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THE EFFECT OF THYMOQUINONE ON THE miRNA PROFILE OF MCF-7 BREAST CANCER CELLS

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ABSTRACT: Background and aim: Thymoquinone (TQ), which is the most bioactive component of *Nigella sativa* (Black cumin), exhibits anticancer characteristics based on cell culture and experimental animal studies. However, molecular action mechanisms of these effects are not clear. MicroRNAs (miRNAs), small non-coding RNAs of approximately 22 nucleotides, are an emerging class of gene expression modulators with relevant roles in several biological processes, including cell differentiation, development, apoptosis, and regulation of the cell cycle. The purpose of this study was to investigate the potential impact of thymoquinone (TQ) on MCF-7 human breast cancer cell miRNAs. **Materials and methods:** The expression levels of miRNAs in MCF-7 cell and TQ treated MCF-7 cells were estimated by miRNA sequencing. The expressions of miRNAs were determined real-time qPCR. **Results:** We detected 10 down-regulated mirRNAs (hsa-miR-1, let 7c-5p, hsa-miR-15b-5p, hsa-mir-202-3p, hsa-miR-214-3p, hsa-miR-210-3p, hsa-miR-31-5p, hsa-miR-424-5p, hsa-miR-497-5p, hsa-miR-98-5p) and 2 up regulated miRNAs (hsa-miR-22-3p, hsa-miR-132-3p) in TQ treated groups, comparing with control group. These findings highlight the effects of TQ miRNA profile and molecular mechanism on MCF-7 cells. **Conclusion:** Finally, according to computational analyses using validated databases PI3 kinase/AKT (hsa04151), Wnt (hsa04310), MAPK (hsa04010) and p53 (hsa 04115) signaling pathways seem to be the key targets of these TQ groups of miRNA.

INTRODUCTION: An estimated 14.1 million new cancer cases and 8.2 million cancer deaths occurred in 2012 worldwide. Breast cancer is the most frequently diagnosed cancers and the leading cause of cancer related deaths in women [8]. All improvement on cancer cell biology, treatment of breast cancer remain problem. To improve the survival of breast cancer patients, we need to develop new anticancer drugs.

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Nigella sativa (Black Seed), is defined as a medicinal herb which has been used for the cure of several diseases for more than 2000 years in most of the middle and far east countries.

Studies had shown that the biological activity of *Nigella sativa* seeds is mainly attributed to its essential oil component which is pre-dominantly (30–48%) thymoquinone (TQ) [4]. The anti-cancer effect of TQ which has been used in traditional medicine for centuries, has been shown by in vivo and in vitro studies made at various cancer series and animal experiments [1, 9].

Micro RNA (miRNA) is a group of small non-encoding RNA molecules of 21 - 23 nucleotides in length, which controls gene expression post-

transcriptionally either via the degradation of target mRNAs or the inhibition of protein translation [2, 3, 7]. The development depressing one or more target genes play an important role in processes like differentiation, proliferation and apoptosis. miRNA's can appear in exonic or intronic zones of protein coding genes or intragene zones. More than 50% of miRNA genes can be seen in cancer associated genom zones or in fragile zones; this points out the important role miRNAs in the pathogenesis of neoplasia.

While some miRNA's are controlled with epigenetic mechanisms, some target directly or indirectly the factores that take part in the epigenetic mechanism. Before the miRNA's functional importance is well understood and their diagnostic and treatment use is materialised, it is necessary to define how they are bound to their targets and they classify gene expression at different levels (Gebeshuber ve ark 2009). Today, where millions of women get breast cancer diagnosis, miRNA's are likely to be an efficient agent in the near future at the diagnosis of breast cancer in earlier stages and not only at the prognosis of the disease but also at the molecular based cancer treatments.

The focus of this study to understand the molecular targets modulated by thymoquinone, and its potential therapeutic implications on breast cancer.

MATERIALS AND METHODS: All cells were cultured at 37 ºC and 5% CO_2 in humidified atmosphere. Human breast cancer cell lines, MCF-7, were obtained from American Type Culture Collection (ATCC, US) MCF-7 cells were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 mg/mL streptomycin. All supplements were purchased from Sigma. TQ was also purcased from Sigma-Aldrich.(U.S)

MCF7 cells were treated with different doses of TQ (5, 25, 50 μM) for 70 hours. Then total RNA was isolated by using Roche High Pure miRNA Isolation Kit (Roche Diagnostics) and cDNA was synthesized from cell lysates. The expressions of 84 miRNAs were determined by The BioMark™ 96.96 Dynamic Array (Fluidigm Corporation) for real-time qPCR. Statistical analysis was performed using the Biogazelle's qbase PLUS 2.0 software. Determinations of relative gene expression values were carried out byusing the 2-ΔΔCt method (normalized threshold cycle (Ct) value of sample minus normalized Ct value of control).

RESULTS: As a result of the statistically analysis, twelve of 84 miRNAs have been differentially expressed compared to control group. 10 miRNAs were down-regulated (hsa-miR-1, let 7c-5p, hsa-miR-15b-5p, hsa-mir-202-3p, hsa-miR-214-3p, hsa-miR-210-3p, hsa-miR-31-5p, hsa-miR-424-5p, hsa-miR-497-5p, hsa-miR-98-5p) and 2 were up-regulated (hsa-miR-22-3p, hsa-miR-132-3p) in TQ's groups, comparing with control group (fold regulation <2, fold regulation>2, $p<0.05$).

TABLE 1: MiRNAs WHİCH ARE SİGNİFİCANT İN MCF-7 CELLS AT 5,25,50MM TQ DOSE

	5μM TQ		25 μM TQ		50 μM TQ	
miRNA	**Fold regulation**	**P value**	**Fold regulation**	**P value**	**Fold regulation**	**P value**
Let-7c-5p	1.006	0.975655	-1.409	0.01258	-2.2548	0.002014
Mir-1	-1.6701	0.00877	-2.2829	0.002438	1.6324	0.00894
Mir-132-3p	-1.15	0.117895	1.3215	0.079581	2.2965	0.00318
Mir-15b-5	-1.0118	0.985616	0.985616	0.000298	-2.4449	0.000067
Mir-202-3p	-3.8425	0.062036	-4.9025	0.00241	-3.0097	0.004535
Mir-210-3p	-1.0917	0.25356	-1.824	0.001036	-2.952	0.000235
Mir-214-3p	-1.4947	0.177834	-2.399	0.009978	-2.1263	0.035804
Mir-22-3p	1.176	0.85124	-1.0636	0.256959	2.1232	0.000021
Mir-31-5p	-2.038	0.017762	-3.2709	0.017762	-2.7727	0.001298
Mir-424-5p	1.3736	0.093315	-2.081	0.00217	-1.2505	0.006009
Mir-497-5p	1.0238	0.741211	-2.1985	0.00218	-3.0216	0.000015
Mir-98-5p	1.1835	0.174818	-1.5644	0.000017	-2.194	0.004163

P value of <0.05

CONCLUSION: Thymoquinone has been investigated for its antioxidant, anti-inflammatory and anticancer activities in both in vitro and in vivo models.

miRNA's are the molecules with increasing importance over the last years after the studies in the last ten years and the discovery of their close connection with cancer. Dysregulation of miRNAs is commonly observed in many cancers, resulting in the up-regulation of oncogenes or the down-regulation of tumor suppressor genes. Numerous studies have confirmed that miRNA plays a critical role in tumor cell survival, invasion and metastasis. The definition of their role in the patogenesis of breast cancer led to the thinking that they could be used for the diagnosis and treatment of breast cancer [6]. The studies about miRNA and breast cancer have therefore gathered pace in the last years (Iorio and friends 2005)

Let-7c is one of the most abundant and highly conserved miRNAs. It has been shown that let-7c inhibits cancer cell survival by regulating cell proliferation and apoptosis [10]. Our finding of Let-7c was down regulation of the TQ 25μM and 50μM [5]. These data also support the antiproliferatif and proapoptotic effect of TQ.

In the study made by Beltran and in 2011, is has been (was) shown that at the MDA-MB-231 breast cancer cell line, the oncogenic miRNA's like miR-1 are down regulated (Beltran *et al.,* 2011; Tutar, Tutar, & Tutar, 2014) We have also observed in our study at the MCF-7 breast cancer cell line, the miR-1 expression was similarly downregulated at (with) 25 μM TQ dose. It was also noted that miR202 and miR 210 increased with breast cancer (Negrini, Ferracin, Sabbioni, & Croce, 2007). We observed that miR 202 at all TQ doses and miR210 at 50μM TQ were down regulated.

According to computational analyses using specialized databases (as DIANA miRPath v. 2, 0); PI3kinase/AKT (hsa04151), Wnt (hsa04310), MAPK (hsa04010) and p53 (hsa 04115) signaling pathways (KEGG pathway number) seems to be the key targets of these miRNA group. These findings highlight the effects of TQ to miRNA profiling of MCF7 cells and may be helpful for further studies. We observed that all findings in our study where we investigated the effect of TQ on miRNA panel were conform to the literatüre and besides, as a difference from other studies, after the TQ application of 3 doses 5 μM, 25 μM and 50 μM for the first time, the miRNA panel was then checked and it was observed that all obtained miRNA's were having an effect on the cell cycle, apopitosis, proliferation and DNA transcription of cancer, at P53, MAPK, PI3K-Akt, WINT signal pathways.

Using high-throughput profiling, dysregulation of miRNAs has been widely observed in different stages of cancer. The upregulation (overexpression) of specific miRNAs could lead to the repression of tumor suppressor gene expression, and conversely the downregulation of specific miRNAs could result in an increase of oncogene expression; both these situations induce subsequent malignant effects on cell proliferation, differentiation, and apoptosis that lead to tumor growth and progress.

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