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ORIGINAL RESEARCH ARTICLE

The effect of thymoquinone on BEAS-2B cell viability and TGF-β1 release

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***Abstract*:** Thymoquinone, one of the essential oil in the structure of cumin, is used for alternative therapy for many diseases from past to present. It was shown to have anti-carcinogenic and anti-inflammatory effects, as well as positive effects on fibrosis. However, there is no study on the effect of thymoquinone on cancer and fibrosis mechanism in bronchial epithelium cell line BEAS-2B. In our study, the effect of thymoquinone on cell viability and transforming growth factor-beta 1 (TGF-β1) level, which has an important role in the regulation of many biological processes including cancer and fibrosis-associated signal transduction, was evaluated. BEAS-2B cells were exposed to thymoquinone at 0–80 μmol/L concentrations for 24-, 48- and 72-hour durations. Cell viability was evaluated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. TGF-β1 level was determined with enzyme-linked immunosorbent assay (ELISA) method from the collected supernatant. Cell viability was found to be increased at all concentrations and durations (10–80 μmol/L; 24, 48 and 72 h) according to the control group (0 μmol/L; thymoquinone in ethanol) ($p < 0.0001$). Moreover, thymoquinone was found to increase the level of TGF-β1 only at 80 μmol/L concentration and 24-hour exposure period (0 μmol/L, 53.41 ± 18.44 pgr/ml TGF-β1; 80 μmol/L, 174.5 ± 80.03 pgr/ml TGF-β1). As a result, thymoquinone was found to increase cell proliferation and encourage TGF-β1 release.

***Keywords*:** BEAS-2B; thymoquinone; fibrosis; cancer; TGF-β1

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Introduction

Cumin, a *Nigella sativa (NS)* species of the *Ranunculaceae* family, is an herbaceous plant that mostly grows in countries bordering Mediterranean. In Turkey, it is called by several names such as black seed, black cumin or blessing piece. There are 0.4%–0.45% essential oils in *NS* seed structure, which differs according to the climate of the region. Thymoquinone (TQ), ($C_{10}H_{12}O_2$, 2-Isopropyl-5-methylbenzo-1,4-quinone), is the most important bioactive compound that forms 18.4%–24% of these essential oils[1,2]. The seed of cumin and its various active ingredients are used commonly as alternative treatment agents in the Far Eastern and some Asian countries for colds, headaches, asthma, diuretic, jaundice, rheumatism and various inflammation from past to present[1]. According to studies, cumin seed and its components have anti-carcinogenic[3], antitumor[4], hepato-protective[5], antioxidant[6], protective[7], anti-inflammatory, immune-modulator, antihistaminic, antimicrobial, gastro-protective, nephro-protective and neuro-protective effects to strengthen the immune system[8]. Moreover, it is shown to have positive effects on cardiovascular diseases, diabetes, reproductive and respiratory diseases, fibrosis and in the treatment of bone complications[9].

There are studies that reported on the chemo-preventive effect of natural products and positive effects of plant-rich diet in cancer, diabetes, cardiovascular diseases and in many diseases including respiratory tract disorders[10]. The effect of thymoquinone on fibrosis mechanism was shown in various diseases such as diabetes and cardiovascular and respiratory disorders[9]. Transforming growth factor-beta 1 (TGF-β1) is a member of TGF-β protein family.

TGF-β1 is a serine/threonine kinase receptor that regulates cell proliferation, motility, survival and apoptosis[11]. This receptor plays an important role in many biological processes including cancer and fibrosis-associated signal transduction. In addition, TGF-β1 suppresses tumor in the early stages of carcinogenesis where it acts as a proto-oncogene in the last stages of metastatic cancer[12].

There are several studies that reported the effect of thymoquinone on various pathologies. However, to the best of our knowledge, there is no study on the effect of thymoquinone on cancer and fibrosis mechanism in human bronchial epithelial cell line BEAS-2B. Thus, in our present study, we aim to evaluate the effect of thymoquinone on cell viability and TGF-β1 release in BEAS-2B cell line.

Materials and methods

Cell culture

The study was performed at Cell Culture Laboratory, Department of Medical Biology, Medical Faculty, Mustafa Kemal University. BEAS-2B cell line was provided from Cell Culture Laboratory, Department of Medical Biology, Medical Faculty, Mustafa Kemal University. BEAS-2B cells was cultured in RPMI (Sigma-Aldrich, St. Louis, MO) which included 1% penicillin (Sigma-Aldrich, St. Louis, MO) and 10% FCS (fetal calf serum) (Sigma-Aldrich, St. Louis, MO) at 5% CO_2 and 37 °C conditions. Culture medium was changed twice per week and the cells were used in studies when they reached sufficient number.

Cell viability

Cell viability was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. BEAS-2B cells were cultured in 24 well plates as 5,000 cell/ml and incubated in keratinocytes (Sigma-Aldrich, St. Louis, MO) at 5% CO_2 and 37 °C conditions. The cells were exposed to different concentrations of thymoquinone (0–80 µmol/L) dissolved in ethanol for 24, 48 and 72 h. At the end of the periods, the culture medium in 24 well plates was collected for TGF-β1 analysis and was kept in -80 °C condition until being analyzed. Subsequently, the remaining cells in the 24 well plates were incubated with 1 mg/ml MTT (Sigma-Aldrich, St. Louis, MO). MTT solution was removed and the cells were exposed to 0.5 ml dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO). Discoloration was evaluated with spectrophotometer (Thermo, Multiskan™ Go, Spectrophotometer, Thermo Fisher Scientific, Finland) at 550 nm.

ELISA

Culture medium samples that were kept in -80 °C were used for ELISA examination. Quantitative analysis of TGF-β1 was performed by using commercial kits (invitrogen, TGF-β1 ELISA kit, Cat. No: KAC1688) and appropriate ELISA device (Thermo Multiskan™ GO Spectrophotometer, Thermo Fisher Scientific, Finland).

Statistical Analysis

In our study, the statistical evaluation was performed by GraphPed Prisim-5 v.5 (GraphPed Software Inc., USA) program. Normal distribution analysis of the data was evaluated with Shapiro-Wilk normality test. The evaluation of cell viability was performed with one-way analysis of variance (ANOVA), while TGF-β1 data were evaluated with t-test and ANOVA. $p < 0.05$ values were accepted as statistically significant.

Results

The cells exposed to thymoquinone at different concentrations (10–80 µmol/L) and durations and are compared to the control group (0 µmol/L; thymoquinone dissolved in ethanol) in terms of cell viability. In all concentrations and durations of exposure, thymoquinone was found to increase the BEAS-2B cell viability ($p < 0.0001$) (Figure 1). Moreover, thymoquinone was found to increase the TGF-β1 level at 80 µmol/L and 24-h exposure duration (0 µmol/L, 53.41 ± 18.44 pgr/ml TGF-β1; 80 µmol/L, 174.5 ± 80.03 pgr/ml TGF-β1) (Figure 2).

Discussion

Cancers such as lung cancer are responsible for the death of millions of people each year worldwide and it is a serious threat for public health and national economies. Despite the advances in modern medicine, alternative treatment options are increasingly preferred in the struggle against cancer. The side effects and complications of the drugs that are used in modern medicine give special importance to alternative treatment options. However, the efficiency and reliability of these treatment agents, which are related with long-term experience and tradition, do not have scientific basis and the studies on them are quite insufficient[13–15]. The most notable preference in alternative treatment are herbal treatments. Thymoquinone, one of the essential oil in the

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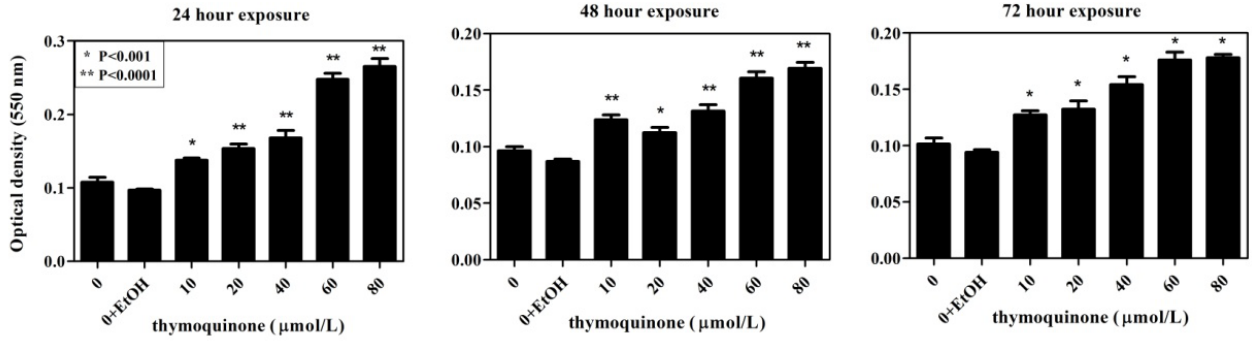

Figure 1. BEAS-2B cell viability. When control group (0+EtOH) was compared to other exposure groups by ANOVA test (Dunnett's multiple comparison test), there was statistically significant difference in all different concentrations and durations (*p < 0.001 and **p < 0.0001).

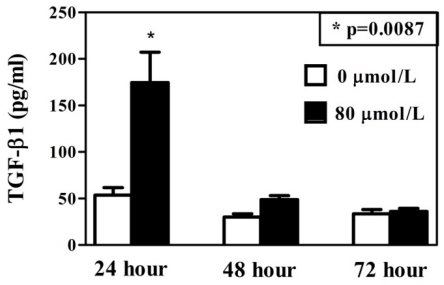

Figure 2. TGF-β1 levels. **Results for ANOVA test**; When control groups (0 group for 24, 48 and 72 h) were compared to each other, there was statistically significant difference between the 24- and 48-h groups (p = 0.0299). Moreover, when exposure groups (80 µmol/L) at 24-, 48- and 72-h exposure periods were compared to each other, there were statistically significant difference between the 24-h group and both the 48- and 72-h exposure groups (p = 0.004 and p = 0.0004, respectively). **Results for t-test**; When control group (0) and exposure group (80 µmol/L) were compared to each other at 24-, 48- and 72-h periods, p values were 0.0087, 0.0173 and 0.8413, respectively.

structure of cumin seed, is one of the herbal source used for the traditional treatment of various diseases from past to present. The effect of thymoquinone on cancer and fibrosis was shown in many diseases[1,9,16–18].

When Kanter[19] evaluated the possible protector effect of thymoquinone in his study, thymoquinone was shown to decrease pulmonary inflammatory response by reducing the peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar exudate, interstitial fibrosis and necrosis. In another study[20] where bleomycin induced rats were used as pulmonary fibrosis model, the effect of thymoquinone on the severity of oxidative stress and anti-inflammatory response was evaluated. While bleomycin significantly increased lung tissue weight, lactate dehydrogenase, total leukocyte and total protein amount, all these effects were meliorated in the group treated with thymoquinone. Moreover thymoquinone was shown to have a reducing effect on emphysema in alveoli, inflammatory cell infiltration, hyperplastic lymphoid cell activation surrounding bronchioles and active form of nuclear factor kappa-β expression. Hydroxyproline measurement was also performed to evaluate fibrosis in the same study and was found to be markedly lower in thymoquinone treatment group. In addition, histopathological examination has verified the anti-fibrotic effect[20]. Ghazwani *et al.*[20] evaluated the effect of thymoquinone on fibrosis mechanism *in-vivo* and *in-vitro* in liver cell line (hepatic stellate cell, or HSCs) and thymoquinone was found to have a protective effect in liver damage-induced mice by CCl4 when compared to controls. There were similar results in *in vitro* studies performed with HSCs and LX2 cells, in which α-smooth muscle actin (α-SMA), *COL1A1*, *COL3A1*, *IL6*, *MCP-1* gene expression was shown to down-regulate[21]. Bai *et al.*[22] elucidated the anti-fibrotic mechanism of thymoquinone in their study. They explained that the palliative effect of thymoquinone on liver fibrosis took place via the blocking of toll-like receptor 4 (TLR4) expression and phosphatidylinositol 3-kinase (PI3K) phosphorylation. In another study[23], the protective effect of thymoquinone was explained in rats having cholestatic liver injury, and thymoquinone was shown to increase anti-oxidative capacity and decrease oxidative damage. Hypoxanthine (HP) and malondialdehyde (MDA) levels were low, while the superoxide dismutase (SOD) and glutathione peroxidase enzyme activities were significantly high[23]. Moreover, in liver fibrosis-induced mice by intra-peritoneal injection of thioacetamide, thymoquinone was shown to have an inhibitory effect on inflammatory infiltration and

accumulation of extracellular matrix proteins. Liver fibrosis was shown to become lighter, which was demonstrated by low protein and expression amount of α-SMA, collagen-I and tissue inhibitor of metalloproteinase-1 (TIMP-I). In addition, thymoquinone was shown to down-regulate TLR4 and pro-inflammatory cytokine levels in the same study[24].

Thymoquinone was shown to have an inhibitory effect on liver and lung fibrosis. In addition, thymoquinone was also shown to down-regulate fibrosis-related genes, decrease fibrosis-related protein, extracellular matrix component levels and oxidative stress, increase anti-oxidative capacity and regulate various signaling pathways. A previous study[16] evaluated the relationship between fibrosis and cancer. Fibrosis was shown to be an increased risk for cancer development. Chronic obstructive pulmonary disease (COPD), which is related to fibrosis, was shown to have a high prevalence in patients diagnosed with lung cancer when compared to control group[17]. Moreover, Park *et al*.[18] demonstrated the increased risk of lung cancer in idiopathic pulmonary fibrosis (IPF). In patients with lung cancer and IPF, squamous cell carcinoma was shown to be the most frequent cell type (35%), followed by adeno carcinoma, small cell carcinoma, and large cell carcinoma.

In our study, thymoquinone was shown to increase BEAS-2B cell viability in all concentrations (0–80 µmol/L) and durations (24–72 h). In addition, TGF-β1 levels were shown to increase in 80 µmol/L concentration at 24-h duration. As a result, our data was correlated with previous studies by Kanter[19] and El-Khouly *et al*.[20] where thymoquinone was shown to have a positive effect on lung fibrosis. Due to the positive relationship between cancer and fibrosis, which was demonstrated in previous studies[16–18], our results give rise to the positive effect of thymoquinone on cancer.

Conclusion

When we consider the increasing importance of alternative treatment options, there are quite insufficient scientific studies associated with this issue. Thus, our study is important in order to scientifically explain the therapeutic effect of thymoquinone, which has been used as alternative treatment agent from past to present. Thymoquinone, which is rich in various phytochemicals and nutritionally essential components, is really a seed proven to provide protection against cancer. We hope our study will shed light to base thymoquinone and other alternative options on a scientific basis in terms of their efficiency and reliability, and will draw the interest of researchers to investigate the potential health benefits of thymoquinone. More studies will be helpful to explain the effect of thymoquinone on various pathologies.

Author Contributions

M Izmirli and H Ecevit prepared the manuscript. All experimental studies were done by B Gögebakan, K Gunduz and N Bilgic.

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Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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