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In vitro antitumor activity in breast cancer cells (SKBR3 and PMC42) of *Ruta graveolens* in homeopathic dilution

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ABSTRACT

Objectives: Given the need to establish less invasive treatments that improve the patient's quality of life and still have antitumour effects, this study aimed to evaluate the behaviour of breast cancer tumour cells (SKBR3 and PMC42) by treatment with ultradiluted *Ruta graveolens* (*Ruta* D35).

Material and Methods: The breast cancer cell lines SKBR3 and PMC42 were treated with ultradiluted *Ruta* D35 and the *in vitro* effects on cell viability was evaluated by MTT cytotoxicity assay.

Results: In the SKBR3 cells, *Ruta* D35 at concentration of 8 µl/ml induced a significant reduction in cell viability when compared to the untreated control. The treatment with *Ruta* D35 in the PMC42 cells reduced cell viability from the treatment at the highest concentrations of 20 µl/ml, 30 µl/ml, 40 µl/ml and 50 µl/ml.

Conclusion: *Ruta* D35 presented *in vitro* cytotoxic activity in SKBR3 and PMC42 breast cancer cells, thereby reducing cell viability. However, further tests are necessary to better understand the cytotoxic action and the potential of *R. graveolens* for treating breast tumour cells.

Keywords: *Ruta graveolens*, Breast cancer, Homoeopathy, Cell viability

INTRODUCTION

Breast cancer is one of the diseases that most affect women. It may be related to age, genetic factors such as women with a family history of breast cancer, lifestyle and oestrogen exposure, among others.[1] The ability to present tumour cell subtypes with different characteristics coexisting in the same tumour environment due to its high heterogeneity is among the characteristics of this type of cancer.[2]

Radiotherapy is usually recommended for the early stages of breast cancer. Other forms of treatment are indicated at the beginning and in more advanced stages of the disease, such as neoadjuvant therapy, which aims to decrease tumour size before definitive surgery. This treatment modality can also be performed with adjuvant therapy to control and eliminate micrometastases that remained after definitive treatment and may also count on endocrine therapy.[3,4] Sometimes, tumour resection and chemotherapy are performed by combining alkylating and antimetabolite agents. However, the treatment is painful and may generate several cytotoxic side effects for patients.[3,5]

In this context, homoeopathy can be used to improve the patient's quality of life and, at the same time, assist in the treatment. This method uses ultradiluted and dynamic natural compounds and varies according to the patient's symptom board.[6] Animal cell studies have shown that plant compounds may have antitumour effects and are less toxic to healthy cells.[7]

Ruta graveolens is a natural compound that has been used as a therapeutic for cancer cases. This plant belongs to the Rutaceae family and is widely distributed in tropical regions.[8] *R. graveolens* has gained more space in the treatment of various diseases in homoeopathy, including inflammatory and skin diseases, among others, due to its active compounds such as flavonoids, phenolic acids, alkaloids and volatile oils.[9,10] *R. graveolens* presents several compounds of interest in medicine, especially in oncology, where previous studies have demonstrated the antiproliferative effects of the plant extract in tumour cells.[11]

We believe that *R. graveolens* has antitumour effects on breast cancer cell lines. This work was conducted due to the need to create cancer treatment tools that could simultaneously help patients' quality of life. We used the ultradiluted *R. graveolens* extract (*Ruta* D35), once this is the most commonly used potency in Brazil, to evaluate it's *in vitro* effects on breast cancer cells viability.

MATERIAL AND METHODS

Preparation of *Ruta* D35

The Mother Tincture was used as the starting point for preparing the tested substance (*Ruta* D35). As described in the Brazilian Homeopathic Pharmacopoeia, the Hahnemannian Decimal Method was used. Using a sterile isotonic solution, one part of the active ingredient was mixed with nine parts of the inert ingredient and succussed 100 times, yielding *Ruta* D1 (1×10^{-1}). Then, one part of *Ruta* D1 was used with nine parts of the inert ingredient and succussed 100 times, yielding *R. graveolens* D2 (1×10^{-2}). The successive dilution continued till *Ruta* D35 was obtained.[12] These products were then bottled in 1.1 ml ampoules.

Cell culture

The breast cancer cell lines used in these tests, SKBR3 and PMC42, were donated by the Biotechnology and Genomic Sciences Laboratory from the Catholic University of Brasilia (purchased by the ATCC and grown according to the protocol). The cell line SKBR3 was cultivated with Dulbecco's Modified Eagle Medium added with 10% foetal bovine serum (all products from the Sigma-Aldrich® brand). The PMC42 cell line was cultivated with RPMI-1640 added with 10% foetal bovine serum (all products from the Sigma-Aldrich® brand).

Cytotoxicity (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide [MTT] assay)

Cells were plated in 75 cm^2 culture flasks and incubated in a controlled atmosphere cell culture oven (37°C, 5% CO_2) until they reached approximately 80% confluence. Cells were then trypsinised with the enzyme TrypLE™ (Thermo-Fisher), counted and plated in 96-well plates at a concentration of 2.5×10^4 cells/ml culture medium.

After 24 h, the medium was replaced with a medium containing *Ruta* D35 at nine concentrations (2, 4, 8, 6, 10, 20, 30, 40 and 50 µl/ml), remaining for 48 h in culture. This procedure was performed in triplicate of wells and plates. The control treatment utilised the same water used in the dynamisation, at the same nine concentrations from 2 to 50 µl of water per ml of medium. Furthermore, control wells were used only with a culture medium, with no addition of any other product to confirm cell growth. After 48 h, the MTT colourimetric assay was performed by adding the reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Catalogue: Sigma N. M2128) in the wells at 0.5 mg/ml followed by incubation for 4 h at 37°C and 5% CO_2. Subsequently, the following steps were performed: Dilution of the formazan crystals with dimethylsulphoxide (Sigma-Aldrich) and plate absorbance reading at 570 nm in a spectrophotometer (Model DR-200BS Kasuaki).

Statistical analysis

Cellular viability was calculated concerning the absorbance obtained in the control group. The percentages obtained were analysed by the Dunnett's multiple comparison test using the GraphPad Prism® 7.04.

RESULTS

The SKBR3 and PMC42 lines treated with *Ruta* D35 at various concentrations (2–50 µl/ml) demonstrated a dose-response effect. *Ruta* D35 had significantly reduced cell viability in the SKBR3 line compared to the control of untreated cells. A significant difference was obtained from the concentration of 8 µl/ml ($P = 0.0391$*). At this concentration, it was already possible to observe a reduction in cell viability. The dose-response effect was more evident in SKBR3 cells treated with higher *Ruta* D35 concentrations (50 µl/ml). At this concentration, cell viability was more expressively reduced than in the control treatment (****$P < 0.0001$), as shown in [Figure 1].

Conversely, *Ruta* D35 significantly reduced cell viability in the PMC42 line at higher concentrations (20–50 µl/ml), as shown in [Figure 2]. Cellular damage was observed in the SKBR3 line, leading to a significant reduction in cell viability at low concentrations (8 µl/ml). In contrast, no significant

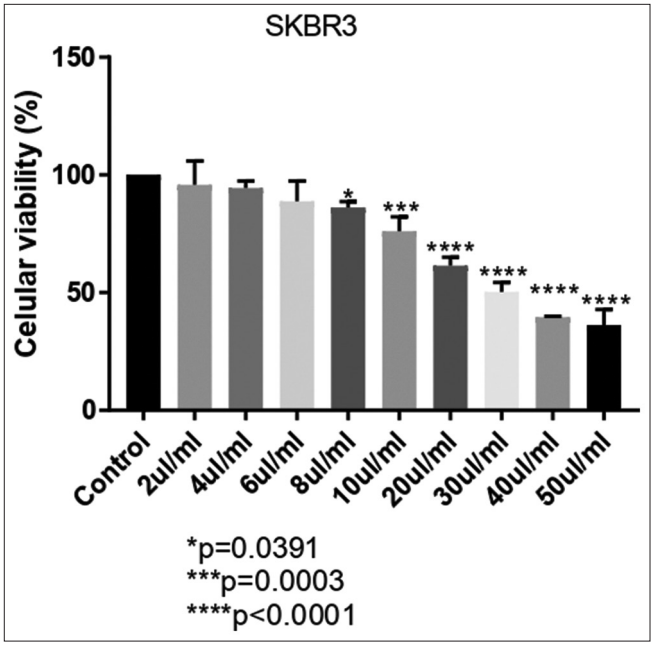

Figure 1: MTT cell viability assay. The X-axis corresponds to the *Ruta* D35 concentration and the Y-axis represents the percentage of cell viability. The untreated SKBR3 cells comprise the control treatment. Cells treated with 8 µl/ml presented cellular damage and consequent reduction of cell viability when compared with the control (**P* = 0.0391). Reduction in viability became more evident at 10 µl/ml (****P* = 0.0003), and following the dose-response effect, cell viability significantly reduced at higher concentrations (20–50 µl/ml; *****P*<0.0001.

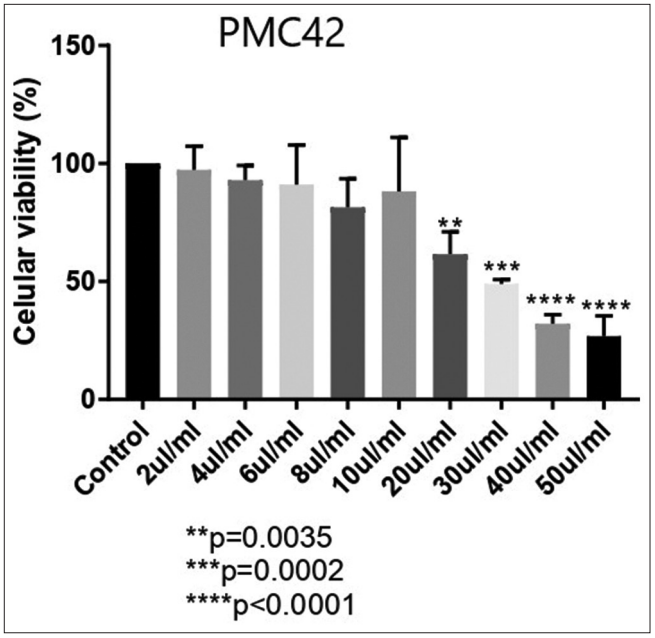

Figure 2: MTT cell viability assay. The X-axis corresponds to the *Ruta* D35 concentration and the Y-axis represents the percentage of cell viability. The untreated PMC42 cells comprise the control treatment. Cells treated with 20 µl/ml presented cellular damage and consequent reduction of cell viability when compared with the control (***P* = 0.0035). Reduction in viability became more evident at 30 µl/ml (****P* = 0.0002), and following the dose-response effect, cell viability significantly reduced at higher concentrations (40 and 50 µl/ml; *****P*<0.0001.

reduction in cell viability was recorded in the PMC42 line when exposed to the same concentration.

DISCUSSION

Homoeopathy appears as a complementary treatment using ultradiluted and dynamic natural compounds. Concerning cancer disease, homoeopathy may assist the patient due to its ability to decrease the side effects generated by conventional treatments.[13,14] Preliminary studies showed that patients with hormone-sensitive breast cancer receiving aromatase inhibitor treatment combined with treatment using *R. graveolens* 5CH and *Rhus toxicodendron* 9CH (homoeopathic compounds) presented reduced joint pain and stiffness.[15]

In the present study, we evaluated the *in vitro* effects of different dilutions of *Ruta* D35 for treating two breast cancer lines (SKBR3 and PMC42). We used the MTT assay, a standard well consolidated and diffused test commonly used to evaluate the metabolic activity of cells *in vitro*. Cell viability levels were compared with the viability of untreated cells. Thus, it was demonstrated that breast cells had a dose-response effect and became sensitive to the treatment with *Ruta* D35. This result suggests that this homoeopathic compound can generate cell death in these cancer lines.

Acridone alkaloids are among the compounds present in *R. graveolens*. Isolated acridone alkaloids tested in different dilutions to treat breast cancer lines showed significant pro-apoptotic and antiproliferative effects compared to cisplatin.[16] Our findings on the effects of *Ruta* D35 on the viability of the SKBR3 and PMC42 breast cancer lines corroborate previous studies in which breast cancer cells, such as MCF-7, presented cell viability reduction, p53 pathway activation and damage to the mitosis process after treatment with *R. graveolens* extract.[11] These results indicate *R. graveolens* potential for reducing the viability and causing cellular damage in tumoural lines.

We observed that the various *Ruta* D35 dilutions produced cytotoxic effects in the breast lines. Similar results were reported in colon cancer lines treated with various potencies of *R. graveolens*. These lines had their cell cycle damaged in the G2/M phase and cell viability was reduced due to the apoptosis process, similarly to solid tumours, which presented reduction when treated with *Ruta* dilution at 200C.[17,18]

Studies reporting the effects of homoeopathic treatments are extremely important due to the limited data on the efficacy of this treatment in cancer patients.[19] Studies on the effects of homoeopathic treatment, especially in the case of *R. graveolens*, have shown promising results regarding antitumour effects on cancer lines. Therefore, it is essential to elucidate the mechanisms behind this effect and the benefits of using this complementary treatment.

CONCLUSION

Ruta D35 presented *in vitro* cytotoxic damage to the SKBR3 and PMC42 breast cancer cells, thereby reducing cell viability. Both cell lines showed sensitivity to the treatment with several *Ruta* D35 dilutions compared with untreated cells. Our results indicate that ultradiluted *R. graveolens* has antitumour capacity. However, further tests are necessary to better understand the cytotoxic action and the potential of *R. graveolens* for treating breast tumour cells.

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Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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