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Original Article

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Evaluation the Anti-proliferative Effect of NVP-AUY922 in Combination with Thymoquinone in Colorectal Cancer Cell Line

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Abstract

***Background & Aims*:** Thymoquinone (TQ) is a natural component and the active herbal complex originate in *Nigella sativa* seed. TQ shows the anti-cancer effects in the previous studies. The effects of TQ, its mechanism on colorectal cancer, and its combination with other newly chemotherapeutic agents are unclear. Heat shock protein 90 (HSP90) has been upregulated in the numbers of malignancies. In this survey, we investigated the impacts of TQ and NVP-AUY922 (a HSP90 inhibitor) on HT-29 colorectal cancer cell line.

Materials & Methods: HT-29 cells were seeded and exposed to TQ and NVP-AUY922 for 24 hours in various concentrations. Cell viability (water-soluble tetrazolium-1) assay was performed. Moreover, in combination cases, various concentrations of both agents examined using cellular viability analysis.

Results: The TQ significantly inhibited cancer cell growth in colorectal cancer cell line in combination with various concentration of NVP-AUY922. Treatment with TQ could augment the cytotoxicity of NVP-AUY922 against the HT-29 as compared with that of NVP-AUY922 alone.

Conclusion: Our findings suggested the anti-proliferative effect of TQ and NVP-AUY922 through cytotoxic pathway to induce cell death.

***Keywords*:** NVP-AUY922, Thymoquinone, Colorectal Cancer Cell Line.

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Introduction

Cancer is one of the main problems and cause of death after cardiovascular diseases in numerous countries (1, 2). Colorectal cancer (CRC) considered as one of the common cancer among the others worldwide (3) 5-fluorouracil based chemotherapy remains the mainstay of treatment for CRC (3-5). Recently, chemotherapy agents including oxaliplatin, irinotecan,

and capecitabine have been developed (3, 6). Regardless of advances in cytotoxic and targeted therapy, ineffective chemotherapy remains as one of the major challenge in long-term managing of lethal metastatic disease and ultimately contributes to higher patient mortality (7). Heat shock protein 90 (HSP90) family are the family ubiquitous molecular chaperone proteins that engaged in folding, activation, maturation, and assembly of numerous proteins, and comprised essential mediators of signal transduction and cell cycle progression. HSP90 proteins have been upregulated in a number of malignancies. HSP90 inhibition can affect multiple oncogenic pathways and related proteins, and consequently could be considered as a smart target for drug development (8). The use of HSP 90 inhibitors has been recently introduced as an attractive anticancer therapy (9-13). NVP-AUY922 is a newly defined resorcinylic isoxazole–based HSP90 inhibitor which displayed potent preclinical efficacy in the treatment of cancer models (13).

Current study proposed the new insights into the usage of natural bioactive compounds to overcome chemoresistance in colon cancer chemotherapy (14). Thymoquinone (TQ) is the active herbal complex originated from *Nigella sativa* (NS) seed. According to previous surveys, numerous medical properties of TQ have been confirmed. In this regards NS has considerable phytochemical and pharmacological properties. Several animal and cell-based surveys have been performed, and some of them confirmed anti-inflammatory, and anti-cancer, effects of TQ (15, 16). However, specific impacts of exposure of CRC cells to TQ in combination with NVP-AUY922 have not been fulfilled in previous studies. This study was conducted to investigate the effects of various modulations of NVP-AUY922 and TQ on cellular toxicity and cellular viability of the HT-29 human colorectal cancer cells.

Materials & Methods

Chemicals and cell culture:

The CRC cell line; HT29 was obtained from the Pasteur Institute (Tehran, Iran). Cells were cultured in DMEM medium (Biowest, France) with the mixture of 10% fetal bovine serum (Biowest, France), 0.1% streptomycin, penicillin, and were maintained at 37°C in a humidified incubator containing 5% CO_2. TQ was obtained from Sigma and the stock solution prepared by dissolving TQ in pure water and diluted with the DMEM.

Cell viability assay:

Cell proliferation was examined using the water-soluble tetrazolium-1 (WST-1) assay, according to the manufacturer's protocols.

Cells were mono-treated with NVP-AUY922 or TQ for 24 h, and co-treated with NVP-AUY922 and TQ in various combinations and concentrations. After 24 h, cellular viability of treated cells in response to TQ and NVP-AUY922 was investigated by WST-1. After plating, treatments were performed for 24 h in various groups (different concentrations) as described below;

I (TQ; 50 µM; NVP-AUY922;50 nM), II (TQ; 40 µM; NVP-AUY922;40 nM), III (TQ; 20 µM; NVP-AUY922; 20nM), IV (TQ; 10 µM; NVP-AUY922; 10nM) and V (TQ; 5 µM; NVP-AUY922; 5nM). VI group (control) was included the untreated control cells.

After treatment for 24 h, WST-1 assay performed as cellular viability test in all treatments. For this, 10 µl of WST-1 stock added to wells. After 3–4 hours at 37°C, cell viability was examined based on the cleavage of the tetrazolium salt, WST-1, to dark red formazan.

The absorbance was read at 420 nm with a reference wavelength > 650 by the enzyme-linked immunosorbent assay (ELISA) microplate reader.

Three or more independent experiments were performed for WST viability assay. The values were shown as the mean ± standard deviation. The statistical significance among different groups was determined using one-way ANOVA.

Results

In this presented study, impacts of single treatments of NVP-AUY922 (1, 5, 10, 20, 50, and 100 nM)- or TQ (5, 10, 20, 25, 50, and 100 µM)- on the viability of HT29 were assessed. Both agents showed the weaker inhibitory impact in lower doses on HT-29, although the

inhibitory ratios for NVP-AUY922 in nM concentrations were higher than those for TQ (µM) (Figure 1A and 1B).

The cytotoxicity of NVP-AUY922 and TQ in single treatments were increased in the dose dependent patterns (Figure 1A, 1B).

The weak cytotoxicity of NVP-AUY922 and TQ against the HT-29 cells at low concentrations were presented.

Consequently, the inhibitory impacts of various combination treatments with exposure to low concentrations of NVP-AUY922 and TQ in HT29 after 24 h were evaluated (Figure 2).

Comparison of inhibitory impacts of various combinations at the same time (TQ and NVP-AUY922), showed that there were more effective cellular inhibitory compared to single treatments (Figure 2). Data indicated that among combination groups [I (TQ; 50 µM; NVP-AUY922;50 nM) II (TQ; 40 µM; NVP-AUY922;40 nM) III (TQ; 20 µM; NVP-AUY922; 20 nM), IV (TQ; 10 µM; NVP-AUY922; 10 nM) and V (TQ; 5 µM; NVP-AUY922; 5 nM). VI group (control)] there were significantly higher toxicity in group I compared to groups II, III, IV, V and VI ($p<0.05$).

Also group II exerted significant toxic effects compared to groups III, IV, and V. Moreover, group II had lower cytotoxic effect versus group I. Besides, group III had more significant inhibitory effects compared to groups V.

Further analysis showed the lower cellular viability in group V versus control group ($p<0.05$).

Treatment with TQ could augment the cytotoxicity of NVP-AUY922 against the HT-29 as compared with that of NVP-AUY922 alone.

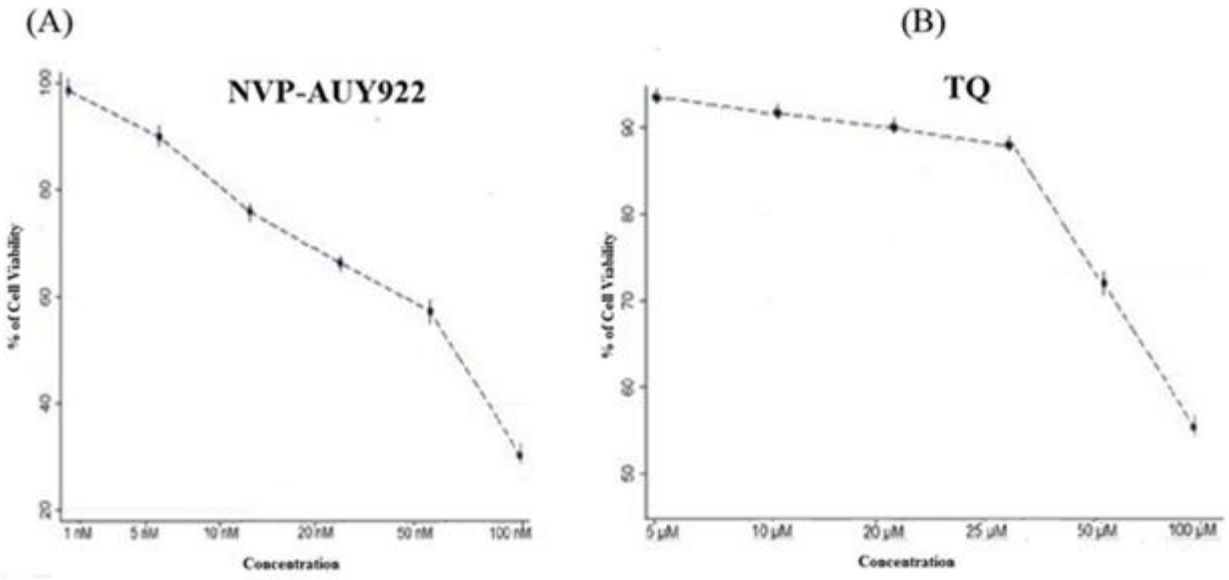

Fig 1: Results of cellular viability assay in HT-29 cells treated with NVP-AUY922 or TQ in single treatments. Growth-inhibitory curves of HT-29 cells treated to gradient concentrations of NVP-AUY922 (A) or TQ (B) in single treatments. Data presented as mean ±standard deviation. TQ: Thymoquinone.

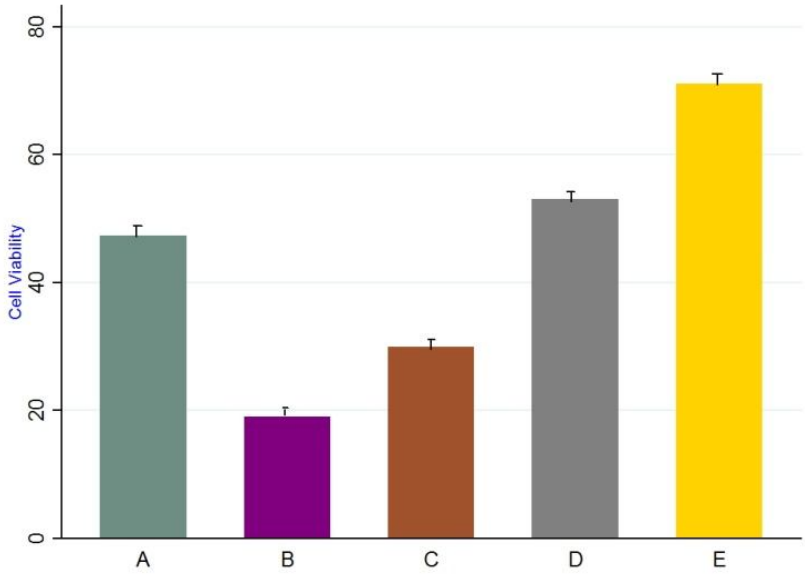

Fig 2: Results of cell viability assay of combination treatments with TQ and NVP-AUY922 in HT-29 cells. Concentrations of NVP-AUY922 in combination with TQ were chosen based on the initial tests described in the methods to assess the cytotoxic impacts of combinations on the HT-29 cell line.
A: (TQ 20 µM; NVP-AUY922 20 nM), B: (TQ 50 µM; NVP-AUY922 50 nM), C: (TQ 40 µM; NVP-AUY922 40 nM), D; (TQ 10 µM; NVP-AUY922 10 nM) and E; (TQ 5 µM; NVP-AUY922 5 nM). Data presented as mean ±standard deviation. TQ: Thymoquinone.

Discussion

Traditional medicines are usually without harmful side effects, and are generally are low-priced. TQ, an ingredient of *Nigella sativa* (NS), considered as one of this safe and efficacious agents (15, 16).

TQ interferes in a wide range of tumorigenic processes and counteracts carcinogenesis, malignant growth, invasion, migration, and angiogenesis. Moreover, TQ can specifically sensitize tumor cells toward conventional cancer treatments (e.g., radiotherapy, chemotherapy, and immunotherapy), and simultaneously minimize therapy-associated toxic effects in normal cells (17).

To better explain its functions, we aimed to assess the anti-proliferative effects of TQ as a natural component in combination with NVP-AUY922 after 24h exposure in HT-29 cell line. For this, we assessed whether treatment with TQ and NVP-AUY922 may increase the cellular toxicity in HT-29 CRC cell line.

Based on results of our study using cellular viability assay, the TQ displayed strong cytotoxicity impacts when combined with low concentration of NVP-AUY922 in nM range.

The results showed that the few ranges of concentrations, from 5 to 50 nM of NVP-AUY922 and 5-50 µM of TQ, gained the considerable possible toxicity and these ranges can be utilized to find the effective concentrations of these agents for treatments of CRC. In these concentrations, significant cytotoxic effects in compared to control group were presented in HT-29 cancer cell line. In this regards, the cellular viability in the TQ and NVP-AUY922 treated cells were lower than the untreated group and single treatments. Therefore, it could be understood that these combinations might had considerable effect in the CRC cell death induction.

In accordance to our data, previous studies showed that after administration of TQ, the significant growth inhibition has been displayed in the breast, gastric, and colon cancer (18-20).

In a study done by Pazhouhi et. al. it was confirmed that TQ synergistically increased the anti-cancer effects

of temozolomide in the glioblastoma cell line (21). Also, Khazaei et. al. described synergistic apoptotic cell death of glioblastoma cells upon treatment with TQ in combination with chemotherapy (22). Likewise, TQ in combination with 5-FU inhibited the expression of pro-cancerous NF-κB, iNOS, VEGF, Wnt, β-catenin, COX-2, and TBRAS with a concomitant elevation in anti-tumorigenic TGF-β1, TGF-βRII, DKK-1, CDNK-1A, Smad4, and GPx expression (23). Similarly, Fröhlich et. al. displayed the elevated ROS production and induction of DNA damage in human colon cancer cells that exposed to TQ and artemisinin (24).

Also, TQ was established to chemosensitize 5-FU in gastric cancer treatment by inducing apoptosis and (19).

We suggest that the ability of TQ to effectively increase the cytotoxic effects of NVP-AUY922 denotes the probable efficacy of these combinations in inhibiting cellular viability of CRC cells. TQ has been shown to have higher toxicity in combined to NVP-AUY922 in different doses.

As TQ significantly decreases the viability of human colon cancer cells in a concentration- and time-dependent manner, and TQ increased apoptosis induction is related to the upregulation of Bax and inhibition of Bcl-2 and Bcl-xl expression, so it has been indicated that TQ also activated caspase-9,-7, and -3 (25).

It seems that it would be an ideal agent to utilize in combination with chemotherapy to increase the efficacy of chemotherapy in CRC.

In conclusion, chemo-sensitizing effects of TQ in CRC cells proposed the potential treatment to the success of CRC chemotherapy via increase of cancer cell growth inhibition.

Indeed, TQ which effectively increased the cytotoxic effects of NVP-AUY922 is likely to inhibit the growth of the chemo-resistance cells. Further studies proposed to evaluate various combinations of NVP-AUY922 and TQ and their effects on reduction of subsequent relapse following chemotherapy.

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Conflict of interest

The authors have no conflict of interest in this study.

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