

Study on Cytotoxic and Apoptotic Potential of *Barringtonia asiatica* Seed Kernel against MCF-7 Cell Line.

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Breast cancer is the commonest carcinoma among women worldwide and the crude breast cancer incidence in Sri Lanka is 19 per 100,000 population, which is on the rise in the younger age groups due to increased life expectancy and urbanization. Thus discovery of novel pharmacological agents for effective therapeutic control and treatment is essential. *Barringtonia asiatica*, mainly grown as an ornamental plant in Sri Lanka is a native of mangrove habitats on the tropical coasts and islands of the Indian Ocean. The present study investigated the cytotoxic potential and mechanisms of cytotoxicity of crude methanolic extract (CME) and an isolated fraction (MPLCBA-3) of CME from *Barringtonia asiatica* seed kernel against MCF-7 cell line. The cytotoxic potential was determined by standard lactate dehydrogenase (LDH) assay and the mechanism of action with Caspase Glo 3/7 assay. Each of results were expressed as the average of replicates. The percentage leakage of cellular LDH to the medium increased with increasing concentrations of both CME and MPLCBA-3 compared to control. The cytotoxic potential of MPLCBA-3 was higher at lower concentrations than with CME. Caspase 3/7 activities significantly ($p < 0.05$) increased in MCF-7 cells after treatment with both CME and MPLCBA-3 at all tested concentrations when compared with the untreated control after 24 hours. Subsequently treated MCF-7 cell DNA on gel electrophoresis showed a smeared pattern indicating DNA fragmentation. According to the results obtained, both CME and MPLCBA-3 indicated cytotoxicity and initiation of apoptosis in the MCF-7 cells, with higher cytotoxic potential present in the isolated fraction.

Key words: *Barringtonia asiatica*, LDH assay, Caspase 3/7 activity, DNA fragmentation.

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Extended Abstract

Introduction

Breast cancer causes significant morbidity and mortality among women worldwide (Guo *et al.*, 2013). Lack of effective therapeutic strategies for control and treatment of breast cancers, pharmacological agents have necessitated the search for newer therapies form of natural products. *Barringtonia asiatica* is a species of *Barringtonia* native to mangrove habitats on the tropical coasts and islands of the Indian Ocean and is grown as an ornamental plant in Sri Lanka. The present study investigated the cytotoxic potential and mechanism of cytotoxicity of crude methanolic extract (CME) and an isolated fraction (MPLCBA-3) from *Barringtonia asiatica* seed kernel against MCF-7 cell line.

Methodology

Thus this study aims to investigate the toxicity and mechanism of the crude methanolic extract (15 g powder / 40 mL MeOH; 24 hrs; dried at 45°C) and MPLCBA-3 fraction obtained by medium pressure liquid chromatography against MCF-7 cell lines. MCF-7 cells were seeded in 24 well plates (2×10^5 Cells/ mL). These were treated with different concentration of CME (10, 25, 50, 75, 100, 125 ppm) and MPLCBA-3 fraction (5, 10, 15, 20, 25, 30 ppm) of *B. asiatica*. After 24 h incubation, LDH activity was determined by using LDH assay kit (Lindamulage and Soysa, 2016). MCF-7 cell line was trypsinized and cell suspensions added (200 μ L, 2×10^5 Cells / mL) to a 96 well plate. Cells were treated with different concentrations of CME (5, 10, 20, 40 ppm) and MPLCBA-3 fraction (2.5, 5, 10, 20 ppm) of *B. asiatica*. After 24 hours, caspase activity was determined using caspase 3/7 assay kit (Sivakumaran *et al.*, 2018). Luminescence was measured using micro plate reader. MCF-7 cell line was trypsinized and cell suspensions added (1 mL, 5×10^4 Cells / mL) in a 24 well plate. Bottom of each well contained cell culture treated cover slips and these were incubated for 24 hours at 37°C in 95% air/5% CO₂ atmosphere, with 95% humidity. Cells were treated with different concentrations of CME (20, 30, 40 and 60ppm) and MPLCBA-3 (5, 10, 15, 20 ppm) of *B. asiatica*. Slides were prepared using standard procedure. The prepared slides were observed under fluorescence microscope (Sivakumaran *et al.*, 2018).

Results and Discussion

Total LDH activity in the medium increased (4.0- 17.74%) with increasing concentration of CME (10-125 ppm). With MPLCBA-3 fraction the activity increased (4.05-8.71%) from 5-30 ppm against MCF-7 cells after 24 hours. LDH activity increased with increasing concentration of the cytotoxic fraction. This confirms cell membrane damage by CME and MPLCBA-3 fraction.

Caspase Glo 3/7 were activated significantly ($P \leq 0.001$) at 5, 10, 20 ppm of CME ($P \leq 0.05$) at thymoquinone, ($P \leq 0.001$) at 2.5, 5 ppm, ($P \leq 0.05$) at 10 ppm of MPLCBA-3 fraction, ($P \leq 0.01$) at thymoquinone against MCF-7 cells When MCF-7 cells were treated with both fractions a dose-dependent activation of caspase Glo 3/7 were observed. All tested concentrations of CME and MPLCBA-3 fraction demonstrated significant percentage of caspase activity in the cells of both cell lines compared to the controls. cell lines the MPLCBA-3 fraction at a lower concentration than positive control indicated high potential in signaling for apoptosis.

AO/EB fluorescence staining was used to confirm that CME and MPLCBA-3 fraction of *B.asiatica* induced apoptosis in MCF-7 cells. AO/EB staining showed uniform green cells in the control of MCF-7 cells whereas apoptotic cells in the early stage were marked by yellow-green (20, 30, 40 ppm of CME, 5, 10 ppm of MPLCBA-3 with MCF-7 cells and apoptotic cells in the late stage which were marked with concentrated and asymmetrically orange nuclei (60 ppm of CME, 15, 20 ppm of MPLCBA-3, 10 ppm of thymoquinone with MCF-7 cells) under fluorescence microscope.

Both MCF-7 and HepG2 cells indicated different stages of apoptosis which was apparent in the color changes in cells observed with CME and MPLCBA-3 fraction. Early-stage apoptotic cells were marked by crescent-shaped or granular yellow-green acridine orange nuclear staining by CME concentrations at 20 ppm (MCF-7) and 10 ppm (HepG2). A similar observation was made in both cell lines with Hoechst stain (figures 4.43 and 4.44). At concentrations of 40, 60 ppm of CME (MCF-7) and 40, 80 ppm CME (HepG2)

Conclusion

Biochemical changes linked with apoptosis included leaching of LDH indicating membrane damage, activation of caspase. The increase in caspase 3/7 activity and the increased LDH activity indicated that these extracts play an important role in apoptosis. Fluorescence microscopic analysis further confirmed that cells undergo apoptosis. According to the results obtained CME and MPLCBA-3 indicated cytotoxicity and apoptotic effects on the MCF-7 cell line.

References

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