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The effects of thymoquinone on pancreatic cancer and immune cells

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SUMMARY

OBJECTIVES: Black cumin is widely used as a spice and as a traditional treatment. The active ingredient in black cumin seeds is thymoquinone. Thymoquinone has shown anticancer effects in some cancers. We planned to investigate its anticancer effect on pancreatic cancer cell lines.

METHODS: Thymoquinone chemical component in various doses was prepared and inoculated on pancreatic cancer cell culture, healthy mesenchymal stem cells, and peripheral blood mononuclear cell culture. IC50 values were calculated by absorbance data and measuring cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide staining of cells incubated with thymoquinone at 24, 48, and 72 h.

RESULTS: There was dose-related cytotoxicity. Maximal cytotoxicity was observed at 24 h and 100 μM thymoquinone concentrations in pancreatic cancer cell culture and mesenchymal stem cells. Any concentration of thymoquinone was not cytotoxic to peripheral blood mononuclear cell. Thymoquinone even caused proliferation at a concentration of 6.25 μM.

CONCLUSIONS: Since the cytotoxic concentration of thymoquinone on pancreatic cancer cell culture and mesenchymal stem cells is the same, it is not appropriate to use thymoquinone to achieve cytotoxicity in pancreatic cancer. However, since thymoquinone provides proliferation in peripheral blood mononuclear cell at a noncytotoxic dose, it may have an immune activator effect. Therefore, in vivo studies are needed to investigate the effect of thymoquinone on the immune system.

KEYWORDS: Cell survival. Mesenchymal stem cell. Pancreatic cancer. Peripheral Blood Mononuclear Cells. Thymoquinone.

INTRODUCTION

Black cumin (*Nigella sativa* L.) is a type of flower, which is common worldwide and in our country. Its seed is widely used as a spice and as a traditional treatment because it is believed to be beneficial for some diseases. The active ingredient in black cumin seeds is thymoquinone (TQ). A limited number of in vitro and in vivo studies suggest that TQ has many beneficial effects, such as anti-inflammatory, antimicrobial, and anticancer properties[1].

TQ is a natural phytochemical compound, and it has bioactivity in cancer cells[2]. TQ affects different molecular targets in various cancer cells, and many mechanisms have been proposed for its anticancer activity. Oxidative stress and inflammation are important mechanisms in cancer development. TQ reduces the oxidative stress with both antioxidant and anti-inflammatory effects and increases the expression and activity of antioxidant enzymes. It also prevents cancer formation by inducing apoptosis[3]. TQ can reduce the risk of cancer by preventing oxidative DNA damage induced by reactive oxygen radicals[4]. It also induces apoptosis by lowering the phosphorylation of NF-κB and IKKα/β. TQ inhibits its metastasis by increasing Janus kinase and p38 activity[5]. In a study, TQ showed anticancer activity in combination with gemcitabine on pancreatic cancer cell lines by suppression of Notch1, upregulation of PTEN, and inactivation of Akt/mTOR/S6 signaling pathways[6]. TQ noncytotoxic dose was found to boost the antiproliferative and apoptotic effects of some chemotherapeutics[7]. TQ has been shown to have immune-modulatory effects in some studies. In particular, it increases the number and activity of immune cells[8].

Black cumin is a well-known spice in our country and cancer patients often use it even without a doctor's recommendation. We observed that patients with end-stage metastatic pancreatic cancer used black cumin even though it was not recommended by us and benefited clinically. The cytotoxic effect of TQ on cancer cell lines has been demonstrated in

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previous studies. However, cytotoxicity on healthy cell lines has mostly not been studied in most of them[9]. Therefore, we planned to show the effects of TQ in pancreatic cancer cell culture (PANC-1), healthy mesenchymal stem cells (MSCs), and peripheral blood mononuclear cell (PBMC) culture. In this way, besides the effect of TQ on pancreatic cancer cells, its cytotoxic effect on healthy cells will be demonstrated. In addition, its effect on the immune cells will be investigated. We planned to determine the toxic effect and dose of different concentrations of TQ chemical components on PANC-1, MSC, and PBMC cells.

METHODS

In this study, after the TQ chemical component was dissolved with 100% DMSO, the concentrations forming the experimental groups were prepared with the complete medium.

Experimental groups

- Only cells (PANC-1, MSC, and PBMC);
- Only cell (medium containing ≤0.1% DMSO);
- 100, 50, 25, 12.5, 6.25, 3.125, 1, 0.1 µM TQ (separately) + cell.

IC50 values were calculated by measuring cell viability by MTT staining of cells incubated with TQ at 24, 48, and 72 h (the concentration-dependent curve was obtained by normalizing only the viability in the cell groups).

MTT-based cell viability analysis

TQ chemical component prepared at 100, 50, 25, 12.5, 6.25, 3.125, 1, and 0.1 µM doses was inoculated into 96-well plates at 10000 cells/well. Toxic effects of TQ at 24, 48, and 72 h were tested with MTT-based absorbance readings. Experimental groups were studied in five repetitions and their averages were taken.

Calculation of IC50 values

The IC50 values of TQ concentrations on PANC-1, MSC, and PBMC cells and the dose-response curve were calculated by entering logarithm values into the "non-linear regression" analysis data of the GraphPad Prism 8 program.

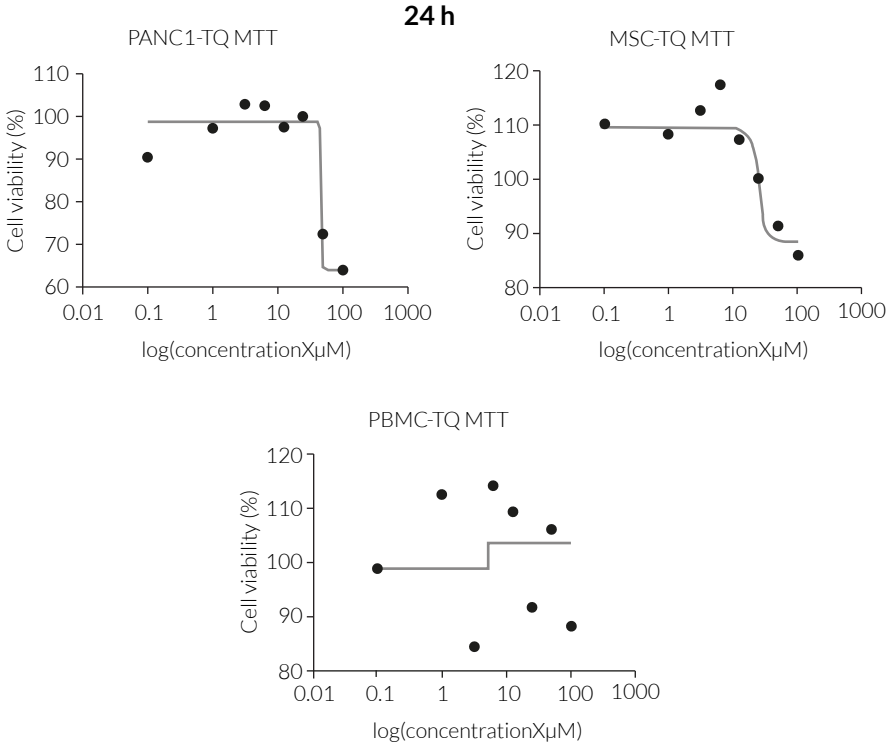
RESULTS

MTT-based cell viability analysis of 100, 50, 25, 12.5, 6.25, 3.125, 1, and 0.1 µM concentrations of TQ chemical components on PANC-1, MSC, and PBMC cells at 24, 48, and 72 h are given in Table 1. Effects of TQ chemical component on PANC-1, MSC, and PBMC cells at 24 and 72 h are given as dose-response curve in Figures 1 and 2. In PANC-1 and MSC, most cytotoxic doses of TQ were 100 µM; the cytotoxic effect decreased through lower doses. In both cell cultures, the maximal cytotoxicity was observed at 24 h, and it was decreased through 48 and 72 h. In PBMC culture, cytotoxicity was not observed. Even cell proliferation was observed at 6.25 µM TQ dose.

Table 1. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide-based cell viability analysis of mean values.

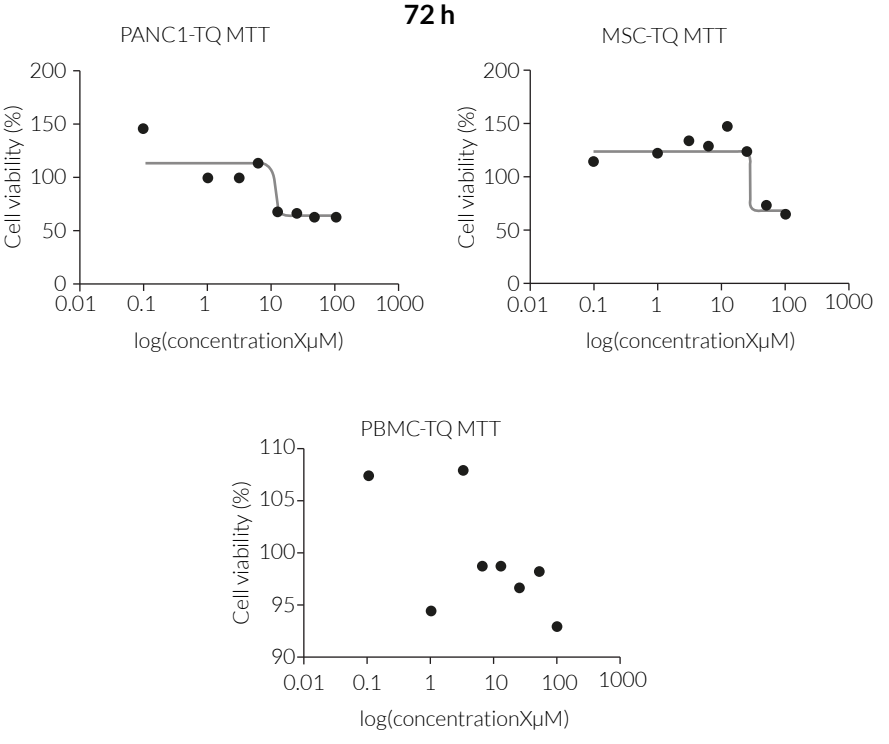
	PANC-1	PANC-1 (≤0.1% DMSO)	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	3.125 µM	1 µM	0.1 µM
24 h	0.3662	0.431	0.274	0.3102	0.4312	0.4198	0.4414	0.443	0.4192	0.3892
48 h	0.5416	0.5496	0.2776	0.3598	0.5504	0.5674	0.5678	0.566	0.5764	0.5224
72 h	0.5036	0.642	0.3242	0.3668	0.6202	0.7414	0.6478	0.6714	0.6122	0.573
	MSC	**MSC (≤0.1% DMSO)**	**100 µM**	**50 µM**	**25 µM**	**12.5 µM**	**6.25 µM**	**3.125 µM**	**1 µM**	**0.1 µM**
24 h	0.336	0.3282	0.2888	0.307	0.3366	0.361	0.395	0.3794	0.3646	0.3708
48 h	0.378	0.3596	0.2866	0.3006	0.3362	0.361	0.4162	0.4014	0.4006	0.429
72 h	0.5008	0.451	0.3124	0.3052	0.3262	0.3372	0.5644	0.4942	0.4974	0.7274
	PBMC	**PBMC (≤0.1% DMSO)**	**100 µM**	**50 µM**	**25 µM**	**12.5 µM**	**6.25 µM**	**3.125 µM**	**1 µM**	**0.1 µM**
24 h	0.2224	0.2136	0.1958	0.2356	0.2036	0.2432	0.254	0.187	0.2502	0.2196
48 h	0.249	0.303	0.3158	0.2716	0.2734	0.2676	0.2884	0.1872	0.3346	0.2274
72 h	0.2044	0.1864	0.19	0.2008	0.1976	0.2016	0.2018	0.2204	0.193	0.2194

DMSO: dimethyl sulfoxide; MSC: mesenchymal stem cells; PANC-1: pancreatic cancer cell culture; PBMC: peripheral blood mononuclear cell; µM: micromolar.



MSC: mesenchymal stem cells; PANC-1: pancreatic cancer cell culture; PBMC: peripheral blood mononuclear cell.

Figure 1. Dose-response curves at 24 h.



MSC: mesenchymal stem cells; PANC-1: pancreatic cancer cell culture; PBMC: peripheral blood mononuclear cell.

Figure 2. Dose-response curves at 72 h.

DISCUSSION

TQ has toxic effects on PANC-1. But cytotoxic doses are also found to be toxic to MSC. The nontoxic TQ dose to MSC had no cytotoxic effect on PANC-1. We concluded that it is not possible to provide a sufficient TQ cytotoxic dose in pancreatic cancer without damaging healthy cells. Mu et al.[6] showed that TQ has cytotoxicity on the PANC-1 cell line at 50 and 25 µmol/L doses. But they did not study the effect of TQ on healthy cells. Tan et al.[10] reported that TQ has a cytotoxic effect on the PANC-1 cell line in various doses but they also did not study on healthy cells. There are very few studies in the literature on TQ that has immunomodulatory and immunotherapeutic potential. Therefore, to investigate the cytotoxic effect of TQ on PANC-1 cells via immune cells, an experimental plan was established for PBMC cells. As a result of this experiment, it was found that no dose of TQ was cytotoxic to PBMC cells. It has even been found to proliferate PBMC cells. As a result of the analyses, we think if TQ has anticancer activity, this effect may not be occurred by direct cytotoxicity but by immune system activation. There are some studies about the immune activator effects of *N. sativa* protein or oil, but there is no any study with TQ. In a study, it was reported that *N. sativa* proteins can increase the production of TNF-alpha either by nonactivated or by mitogen-activated PBMC[11]. In another study, *N. sativa* proteins achieved the secretion of IL1-beta and IL-3 from PBMC[12]. At present, there is no any in vivo study to show the immune-activator effect of the TQ or *N. sativa*. Anticancer activity of *N. sativa* has been shown in some in vivo studies and this effect has been attributed to the anti-inflammatory and antioxidant properties of TQ[8].

To understand this immune activator mechanism of action, in vivo studies supported by different doses of TQ and control groups are needed. For this reason, we think in vivo animal studies are necessary to elucidate the anticancer mechanism of TQ.

CONCLUSIONS

Although black cumin is a plant that is frequently used by cancer patients without the knowledge of the doctor, its effect on cancer is not known exactly. In our study, there are some clues that it may have its main anticancer effects through the activation of the immune system rather than its direct cytotoxic effects. These results encouraged us to investigate the relationship between TQ and the immune system. More in vitro and animal experiments are needed to investigate the anticancer effects of TQ via immune cells.

AUTHORS' CONTRIBUTIONS

CA: Conceptualization, Data curation, Formal Analysis. **DDK:** Data curation, Formal Analysis. **GSK:** Data curation, Formal Analysis. **DÇ:** Data curation, Formal Analysis. **EO:** Data curation, Formal Analysis. **EK:** Data curation. **EY:** Data curation. **FÖ:** Data curation.

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