Anti-Breast Cancer Effect of Selective Qatari Medicinal Plant Crude Extracts

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ABSTRACT

Breast cancer is the most commonly diagnosed cancer among women around the world, including Qatar. Although numerous treatments exist, these conventional treatments are often ineffective over time, are toxic to normal cells, and have severe side effects. Therefore, Complementary and Alternative Medicine (CAM) are gaining attention due to their safety and effectiveness. This study aims to determine the anti-breast cancer effect of some Qatari medicinal plants. We tested the alcoholic crude-extract of Nigella sativa (NS) seeds, Senna italica, Glossonema edule, and Glossonema glutinosa leaves against breast cancer cell survival, proliferation, and migration. Our data revealed that crude extract of Glossonema glutinosa did not have any significant effect on breast cancer cell killing. However, crude extract of Senna italica and Nigella sativa had minimal breast cancer cell killing effect. Interestingly, crude extract of Convolvulus glomeratus showed strong breast cancer killing effect. Surprisingly, scratch wound migration assay showed that all the crude extracts tested had the capacity to inhibit breast cancer cell migration. We further analyzed the molecular mechanisms of anti-breast cancer effect of Convolvulus glomeratus as it showed the best effect. Pro-apoptotic effect of Convolvulus glomeratus crude extract was confirmed by fluorescence activated cell sorting (FACS) using Annexin V/PI staining, and by western blot for pro-apoptotic marker caspase 3. Further fractionation studies are required to determine the active compounds of these medicinal herbs, which exert its anti-breast cancer effect. After successful animal and human studies, this Qatari native plant could be a promising natural remedy against breast cancer.

OBJECTIVES

- To determine the effect Nigella sativa (NS) seeds, Senna italica, Glossonema edule, and Glossonema glutinosa Choissey leaves against breast cancer cell survival, proliferation, and migration.
- To examine the molecular mechanisms behind the effect of Convolvulus Glomeratus Choissey leaves against MDA-MB 231 breast cancer cells.

INTRODUCTION

Breast cancer (BC) is the type of cancer that affects the tissues of the breast. It’s one of the most prevalent invasive cancers that affects 1 in 7 women worldwide and is expected to increase to 376 by 2025 from the over 508,000 deaths reported in 2011 (1). BC cases in Qatar has shown a rapid increase about 22 times higher in 2012 from 2011 (2). The lethal and uncontrolled proliferation of the breast tissue cells could arise due to exposure to ionized radiations, old age, obesity, alcoholism but the most major risk factors are gender and genes – family history (3). MDA-MB 231 is ‘Basal-like’ BC cell line which is Triple Negative and is more sensitive to lethal and uncontrolled proliferation of the breast tissue cells that affects 1 in 7 women worldwide and is expected to increase to 376 by 2025 from the over 508,000 deaths reported in 2011 (1). BC cases in Qatar has shown a rapid increase about 22 times higher in 2012 from 2011 (2). The lethal and uncontrolled proliferation of the breast tissue cells could arise due to exposure to ionized radiations, old age, obesity, alcoholism but the most major risk factors are gender and genes – family history (3). MDA-MB 231 is ‘Basal-like’ BC cell line which is Triple Negative and is more sensitive to lethal and uncontrolled proliferation of the breast tissue cells. These results suggest great potential in the use of natural, Qatari medicinal plants as a treatment for BC cells.  Additionally, we found that alcoholic extracts kill breast cancer cells more efficiently than acetone extracts. This is due to salivary extracts having high antioxidant activity, and phenolic compounds. In figure 2, the scratch and wound assay depicts the effect of the treatment of Senna italica plant extract on the migration of MDA-MB 231 BC cell line. Treatment at high concentrations (1000µg/ml and 2000µg/ml) had the most inhibitory effect after 24hr. On the other hand, low treatment concentrations (62.5µg/ml, 125µg/ml, 250µg/ml) had very low inhibitory effect as the cells showed migration towards the wounded area. When compared to the untreated cells (control), the treated cells showed high inhibition of migration. This is especially in the case of the treatments at higher concentrations. Similar results were observed for NS and Glossonema extracts. In figure 3 and 4, the Western blot showed the expression of Caspase-3, Cleaved (C) Caspase-8 and -6. The methanolic extracts of Convolvulus glutinosa Choissey was depicted to kill breast cancer cells effectively as shown by the FACS analysis study that used Annexin V/PI stain. Moreover, Caspase 3, apoptosis inducing protease was expressed. Cellular proteins such as Gelsolin, Icat/Cd, -frin and others were activated in the presence of Caspase 3 to aid and facilitate apoptosis.

METHODOLOGY

EXTRACTION

First, the sample was harvested and freeze dried. After grinding, the sample was dissolved in organic solvent either in acetone or alcoholic extract, then placed in a Tissue Lyser (Qiagen), then dried under the Merckies evaporator. Lastly, the sample was vacuum dried and stored at -4℃ until use.

CONCLUSION

In this study, anti-breast cancer tumor characteristics expressed by alcoholic extracts of Convolvulus glutinosa. Glossonema edule, Senna italica and Convolvulus glutinosa Choissey were tested on MDA-MB 231 BC cell line against proliferation and migration. While Convolvulus demonstrated significant inhibitory effects on cell proliferation, Nigella sativa and Senna italica had minimal cytotoxic effects. However, they both showed significant and strong inhibition of migration, and Glossonema gave no significant effect on BC cell killing along with minimal inhibition of migration of cells. In addition, flow cytometry and western blot analysis showed that treatment with Convolvulus extract had significant pro-apoptotic effect. Further studies for fractionation are required to determine the active compounds of these medicinal herbs, which exert its anti-breast cancer effect. These results suggest great potential in the use of natural, Qatari medicinal plants as a treatment for BC cells. Future studies using animal and human models would be next step in establishing such CAM plants as an anti-cancer drug. We acknowledge QNRF UERP22-143-024 for funding this research project.

REFERENCES


RESULTS

Table 1. Effects of (A) Nigella sativa (NS) by AlamarBlue. (B) Glossonema edule, and (C) Senna italica by MTT on the proliferation of MDA-MB 231 Breast Cancer cell line. (D) The effect of Acetone and methanol extract of Convolvulus glutinosa Choissey on MDA-MB 231 after 24 hrs and 48 hrs P-value 0.05 is considered significant as compared to DMSO Ctrl. *p<0.05

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Figure 2. The inhibitory effect of the treatment of Senna italica on the migration of MDA-MB231 cell line.

Figure 3. Pro-apoptotic effect of methanol extracts on MDA-MB 231 after 48 hrs using the Annexin V FITC and PI stain.

Figure 4. (A) Expression of Caspase 3, (B) Caspase 8 and (C) Caspase 9 in MDA-MB 231 (Ethanol treated 48 hrs).

Flow Cytometry

Figure 2 The inhibitory effect of the treatment of Senna italica on the migration of MDA-MB231 cell line.

Flow Cytometry

Figure 3. Pro-apoptotic effect of methanol extracts on MDA-MB 231 after 48 hrs using the Annexin V FITC and PI stain.

Figure 4: (A) Expression of Caspase 3, (B) Caspase 8 and (C) Caspase 9 in MDA-MB 231 (Ethanol treated 48 hrs).