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Anti-proliferative and Anti-metastatic Effect of Aqueous Extract of *Origanum Syriacum* on Aggressive Human Breast Cancer Cells

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Around the world as well as in Qatar, breast cancer is characterized among the highest rates of mortalities that are cancer related. Alarmingly, statistics have shown that the incidence of breast cancer is slightly higher in Qatari women than other Arab countries in the region. This evidence suggests the importance of focusing research on understanding and treatment of breast cancer in Qatar. Current treatment options for breast cancer includes; chemotherapy, surgery, and radiotherapy. Chemotherapy and radiotherapy are associated with undesired side effects; thus, many people tend to look for alternative treatments. Herbal treatments have been used as an alternative to traditional cancer therapies in recent years. Several studies have shown that herbs contain bioactive compounds, including flavonoids, steroids, as well as others, which exert anti-oxidative, anti-proliferative, and anti-inflammatory properties. One of the most commonly used herbs in the Arabian Gulf region is *Origanum syriacum*. It is known to have anti-oxidative effect. Unfortunately, studies are extremely limited in terms of understanding its anti-carcinogenic effect. Therefore, this study was carried out to determine the effect of *O. syriacum* aqueous extract (OSE) on an aggressive type of breast cancer (MDA-MB-231) cells. *O. syriacum* extract was prepared by dissolving the ground dried leaves in water and then drying it using a rotarvapor. Viability of MDA-MB-231 cells in the presence or absence of increasing concentration of OSE was examined by MTT assay. The flow cytometry was used to test the cell cycle progression in the presence of OSE. Moreover, the migratory capacity of MDA-MB-231 cells was determined by Boyden chamber and scratch assay. The invasiveness of MDA-MB-231 in the absence and presence of OSE was investigated using the Matrigel coated wells. Furthermore, adherence of MDA-MB-231 cells to fibronectin was tested with and without OSE. The oxidative stress of the different concentrations of OSE against MDA-MB-231 was determined using ROS-glo assay. Finally, western blot analysis was performed to test the metastatic ability (occludin expression) as well as autophagy marker (LC3A/B expression). The results indicated that OSE decreased the proliferation of MDA-MB-231 cells in a time and a concentration dependent manner. The highest anti-proliferative

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effects of OSE were observed at concentrations of 0.8 mg/ml and above after 24, 48 and 72 hrs of exposure. Furthermore, OSE arrested cells in G1 phase of cell cycle. Also, migratory capacity of MDA-MB-231 cells declined in the presence of OSE at a concentration of 1.2 mg/ml. Moreover, OSE inhibited the adhesive property along with the invasiveness of aggressive breast cancer cell line. Supporting the above results, an increase in occludin expression was observed in cells treated with OSE indicating that *O. syriacum* extract has an anti-metastatic capacity. Additionally, the production of ROS as well as the expression of LC3A/B proteins increased in MDA-MB-231 cells treated with OSE at concentration of 1.2 mg/ml. Our results demonstrate that Origanum syriacum may have a potential to be used as a supplemental therapy for patients suffering from malignant breast cancer. Further insight into understanding molecular mechanisms and safety of OSE using *in vivo* studies should be carried out to fully understand its activity at the molecular level and determine its safe use in the treatment/prevention of breast cancer.