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Urtica dioica extract suppresses *miR-21* and metastasis-related genes in breast cancer

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ABSTRACT

Background: Breast cancer has a high prevalence among women worldwide. Tumor invasion and metastasis still remains an open issue that causes most of the therapeutic failures and remains the prime cause of patient mortality. Hence, there is an unmet need to develop the most effective therapeutic approach with the lowest side effects and highest cytotoxicity that will effectively arrest or eradicate metastasis.

Methods: An MTT assay and scratch test were used to assess the cytotoxicity and migration effects of Urtica dioica on the breast cancer cells. The QRT-PCR was used to study the expression levels of *miR-21*, *MMP1*, *MMP9*, *MMP13*, *CXCR4*, *vimentin*, and *E-cadherin*.

Results: The results of gene expression in tumoral groups confirmed the overexpression of *miR-21*, *MMP1*, *MMP9*, *MMP13*, *vimentin*, and *CXCR4*, and the lower expression of *E-cadherin* compared to control groups ($P < 0.05$). Moreover, the results of the MTT assay show that Urtica dioica significantly inhibited breast cancer cell proliferation. Moreover, findings from the scratch assay exhibited the inhibitory effects of Urtica dioica on the migration of breast cancer cell lines.

Conclusion: Urtica dioica extract could inhibit cancer cell migration by regulating *miR-21*, *MMP1*, *MMP9*, *MMP13*, *vimentin*, *CXCR4*, and *E-Cadherin*. Moreover, our findings demonstrated that the extract could decrease miR-21 expression, which substantially lessens the overexpressed *MMP1*, *MMP9*, *MMP13*, *vimentin*, and *CXCR4* and increases *E-cadherin* in the tumoral group.

1. Background

Breast cancer is the most common malignancy after skin cancers and is the second cause of death among women worldwide. The peak incidence of breast cancer occurs between the ages 20 and 59 years [1,2].

Depending on the stage of diagnosis, breast cancer is treated with a multidisciplinary approach involving surgery, radiation and chemotherapy, hormonal therapy, immunotherapy, and other novel treatment strategies such as gene silencing [3,4]. Several new studies have proposed that most breast patients should be treated with one or several complementary and alternative medicines (CAM) including herbal treatment [5]. In this connection, herbal medicine is the most commonly used treatment among numerous cultures in Asia, Africa, Europe, and America, and is regarded as the oldest system of medicine in use [6].

There are various types of herbal medicines that originate from different cultures around the world. Urtica dioica (stinging nettles) is one of the most well-known plants used as an herbal treatment, especially in the southwest of Asia. Urtica dioica is a medicinal plant with 40 genera and about 500 subspecies; most of which are native to America, India, Malaysia, and tropical countries. Urtica dioica species are rarely grown in Africa and Europe [7]. The constituent profile of Urtica dioica varies depending on the plant part in question; however, the main components include acetylcholine, histamine, 5-HT (serotonin), moroidin, leukotrienes, and possibly formic acid [8,9] compounds and a type of glucoside with reddening skin effects. Orticine, as a colored substance, can be isolated from the stinging hairs of this plant. Our previous studies on the anti-cancer effect of Urtica dioica extract demonstrate its apoptosis activation effect on several cancer cells including breast cancer cells [10,11]. Our recent study also shows that Urtica dioica

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Table 1
Primer sequences.

Gene	Sequences	
GAPDH	F	5′- CAAGATCATCACCAATGCCT −3′
	R	5′- CCCATCACGCCACAGTTTCC-3′
CXCR4	F	5′-AACTTCAGTTTGTTGGCTGC-3′
	R	5′-TTTAACATGTACTTTTATTA-3′
MMP1	F	5′-GCGCACAAATCCCTTCTACC-3′
	R	5′-ATCCGTGTAGCACATTCTGTCC-3′
MMP9	F	5′-ATTCATCTTCCAAGGCCAATCC-3′
	R	5′-CTTGTCGCTGTCAAAGTTCG-3′
MMP13	F	5′-GACAAGTAGTTCCAAAGGCTACAA-3′
	R	5′-GGGTTGGGGTCTTCATCTC-3′
E-Catherine	F	5′-TGCCCAGAAAATGAAAAAGG-3′
	R	5′-GTGTATGTGGCAATGCGTTC-3′
Vimentin	F	5′-CAGGCAAAGCAGGAGTCCA-3′
	R	5′-AAGTTCTCTTCCATTTCACGCA-3′
miR-103-3p	Target sequence	5′-AGCAGCAUUGUACAGGGCUAUGA-3′
miR-21	Target sequence	5′-CAACACCAGUCGAUGGGCUGU-3′

extract could inhibit growth and migration of cancer cells, as well as increasing sensitivity to paclitaxel in breast cancer cells [12].

Invasion and migration of cancer cells to other tissues are crucial stages for the expansion of malignancies, which in turn leads to potentially life-threatening metastasis. Generally, a cellular invasion is controlled by the cascade of consonant molecular events causing tumor cells to detach from their primary site and scatter to an even distant site in the body. Crucial genes involved in this process include MMP1, MMP9, MMP13, vimentin, CXCR4, and E-cadherin. In this regard, the deregulation of these genes is the most important reason for the development of epithelial-mesenchymal transition (EMT) in changing adhesion junctions and the migratory ability of cells [13,14].

MicroRNA (miRNA) expression changes in breast cancer lead to detecting some specific miRNAs involved in the development. Studies show that metastasis-related genes and the miRNA related to these genes undergo a change in invasive and migration cancers. MicroRNA-21 is known to be an important oncomir that is overexpressed in advanced tumor stage and lymph node metastasis.

In the present study, we evaluate *miR-21* and its metastasis-related gene expression in tumoral and normal marginal tissues of the breast cancer. Also, we studied the cytotoxic and anti-metastatic effect of a dichloromethane extract of Urtica dioica in three different metastatic breast cancer cell lines and compared these with the normal cell line.

2. Methods

2.1. Ethics statement

To use clinical samples, informed consents were taken from each patient. The methodology used in this study and using tissues of the patients were granted by the ethic committee of Tabriz University of Medical Sciences (statement no. 92/74). Forty-five pathologically diagnosed with stage 2 and 3 of breast cancer samples and marginal normal tissues were collected from patients with the breast cancer. Breast cancer patient tissues were instantly transported into liquid nitrogen and were stored at −80 °C until further use. Cancer tissue samples were obtained from the Imam Reza Hospital affiliated to Tabriz University of Medical Sciences.

2.2. Preparation of herbal extract

The Urtica dioica plant was obtained from the wild natural environs of the Tabriz-mountains (Eastern Azerbaijan Province, Iran). The extraction was carried out on dried leaves using dichloromethane solvent for 24 h in a 10-l extraction reactor equipped with rotation and temperature sensor. To reach the highest purity percentage, the extract was passed through Whatman filter paper no.40 and the resultant filtrate was removed under reduced pressure by a rotary vacuum evaporator.

2.3. Cell culture

MCF-7(NCBI code: C135) and MDA-MB-231 (NCBI code: C578), 4T1 (NCBI code: C604) and HFFF2 (NCBI code: C163) were purchased from the National Cell Bank of Iran (Pasteur Institute, Iran-Tehran). Cells were maintained as monolayers in a humidified incubator with a 5% CO2 at 37 °C in RPMI-1640 containing 10% FBS, 100 units/ml of penicillin, 100 μg/ml of streptomycin (Gibco, Grand Island, NY). For wound healing assays, cells were plated at the concentration of 5 × 104 cells/well for 18–24 h (confluency 70 percent).

To evaluate the efficiency of Urtica dioica extract, the experiment was planned by time and dose factors. Breast cancer cells and normal cells were exposed to a wide concentration range of the plant extract (10–70 μg/ml) for 24 and 48 h.

2.4. Cell viability assay

In order to show whether treatment with Urtica dioica extract has a cytotoxic effect on tumor cells, we performed MTT assay. MMT assay was performed on MCF-7, MDA-MB-231, 4T1 and HFFF2 cell lines following treatment with Urtica dioica extract.

Table 2
Differential gene expressed between marginal and tumoral breast tissue.

	Marginal tissues			Tumoral tissues			In vivo Tumor			Breast cell lines			Normal cell lines		
	M	R		M	R		M	R		M	R		M	R	
	N	Min	Max	N	Min	Max	N	Min	Max	N	Min	Max	N	Min	Max
miR-21	0.82	0.5	1.65	1.73	0.93	6.96	1.56	1.41	1.65	1.27	1.05	1.49	0.72	0.53	0.88
MMP1	0.72	0.26	1.55	1.51	1.05	3.92	1.09	1.06	1.15	1.16	1.12	1.22	0.88	0.83	0.93
MMP9	0.73	0.51	1.06	1.30	1.01	2.04	1.17	1.08	1.23	1.18	1.08	1.23	0.79	0.69	0.88
MMP13	0.61	0.38	0.97	1.40	1.06	2.51	1.34	1.26	1.43	1.45	1.16	1.85	0.47	0.35	0.57
CXCR4	0.79	0.33	1.53	1.44	1.07	2.87	1.3	1.23	1.34	1.24	1.12	1.35	0.96	0.74	1.18
Vimentin	0.67	0.36	0.96	1.51	1.08	2.60	1.21	1.1	1.38	1.23	1.14	1.33	0.99	0.83	1.25
E-Catherine	1.32	1.07	1.85	0.70	0.30	0.89	0.59	0.30	0.74	0.59	0.37	0.73	0.90	0.63	1.09

M: Median; R: Range; N: Normalized.