer cell line MDA-MB-231.

Methods. To evaluate the cytotoxic effect of *D. bicolor*, different extracts and the essential oil (EO) were obtained. The effect of each extract was analyzed by MTT viability assays on breast cancer cells MDA-MB-231. Also the IC_{50} value was calculated. And at the end, the essential oil was selected because it presented the major cytotoxic activity. So, the effect of the essential oil was studied by analyzing characteristics of apoptosis such as morphological changes (H&E staining), DNA fragmentation (TUNEL assay) and expression of caspases (Western Blot).

Results. Different extracts (aqueous, ethanolic, acetonic, hexanic) and EO were obtained and they were assessed by MTT viability assays using different concentrations (20-400 µg/ml) and times of incubation (24, 48 and 72 h). EO was the most active inducing citotoxicity on breast cancer cells. It showed an IC_{50} value of 53.81±1.69 µg/ml (Table 1). Also, MCF10A epithelial mammary cell line was exposed to EO obtaining an IC_{50} of 207.51±3.26 µg/ml, showing the EO a selective activity on cancer cells.

Table 1. IC50 value obtained for each extract on MTT assays.

Extract	IC50(µg/ml)
Aqueos	----------
Ethanolic	128.20 ± 2.035
Acetonic	203.2 ± 2.30
Hexanic	450.7 ± 2.657
EO	53.81 ± 1.691

So, in order to demonstrate that EO induced an apoptotic process in breast cancer cells MDA-MB-231; first cancer cells were treated with EO using IC_{50} value and analyzed by an H&E staining during 3 to 24 h, results indicated the presence of membrane blebbing, cell shrinkage, cell size reduction and apoptotic bodies, representing the induction of apoptotic process (Fig 1).

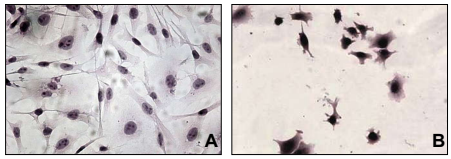

Fig.1 Breast cancer cells stained with H&E staining. A) untreated cancer cells; B) cancer cells treated with the EO using IC50 value during 12 h of incubation.

After that, to confirm the apoptotic process, breast cancer cells were treated with EO using IC_{50} value and incubated with an Annexin-V FITC binding assay, results revealed that the activation of apoptosis process were induced by the essential oil after 0.5 to 24 h of incubation showing a 50% and 90% of apoptosis respectively. Also a TUNEL assay was performed to evaluate DNA fragmentation on breast cancer cells; results indicated the activation of DNA fragmentation after 0.5 to 24 h of exposure with EO (Fig. 2).

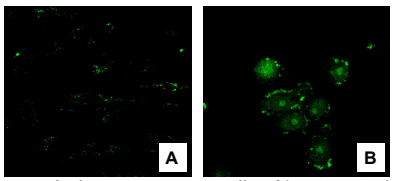

Fig. 2 TUNEL assay in breast cancer cells. A) untreated cancer cells; B) cancer cells treated with the EO using IC_{50} value after 12 h of incubation.

At the end, the analysis of procaspases proteins were performed to demonstrate a pathway apoptosis activation induced by EO; results indicated that expression of procaspases 9 and 3 decreased after 0.5 to 24 h of incubation with EO, thus the intrinsic apoptotic pathway was induced.

Conclusions. EO demonstrated an important cytotoxic activity on breast cancer cells MDA-MB-231 by inducing and apoptotic process with an IC_{50} value of53.81±1.69 µg/ml

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