

CYTOTOXIC EFFECT OF DILLAPIOLE ON HUMAN BREAST CANCER MCF-7 CELLS

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ABSTRACT

Plant secondary metabolites and their derivatives play a significant role in anticancer drug therapy since they are effective against specific characteristics while reducing side effects. Dillapiole is a phenylpropanoid that holds several bioactivities like anti-fungal, anti-inflammatory, anti-leishmanial, and anti-cancer. This study aims to investigate the possible cytotoxic effect of dillapiole on human breast cancer, MCF-7 cells. Cells were cultured in complete RPMI media and incubated at standard culture conditions. After the cells reached 80% confluency, cells were treated with various concentrations (ranging from 0 μ M to 150 μ M) of dillapiole and tamoxifen as a positive control. Cells were later incubated at 48 and 72 h. Using WST-1 assay, the cytotoxic effect was determined for both incubation times. Results show tamoxifen inhibited the MCF-7 cells with the IC₅₀ at 75.9 μ M and 39.8 μ M for 48 and 72 h respectively. Parallel with the positive control results, there was a significant cytotoxic effect of dillapiole against MCF-7 cells at 48- and 72-hr incubation with the IC₅₀ at 92.1 μ M and 63.1 μ M respectively. Based on these results, dillapiole was cytotoxic against MCF-7 cells and its cytotoxic activity was both in a time and dose-dependent manner (<0.05). The morphological analysis supported the WST-1 assay. Our preliminary finding is in agreement with other previous studies, suggesting that dillapiole appears to be a promising anti-cancer agent and opens a wider possibility of downstream analysis on its underlying cytotoxicity mechanism.

Key words: Anti-cancer, cytotoxic, Dillapiole, MCF-7 cells, and WST-1 assay

INTRODUCTION

With an anticipated 2.3 million new cases, female breast cancer has surpassed lung cancer as the most commonly diagnosed disease in women. Breast cancer is the most commonly diagnosed cancer in women and the most prevalent cause of cancer mortality (Sung *et al.*, 2020). MCF-7 cells were constructed at the Michigan Cancer Foundation in 1973. It is the most studied cancer cell line in the world (Lee *et al.*, 2015). There are two types of breast cancer about estrogen-receptor expression. There is ER (estrogen-responsive) positive and ER-negative. About 60-65% of breast cancers are ER-positive. ER-negative tumors are less affected by cancer prevention and treatment compared to ER-positive tumors which have a better prognosis (Pan *et al.*, 2011). MCF-7 is an estrogen receptor-positive human breast cancer cell line (Gunduz & Gunduz, 2011). MCF-7 cells are useful for in vitro breast studies because they retain several ideal mammary epithelial characteristics, such

as estrogen processing in the form of estradiol via estrogen receptors in the cell cytoplasm (Comsa *et al.*, 2015). It's the first breast cancer cell line to respond to hormones, thus making it ideal for therapeutic testing. The cells can form domes and develop in monolayers when produced in vitro (Russo *et al.*, 1977).

The cytotoxicity effect is a mechanism where a compound can kill and destruct the cells. Thus, it is becoming a fundamental aspect in the screening of anti-cancer therapy for *in vitro* cytotoxicity testing. This testing is considered simple, cost-effective, and an efficient predictive tool in characterizing any toxic potential of a new drug. All the chemical entities tend to become poisonous depending upon dosage. Therefore, toxicity testing becomes a necessity and crucial not only in the research field but more importantly in the industrial scale setting (Niles *et al.*, 2009).

Conventional treatment of cancer like chemotherapy is not selective against cancer cells and can be toxic to both cancer and normal cells. Killing both cancerous and normal cells can lead to

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adverse side effects (Benjamin, 2021). Tamoxifen has an anti-estrogenic effect and has been used as a first-line treatment for estrogen-positive breast cancer patients for over 30 years after its introduction. Even though tamoxifen significantly reduced the recurrence and mortality rates of the disease, a considerable number of reported cases prove that tamoxifen itself is carcinogenic in gynecological reproductive organs (Abd-Alhussain *et al.*, 2020).

Over a decade ago, medications are usually produced from natural sources like plants. The plant itself or a certain compound will be extracted from the source and act as the main ingredient of a medication. Some plants had been proven to play a certain role in chemotherapy treatment for cancer (Gao *et al.*, 2011). It is proven from a study that a particular cytotoxicity agent succeeded in killing a few human cancer cell lines but is not toxic to normal cells (Soprano *et al.*, 2016). There were many active compounds from plants that have been demonstrated to show cytotoxic effects on MCF-7 cell lines with a promising apoptosis involvement in its inhibition mechanism (Ghandourah *et al.*, 2017).

Peperomia pellucida for example is a plant that has been used for years to improve one's health. The plant is commonly found in Africa, South America, and Southeast Asian countries (Tamokou *et al.*, 2017). It is a traditional medication for treating several diseases like gastric ulcers, skin problems arthritis, and high cholesterol level. The main component of *Piper aduncum*, a species of *Peperomia pellucida* is dillapiole, a compound that potentially can treat diseases (Alves *et al.*, 2019). Dillapiole has been found to have an anti-proliferative effect on several cancer cells including oral and ovarian cancer cells (Chan, 2014). Recently, it was reported that dillapiole has shown anti-cancer properties in breast cancer cell line, the MDA-MB-231 (Ferreira *et al.*, 2014). However, the anti-cancer property of dillapiole towards MCF-7 cell lines is yet to be discovered. Therefore, this study was intended to analyze the cytotoxicity potential of dillapiole in the breast cancer cell line, the MCF-7 cells.

MATERIALS AND METHODS

Sample preparation

For human breast cancer MCF7 cells were cultured in RPMI 1640 medium supplemented with 10% of fetal bovine serum and 100 units/mL penicillin and 100 µg/mL streptomycin in standard cell culture condition (37 °C, 5% CO₂ & 80% humidity). Dillapiole (Extrasynthese, France) and tamoxifen were dissolved in dimethyl sulfoxide (DMSO) at 50 mM concentration as the main stock solution.

Cytotoxic effects using WST-1 assay

Cells were seeded at the concentration of 2×10^5 per well in the 96-well culture plates for 24 h. Next, the culture medium is replaced with a fresh medium containing dillapiole and tamoxifen respectively at different concentrations (ranging from 10 µM, 25 µM, 50 µM, 75 µM, 100 µM, 125 µM & 150 µM) in triplicates. The negative control group was prepared using the vehicle alone. Cells were incubated with both compounds for both 48 and 72 h respectively in standard culture conditions. After the incubation times, the cytotoxicity activities of the compounds were assessed using WST-1 assay (Roche, Switzerland). After treatment, 10 µL of WST-1 labeling reagent was added to each well and incubated again for 90 min in 5% CO₂ at 37 °C. The plate was shaken for 1 min to mix the contents and its absorbance at $\lambda=450$ nm as measured against background control using an ELISA reader. The percentage of the viability of the cells was calculated using the following formula:

Equation 1:

$$\% \text{ Viability} = \frac{\text{Mean absorbance of pure compound-negative control}}{\text{Positive control-negative control}} \times 100$$

From the calculation generated using the formula, the graph is produced based on the percentage of cell viability against the different concentrations of dillapiole (Figures 1 & 2) and tamoxifen (Figures 3 & 4) at 48- and 72-h incubation time.

Morphological observation

Morphological observation of the treated MCF-7 cells was carried out concurrently during the WST-1 assay procedure. After the incubation times, cells were immediately observed under the inverted phase contrast microscope at 100x magnification. Viable and non-viable cells were identified and analyzed. Cells have then proceeded to next the step of the WST-1 assay following the manufacturer's protocol.

Statistical analysis

All data from experiments were reported as the mean \pm standard deviation (SD) using SPSS software (IBM version 20.0). The differences in percentage resulting from different concentrations of IONPs for antibiofilm and cytotoxicity assays were analyzed by the Kruskal Wallis H test followed by posthoc test, Mann Whitney U. Value $P < 0.05$ was regarded as statistically significant (Khoramian *et al.*, 2015).

RESULTS AND DISCUSSION

Cytotoxicity effect of dillapiole against MCF-7 cells

The results show that dillapiole exhibited a cytotoxic effect on MCF-7 cells by reducing the cell

viability at both 48 and 72 h (Figures 1 to 2) of incubation time. It is observed that the cytotoxic effect of dillapiole was increased upon the increase of the compound concentration and incubation time. The present results revealed that the cytotoxic effect is dose and time-dependent (<0.05) with optimum IC_{50} observed at 72 h. Tamoxifen (Figures 3 to 4), an established drug treatment particularly for estrogen-positive breast cancer patients is used as a positive control in this experiment. The main principle of treatment that can be considered cytotoxic includes when the cytotoxic compound can prevent cellular attachment, adversely affects cell replication cause significant morphological changes with cell disruption effects that eventually lead to the reduction of overall cell viability (Niles *et al.*, 2009). Based on this data (Table 1), both compounds showed parallel cytotoxic

effects, suggesting that dillapiole could be a potent candidate for managing breast cancer patients

This preliminary finding also is in agreement with other studies done using a different panel of cancer cells such as the triple-negative breast cancer MDA-MB-231 cell and the melanoma (Mel-85; SK-MEL-28, Sbc1-2;) cell lines. However, IC_{50} obtained in the present study was much higher compared to the reported IC_{50} in other cell lines. According to Ferreira *et al.*, IC_{50} obtained from different panels of cancer cell lines such as in breast and melanoma cancer (MDA-MB-231; Mel-85; SK-MEL-28 & Sbc1-2) is within the range of 25 to 27 μM (Ferreira *et al.*, 2014). The difference might be partly influenced by the absence of estrogen-receptor in MDA-MB-231 cells or different types of cell origin for melanoma cells.

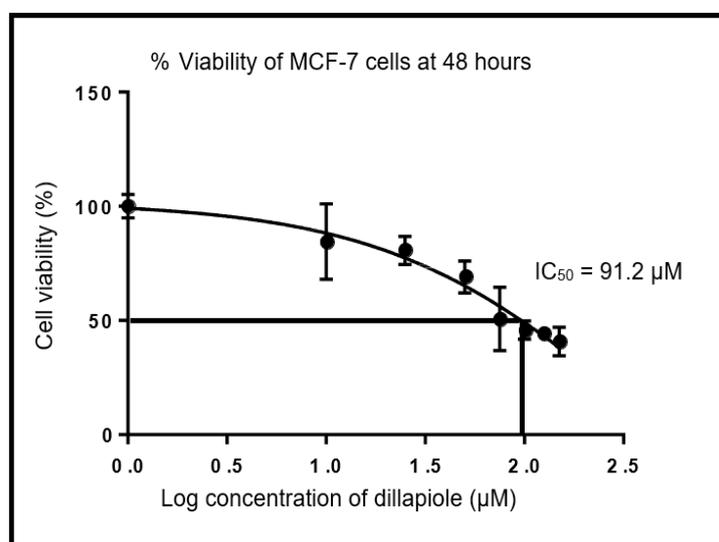


Fig. 1. Graph for percentage viability of MCF-7 cells treated with dillapiole at 48 hours.

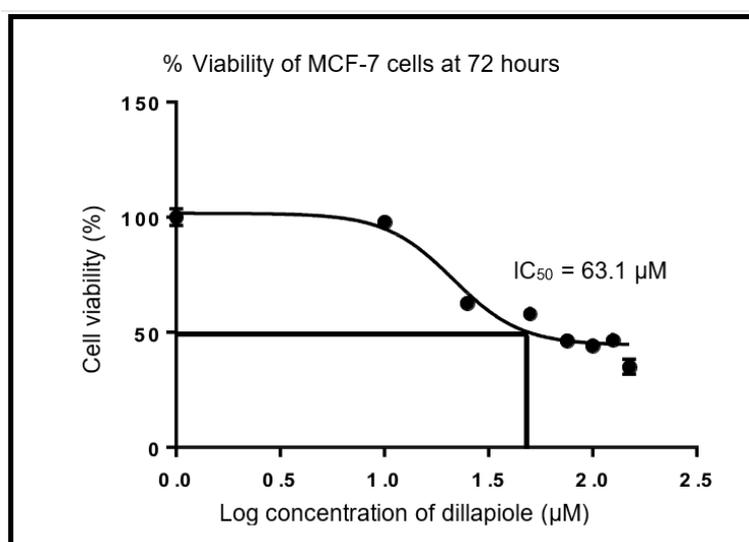


Fig. 2. Graph for percentage viability of MCF-7 cells treated with dillapiole at 72 hours.

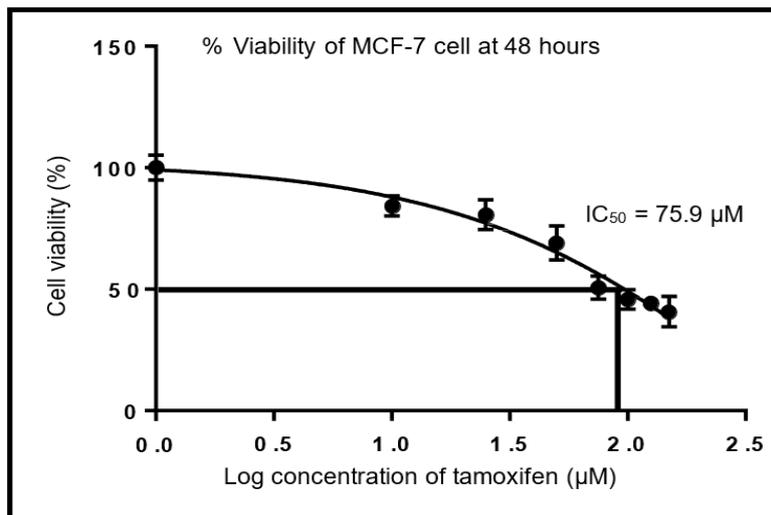


Fig. 3. Graph for percentage viability of MCF-7 cells treated with tamoxifen at 48 hours.

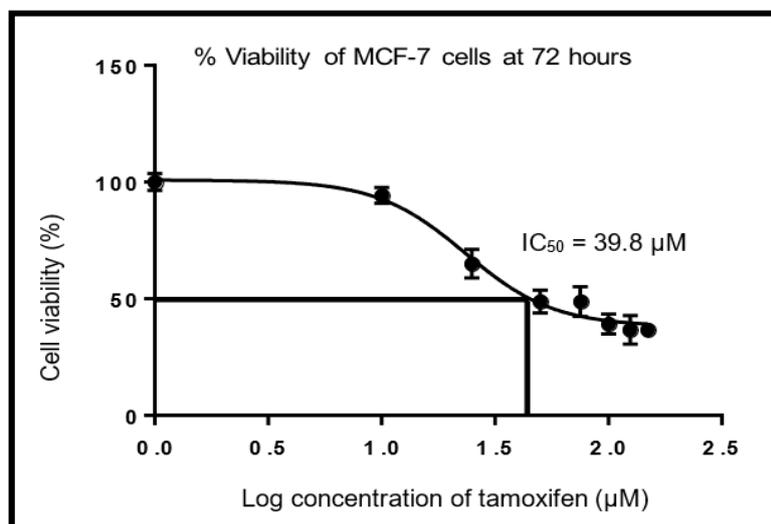


Fig. 4. Graph for percentage viability of MCF-7 cells treated with tamoxifen at 72 hours.

Table 1. IC₅₀ concentrations for dillapiole and tamoxifen at 48 and 72 hour of incubation time

| Compound | Incubation time | |
|------------|-----------------|--------------|
| | 48 hour (µM) | 72 hour (µM) |
| Dillapiole | 91.2 ± 0.58 | 63.1 ± 0.46 |
| Tamoxifen | 75.9 ± 0.37 | 39.8 ± 0.29 |

Morphological analysis

Observation of morphological changes (Figure 5) demonstrated a similar trend with increasing inhibition patterns seen upon higher dillapiole concentration and incubation time. However, detailed morphological changes of the cells were not comprehensively studied here and more data collection in the future is necessary to reveal the mode of inhibition of MCF-7 cells. Previously, a study done both *in vitro* and *in vivo* showed that dillapiole induced apoptosis in mouse

blastocysts in a dose-dependent manner (Chan., 2014).

Interestingly, the cytotoxicity effect of dillapiole on both estrogen-positive MCF-7 as well as in estrogen-negative MDA-MB-231 indicates the possible cytotoxic mechanism is not depending on the regulation of hormone receptors. This suggests the broad capacity of the cytotoxic properties of dillapiole on a different panel of breast cancer cell lines. Based on that fact, dillapiole could also be cytotoxic to

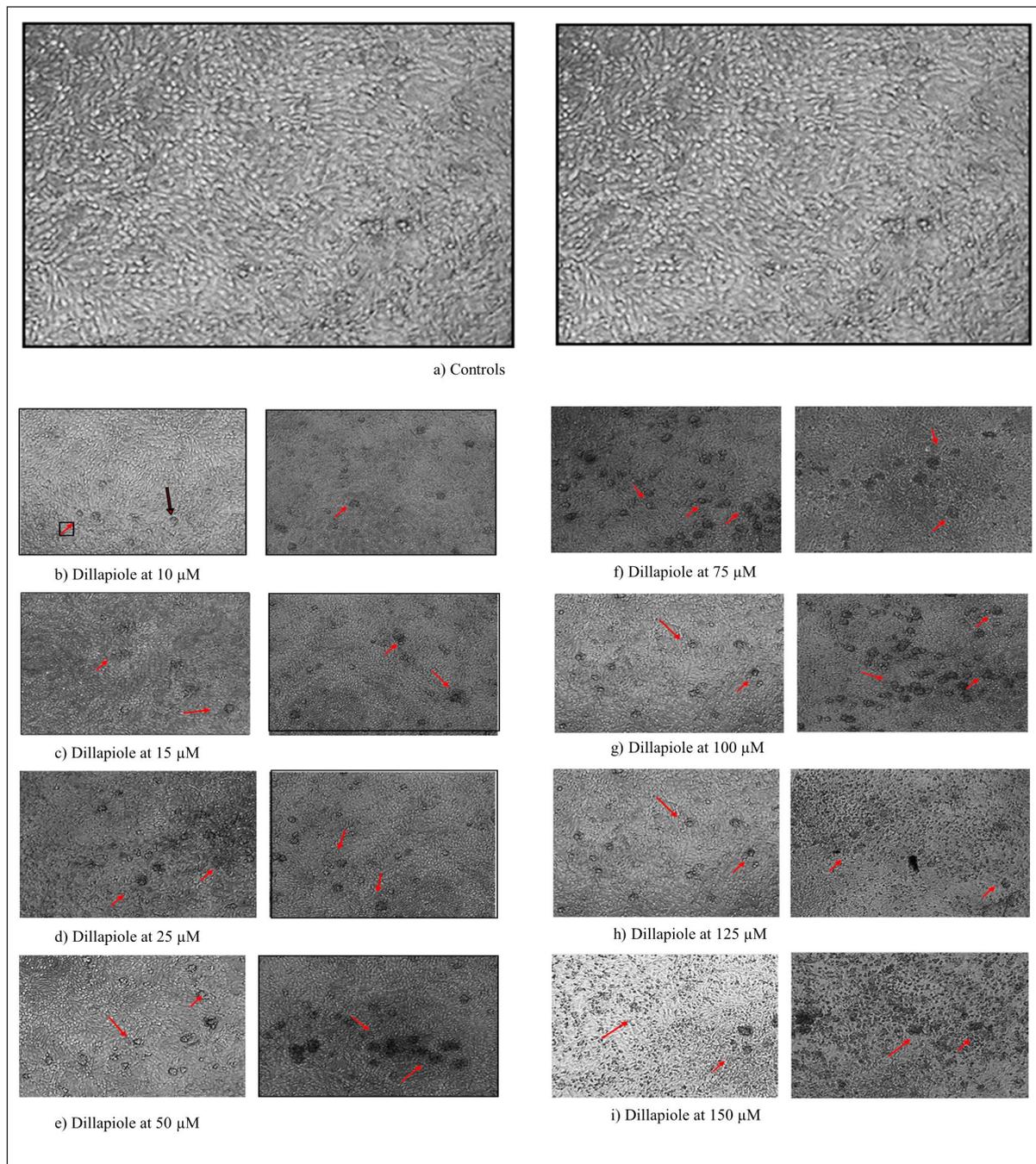


Fig. 5. Representative photomicrograph shows morphological changes of MCF-7 cells using an inverted phase-contrast microscope (100 \times). Cells were treated with dillapiole for 48 hours (left) and 72 hours (right) at different concentrations respectively (a – i). The increase of detached cells shown is parallel with the increase of dillapiole treatment. Red arrows indicate the detached cells.

other types of breast cancer cells such cells as HER2-enriched breast cancer. However, this claim requires more explanation and could be a good baseline for future studies. On the other hand, this finding also paved the way for other studies by looking at the underlying cytotoxic mechanism. It is reported in other studies that dillapiole exhibited anti-cancer activities by arresting cells at the G0/G1 phase. It is also shown that the anti-migration effects of dillapiole can be associated with the disruption of actin filament which prevents tumor cell proliferation.

CONCLUSION

In conclusion, we have successfully demonstrated the cytotoxic effect of dillapiole against MCF-7 cells. Our preliminary findings indicate that the cytotoxic activity of dillapiole against MCF-7 cells is dose and time-dependent manner. The optimum IC_{50} of dillapiole (IC_{50} : 63.1 μ M) obtained in this experiment was achieved after 72 h of treatment incubation. Further studies are required to elucidate the mechanism of inhibition of dillapiole against MCF-7 cells. This study also suggests that the mode of cytotoxic effect in dillapiole is independent of the hormonal regulation effect, therefore recommending a possibility of investigation on other panels of cancer cells such as HER2 enriched breast cancer type.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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