

Full Length Research Paper

Thymoquinone rich fraction from *Nigella sativa* and thymoquinone are cytotoxic towards colon and leukemic carcinoma cell lines

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Nigella sativa has been used for centuries in Asia, Middle East and Africa to promote health and fight diseases. In this study, the anti-cancer effects of thymoquinone rich fraction (TQRF) extracted from *N. sativa* seeds using supercritical fluid extraction (SFE) system and commercially available thymoquinone (TQ) on colon cancer (HT29), lymphoblastic leukemia (CEMSS) and promyelocytic leukemia (HL60) cells lines were investigated. The concentration that gave 50% inhibition of cell viability (IC₅₀) of HT29, CEMSS and HL60 cells treated with TQRF were 400, 350 and 250 µg/ml, respectively. Meanwhile, the IC₅₀ of TQ was 8, 5 and 3 µg/ml, respectively. Cell cycle analysis shows the increment of apoptosis in a time-dependent manner. However, both TQRF and TQ were not able to arrest the cell cycle phases of the cells. Apoptosis was the main mode of HT29, CEMSS and HL60 cells death induced by both TQRF and TQ. Our findings support the potential use of TQRF and TQ for the treatment of colon cancer and leukemia.

Key words: *Nigella sativa*, thymoquinone, supercritical fluid extraction system, colon cancer, lymphoblastic leukemia, promyelocytic leukemia, cell cycle.

INTRODUCTION

Spice-derived phytochemicals have an enormous potential in the prevention and treatment of cancer through multiple targets and yet pharmacologically are

highly safe (Aggarwal et al., 2008). *Nigella sativa* L. (Ranunculaceae family) seeds, commonly known as black seed or black cumin, not only employed for thousands of years as a spice and food preservative, but also used as a protective and curative remedy for numerous diseases. Generally, *N. sativa* seeds contain more than 30% fixed oil and 0.40 to 0.45% volatile oil (Ali and Blunden, 2003). Thymoquinone represents 18.4 to 24% of the *N. sativa* volatile oil (Arslan et al., 2005). The pharmacological investigations of the seed extracts reveal a broad spectrum of activities including antioxidant (Mariod et al., 2009), antidiabetic (Arayne et al., 2007), nephrotoxicity (Yaman and Balikci, 2010), neuroprotective (Ismail et al., 2008; Mousavi et al., 2010), intestinal ischemia-reperfusion injury (Terzi et al., 2010), antiasthmatic (Boskabady et al., 2010), anti-inflammatory

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Abbreviations: TQRF, Thymoquinone rich fraction; SFE, supercritical fluid extraction; TQ, thymoquinone; HT29, colon cancer; HL60, promyelocytic leukemia; CEMSS, lymphoblastic leukemia; MTS, methyl thiazole tetrazolium; TC, total cholesterol levels; LDLC, low density lipoprotein cholesterol; LDLR, low density lipoprotein receptor; 3-HMG-COAR, hydroxy-3-methylglutaryl-coenzyme A reductase.

(Ragheb et al., 2009) and antimicrobial (Allahghadri et al., 2010). Many of the pharmacological activities have been attributed to the quinone constituents of the seed (Ali and Blunden, 2003).

There is a growing interest in the therapeutic potential of TQ in cancer therapy. TQ has been reported to inhibit the proliferation of several types of human colon cancer cells (Caco-2, HCT-116, LoVo, DLD-1 and HT-29) (El-Najjar et al., 2010), SW-626 human colon cancer cells (Norwood et al., 2007) and HCT-116 human colon cancer cells (Gali-Muhtasib et al., 2008a) without exhibiting cytotoxicity to normal human intestinal FHS74Int cells (El-Najjar et al., 2010). TQ reduces mouse colon tumor cell invasion and inhibits tumor growth in murine colon cancer models (Gali-Muhtasib et al., 2008b). TQ induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells (El-Mahdy et al., 2005). The anticancer and anti-inflammatory activities of TQ may be partly mediated through the suppression of the various carcinogens and inflammatory stimuli, NF- κ B activation pathway, and thus may be used in the treatment of myeloid leukemia and other cancers (Sethi et al., 2008). However, to our best of knowledge, there is no study showing anticarcinogenic effects of SFE extract of *N. sativa* on colorectal cancer and leukemia.

On the other hand, treatment with TQ downregulated glycoprotein mucin 4 (MUC4) expression in pancreatic cancer that contributes to the regulation of differentiation, proliferation, metastasis and the chemoresistance of pancreatic cancer cells (Torres et al., 2010). Antitumor activity of gemcitabine and oxaliplatin is augmented by TQ in human pancreatic cancer cell lines BxPC-3 and HPAC (Banerjee et al., 2009). TQ also was cytotoxic towards the human cervical carcinoma cells (HeLa) in a dose-, and time-dependent manner. In addition, TQ induced apoptosis in HeLa cells by the upregulation of the expression of p53 (Yazan et al., 2009).

Combinatorial effects of TQ on the anti-cancer activity of doxorubicin were tested for cytotoxicity on human cells of HL-60 leukemia, 518A2 melanoma, HT-29 colon, KB-V1 cervix, and MCF-7 breast carcinomas. TQ has improved the anti-cancer properties of doxorubicin in a cell line specific manner. A significant rise of the growth inhibition by doxorubicin in HL-60 and multi-drug-resistant MCF-7/TOPO cells was observed when TQ was added. The mode of action of both drugs and of their mixture was mainly apoptotic (Effenberger-Neidnicht and Schobert, 2010). TQ has shown a cytotoxic effect on HepG2 cells, triggering effects on caspase activation and apoptotic cell death and cell cycle arresting activity. These results suggest that TQ can be a promising anti-cancer therapeutic agent for hepatocellular carcinoma while preventing chemotherapy induced damages on non-tumor tissues (Hassan et al., 2008).

On the other hand, ethanol extract of *N. sativa* seeds generates antioxidants, possesses antitumor activity and prolong the life span of mice bearing Ehrlich ascites

tumor (EAT) (Musa et al., 2004). The cytotoxicity of essential oil, ethyl acetate extract and butanolic extract of *N. sativa* depends on the tumor cell types (P815, Vero, BSR, ICO1) (Mbarek et al., 2007). *N. sativa* extract has cytotoxic effects against different types of cancer cell lines *in vitro* and *in vivo*. Salomi et al. (1992) reported that a number of cell lines, such as Dalton lymphoma, Ehrlich ascites carcinoma and Sarcoma-180 exhibited coordination or sensitivity to *N. sativa* extract when administered intraperitoneally (Musa et al., 2004). Strong cytotoxic activity of the aqueous extract of *N. sativa* seeds is in agreement with previous investigations using ethanolic (Salomi et al., 1992) or methanolic (Swamy and Tan, 2000) extracts of these seeds. The decoction prepared from a mixture of *N. sativa* seeds, *Hemidesmus indicus* roots and *S. glabra* rhizome has powerful cytotoxic properties towards human liver cancer cells (HepG2) *in vitro* (Thabrew et al. 2005).

Supercritical fluid extraction of lipid has received attention as an alternative method to organic solvent extraction and has been shown to be an ideal method for extracting and fractioning oils (Vagi et al., 2002). Supercritical CO₂ is non-toxic, non-flammable, and simple to use when compared to conventional organic solvents. Furthermore, SFE fractionation allows the pool of target compounds in the oil fraction. These advantages may make supercritical CO₂ extraction ideal in the food and pharmaceutical industries (Pourmortazavi and Hajimirsadeghi, 2007). Recently, we have identified TQRF as a fraction prepared from the seeds of *N. sativa* using SFE system with high content of TQ (2%) (Al-Naqeeb et al., 2009).

In addition, we found that TQRF and TQ are effective in regulating Apolipoprotein A-1 and Apolipoprotein B100 genes on cholesterol metabolism in HepG2 cells. The *in vitro* study suggested a possible use of TQRF in the treatment of hypercholesterolemia (Al-Naqeeb and Ismail, 2009). More recently we have shown that TQRF and TQ are effective in reducing plasma total cholesterol (TC) levels and low density lipoprotein cholesterol (LDLC) in rat. Both TQRF and TQ were shown to up-regulate mRNA level of low density lipoprotein receptor (LDLR) and down-regulate of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-COAR) significantly *in vitro* and *in vivo* (Al-Naqeeb et al., 2009).

In this study, we examined the anti-cancer effects and mode of cell death of TQRF in comparison with TQ on HT29, CEMSS and HL60 cell lines.

MATERIALS AND METHODS

Materials and chemicals

N. sativa seeds were purchased from local market in Yemen and stored as a voucher specimen in Nutrigenomics and Nutricosmeceuticals Programme, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia. Colorectal (HT29), promyelocytic leukemia (HL60) and

lymphoblastic leukemia (CEMSS) cell lines were kindly provided by Biodiagnostic Technology Programme, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia. The chemicals used in this study were TQ, RPMI 1640, penicillin, streptomycin, phosphate buffer saline (PBS) tablet, sodium azide, triton X-100, EDTA, propidium iodide and RNase (Sigma-Aldrich Co., St. Louis, Missouri, USA); methyl thiazole tetrazolium (MTS) (Promega, Southampton, UK); fetal bovine serum (FBS) (Gibco BRL Life Tech, Grand Island, USA).

Supercritical fluid extraction of thymoquinone rich fraction

Thymoquinone rich fraction was extracted from *N. sativa* seeds using SFE system as reported by our previous study (Al-Naqeeb and Ismail, 2009). Briefly, *N. sativa* seeds were cleaned and dried in an oven at 40°C until a constant weight was obtained. Hundred grams of the seeds were ground into a powder for 1 min using an electrical grinder (Waring Blender, Tokyo, Japan) just before the SFE extraction was initiated. Then 100 g *N. sativa* powder were placed in the 1 L SFE extraction vessel (Thar 1000 F, Tokyo, Japan). Extraction was performed using CO₂ supplied to an extraction unit. The unit was equipped with an extractor vessel and three cyclone separators. The extraction vessel was tightly sealed and the desired extraction temperature at 40°C and pressure at 600 bar were set. SFE flow-rate was maintained at 30.00 ml/min and regulated by an automated back pressure regulator. The entire extraction process required 3 h and the resulting oil samples were collected from the system collection vessel, weighed and kept at -20°C in amber bottles for further analysis.

Cytotoxicity and cell viability test

The HT29, CEMSS and HL60 cells were plated in 96-well plates at a density of 1×10^5 cells/ml and were incubated overnight at 37°C, 5% CO₂ humidified incubator. Cells were cultured using RPMI 1640 supplemented with 10% FBS, 100U of penicillin/ml and 100 µg of streptomycin/ml. A 2-fold serial dilution was performed in 96-well plate starting from a concentration of 500 µg/ml of TQRF and 100 µg/ml of TQ in 100 µl of complete medium and incubated for 72 h at 37°C, 5% CO₂. For control, 100 µl PBS was used instead. After 72 h incubation, the mitochondrial function of cells was measured by MTS conversion assay, which served as a general indicator of cell viability for each treatment condition. The CellTiter 96® AQueous One Solution Reagent (MTS solution) (Promega Southampton, UK) was thawed in a water bath at 37°C for 10 min. Twenty microliters of MTS reagent was pipetted into each well containing the samples in 100 µl culture medium. Plates were then incubated for 4 h at 37°C, 5% CO₂. The cleavage of MTS to formazan by metabolically active cells was quantified and the optical density (OD) of the wells was determined using an ELISA reader (Opsys MR, Thermo LabSystems, Franklin, MA, USA) at 490 nm wavelength. The dose-response curve was plotted and the concentration that gave 50% inhibition of cell growth (IC₅₀) was calculated.

Cell cycle analysis

HT29, CEMSS and HL60 cells at a concentration of 1×10^5 cells/ml in 2 ml culture medium containing 10% FBS was seeded into 6 wells plate and treated with TQRF and TQ at IC₅₀ concentrations. Then, the plates were incubated in atmosphere of 5% CO₂ at 37°C for 24 and 48 h. After 24 and 48 h incubation, the cells were spun down at 1000 rpm for 10 min. The supernatant was discarded and the pellet was washed with PBS twice. Cell pellets were fixed with 500 µl of 80% cold ethanol and 250 µl of cell suspension and kept

for at least 2 h at -20°C. Cells were then spun down at 1000 rpm for 10 min and the ethanol was decanted. The cell pellet was washed with 1 ml PBS and sodium azide twice. Cell pellet was resuspended with 1 ml PBS containing 0.1% triton X-100, 10 mM EDTA, 50 µg/ml RNase and 2 µg/ml propidium iodide. After which, the cell was incubated for 30 min to 1 h at 4°C. Finally, the cell cycle was measured by flow cytometer (Cyan ADP, Beckman Coulter, USA) using the Summit V4.3 software.

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA)-one way analysis of variance. A $p < 0.01$ are considered as significant differences.

RESULTS

Cytotoxicity and cell viability

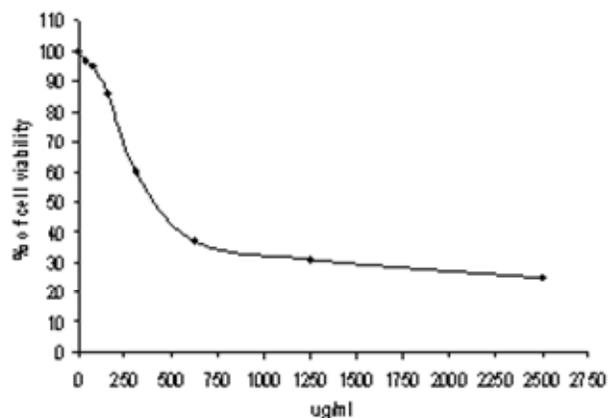
The MTS assay shows that TQRF and TQ exhibit cytotoxic effects against HT29, CEMSS and HL60 cells lines. TQRF shows cytotoxicity effect with IC₅₀ values at 400 µg/ml, 350 µg/ml and 250 µg/ml on HT29, CEMSS and HL60 respectively, compared to untreated control cells after 72 h of treatment (Figure 1). On the other hand, the IC₅₀ of TQ against HT29, CEMSS and HL60 cells compared to untreated control cells after 72 h of treatment were 8, 5 and 3 µg/ml, respectively (Figure 2).

Cell cycle analysis

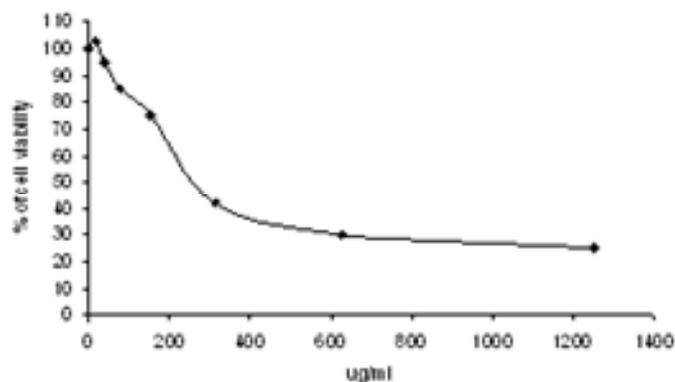
Figures 3 and 4 show the effects of TQRF and TQ on the cell cycle of HT29, CEMSS and HL60 cells for 24 and 48 h. We found that 24 h treatment of HT29, CEMSS and HL60 cells with TQRF at IC₅₀ value of MTS assay were able to induce 6, 58 and 88% of apoptotic cells, respectively. The number of apoptotic cells increased gradually after 48 h treatment, TQRF induced 12, 90 and 91% of HT29, CEMSS and HL60 apoptotic cells, respectively. A similar trend was also found in cells treated with TQ at IC₅₀ value of MTS assay. Treatment of TQ for 24 h had induced 5, 74 and 95% of apoptotic cells while 48 h treatment increased the apoptotic cells to 9, 90 and 98% of HT29, CEMSS and HL60 cells, respectively. The percentages of apoptosis increased in a time-dependent manner in HT29, CEMSS and HL60 after being treated with both TQRF and TQ. However, the apoptotic cells increment between 24 and 48 h treatment with TQRF and TQ were only significant ($p < 0.01$) on CEMSS cells.

On the other hand, there are more significant ($p < 0.01$) apoptotic cell deaths in CEMSS and HL60 compared to HT29 for both TQRF and TQ treated cells. The decrements ($p < 0.01$) in percentages of G0/G1, S and G2/M phases of CEMSS and HL-60 suggesting that TQRF and TQ were able to retard the growth of leukemia cells. However, G0/G1, S and G2/M phases of HT29

(A) HT29



(B) HL60



(C) CEMSS

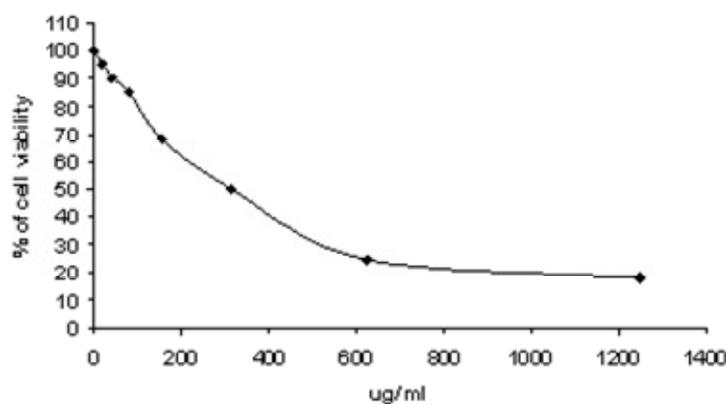


Figure 1. Reduction of percentage of viable cell population treated with thymoquinone rich fraction (TQRF) on (A) HT29 (B) HL60 (C) CEMSS cell lines. The cells were treated for 72 h with various concentrations of TQRF. Levels of cell viability were measured using the MTS assay. The viability of untreated control cells was defined as 100%. The concentration that gave 50% inhibition of cell growth (IC_{50}) was calculated.

showed no significant different with the untreated cells.

DISCUSSION

There has been increased interest in the use of naturally occurring compounds with chemopreventive and chemotherapeutic effects in the treatment of cancers. Apoptosis is an essential process in the pathogenesis of cancer. Recently, medicinal herbal provide new insights into the molecular mechanisms underlying the apoptotic process in cancer progress (Kwon et al., 2007). *N. sativa* conventionally prepared crude extract and TQ standard has shown significant effect *in vitro* and *in vivo* against different cancer cell lines. However, the effects of its SFE extract against cancer cells are yet to be determined. SFE is an attractive alternative to conventional methods of extraction due to its use of environmentally compatible

fluids, reduced solvent consumption, oxygen-free extraction environment and shorter extraction time (Machmudah et al., 2005). These advantages of supercritical CO_2 extraction may provide a suitable and better extraction process in production of food and pharmaceuticals.

In this study, we investigated the effects of TQRF extracted by using SFE and TQ on HT29, CEMSS and HL60 cell lines. The results of cell viability showed that TQRF and TQ were cytotoxic to colorectal HT29 and both leukemic HL60 and CEMSS cells. We recently, showed that TQRF and TQ exhibit cytotoxic effects against HepG2 cells with IC_{50} at 100 μ g/ml for TQRF and 3 μ g/ml of TQ (Al-Naqeeb and Ismail, 2009). TQ content in TQRF (20 mg TQ/g TQRF) is one of the suggested major bioactive that contributes to apoptotic effect on the tested cell lines. Notably, 100 μ g/ml of TQRF contained about 2 μ g/ml of TQ. We observed that less or equal equivalent

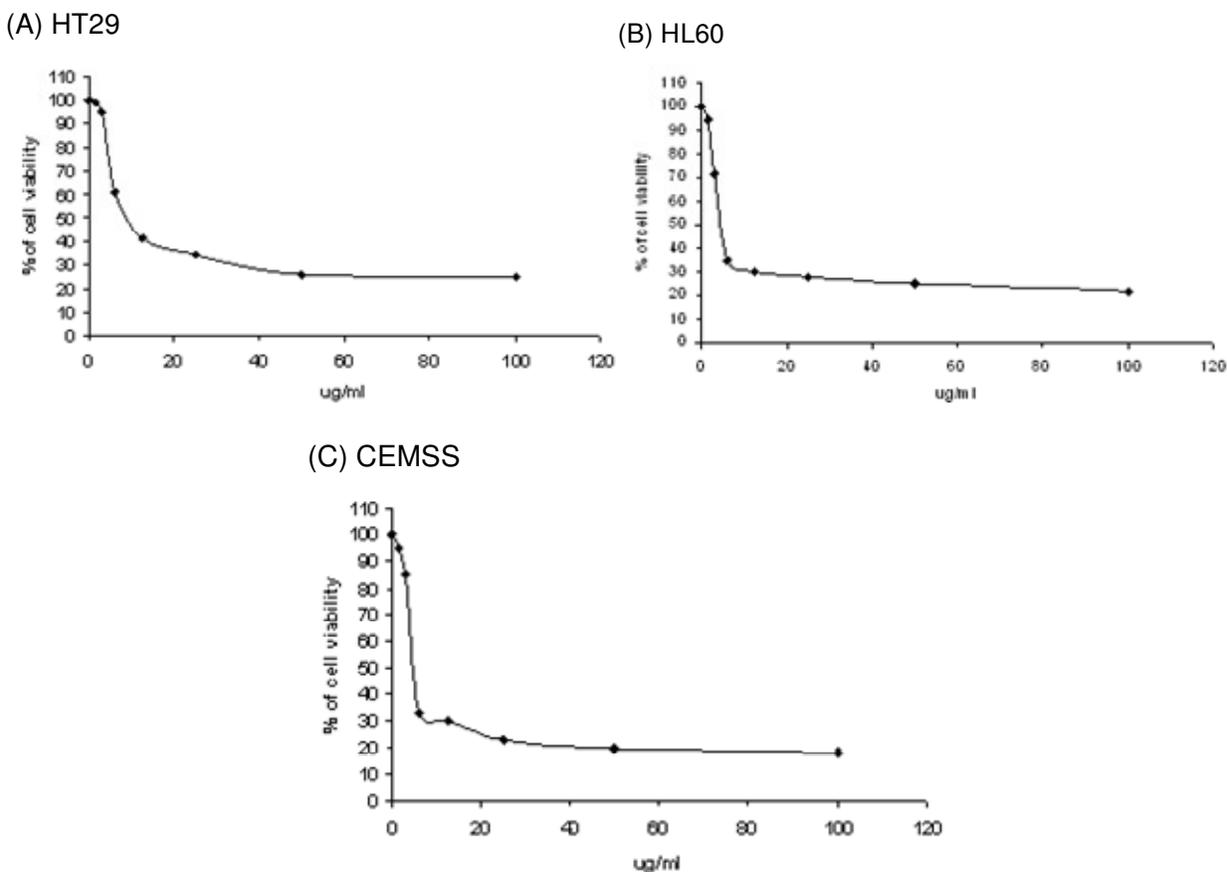


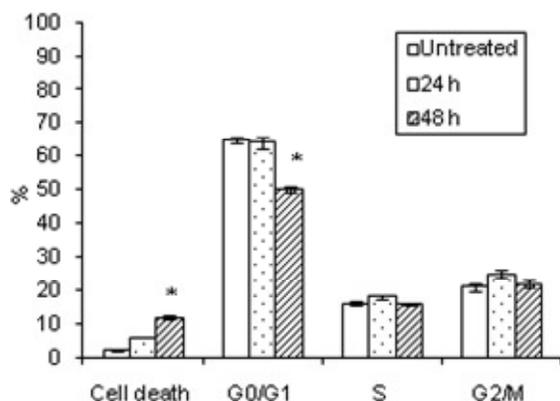
Figure 2. Reduction of percentage of viable cell population treated with thymoquinone (TQ) on (A) HT29 (B) HL60 (C) CEMSS cell lines. The cells were treated for 72 h with various concentrations of TQ. Levels of cell viability were measured using the MTS assay. The viability of untreated control cells was defined as 100%. The concentration that gave 50% inhibition of cell growth (IC_{50}) was calculated.

concentrations of TQ in TQRF showed almost equal rate of apoptotic cells compared to pure TQ, thus suggesting the apoptotic effect of TQRF on tested cells mainly due to the TQ content. The IC_{50} of TQRF against HT29 (450 $\mu\text{g/ml}$), CEMSS (350 $\mu\text{g/ml}$) and HL60 (250 $\mu\text{g/ml}$) is much higher than in HepG2 cells (100 $\mu\text{g/ml}$). These data were supported by the findings of El-Najjar et al. (2010) who found that HT-29 cell line was the least sensitive to TQ-induced growth inhibition compared to HCT-116, DLD-1, Lovo, and Caco-2.

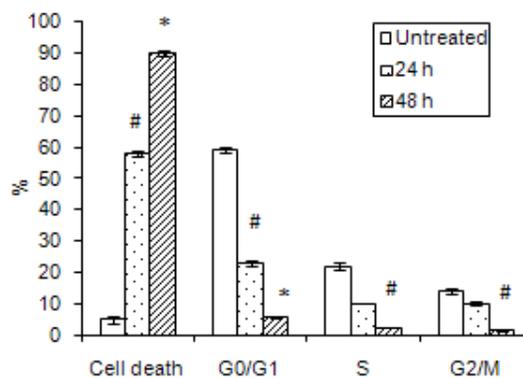
These findings suggest that TQRF might be targeting the different features available in several types of cancer cells. TQ induced apoptosis in HeLa cells by the upregulation of the expression of p53. Therefore, the study suggested TQ induced apoptosis through p53 dependent pathway in HeLa cells (Yazan et al., 2009). On the other hand, water extract of *N. sativa* had IC_{50} at 6 mg/ml against HepG2 cells (Thabrew et al., 2005). However, ethyl acetate fraction of *N. sativa* at concentration of 100 $\mu\text{g/ml}$ completely killed all the HepG2 cells (Swamy and Tan, 2001).

In the cell cycle analysis, both TQRF and TQ effectively killed the leukemic cells up to 98%. In contrast, HT29 has slightly increased of apoptotic cells to 12%. The present study showed that HL60 cells were more sensitive to TQRF and TQ treatment in both MTT and cell cycle assays in comparison to CEMSS and HT29. This indicates that TQRF and TQ response to the cancer cells in a cell-type dependent manner. In addition, these treatments were not able to arrest HT29, CEMSS and HL60 cells at specific cell cycle phases suggesting that TQRF and TQ killed HT29, CEMSS and HL60 cells at different cell cycle stage. Therefore, these results showed that apoptosis was the main mode of HT29, CEMSS and HL60 cells death induced by TQRF and TQ. However, HT29 may need longer exposure to TQRF and TQ treatment to have the significant effects. Moreover, TQRF and TQ might have cytostatic effects to HT29 instead of cytotoxic effects. If the exposure of TQRF and TQ on HT29 was prolonged, cell cycle arrest maybe observed. In contrast, TQRF and TQ showed cytotoxic effects to HL60 and CEMSS cells as within 24 h, both treatments

(A) HT29



(B) CEMSS



(C) HL60

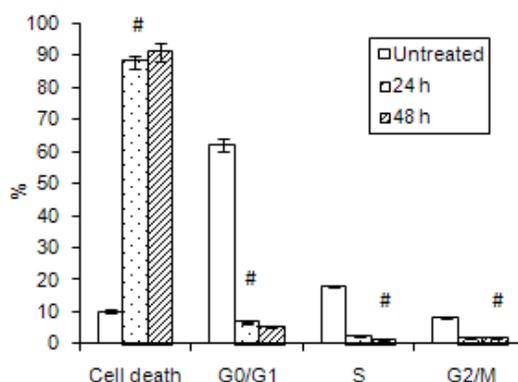


Figure 3. Effects of thymoquinone rich fraction (TQRF) on cell cycle in (A) HT29 (B) CEMSS and (C) HL-60 cells determined by flow cytometric analysis. The cells were treated with IC_{50} value of MTS assay for 24 h and 48 h. Percentage of cell population in each cell cycle phases were determined using the Summit V4.3 analysis software. * $p < 0.01$ was considered significant compared to 24 h treatment. # $p < 0.01$ was considered significant compared to untreated cells

were able to induce apoptosis.

In cell viability assay, 400 $\mu\text{g/ml}$ of TQRF reduced 50% of cell viability in HT29 for 72 h of treatment. In contrast, only 6% and 12% of cell death was noticed in cell cycle analysis of 400 $\mu\text{g/ml}$ TQRF treated for 24 h and 48 h, respectively. The cell death was noticed to be doubled in duration of 24 h. Therefore, for 72 h of treatment with TQRF, the cell death of cell cycle analysis may around 18% only. The findings indicate that there are no correlation between MTS assay and cell cycle analysis. This is due to different parameters measured in each assay. Cell viability assay measured the total number of cell death, whereby cell cycle assay differentiated between cell death and arrested cells (Ho et al., 2009). On the other hand, the explanation for the difference of percentage proliferation measured using these two assays may be that TQRF and TQ induces proliferation of HT29 but does not enhance the mitochondrial activity. Gerlier and Thomasset (1986) observed that the increase

in mitochondrial activity which is determined by MTS assay was independent of the increase in DNA synthesis measured by cell cycle analysis using flow cytometer, thus, the proliferative events can be independent from mitochondrial activity (Wang and Zheng, 2002).

In conclusion, this study indicates that TQRF and TQ possess cytotoxic properties against colorectal cancer and showed more prominent cytotoxic effects on leukemic cell lines. In spite of TQ, TQRF was also has potential to be used as anti-cancer treatment. Therefore, TQRF from *N. sativa* does have the potential to be developed as a nutraceuticals for preventing the progression of cancer.

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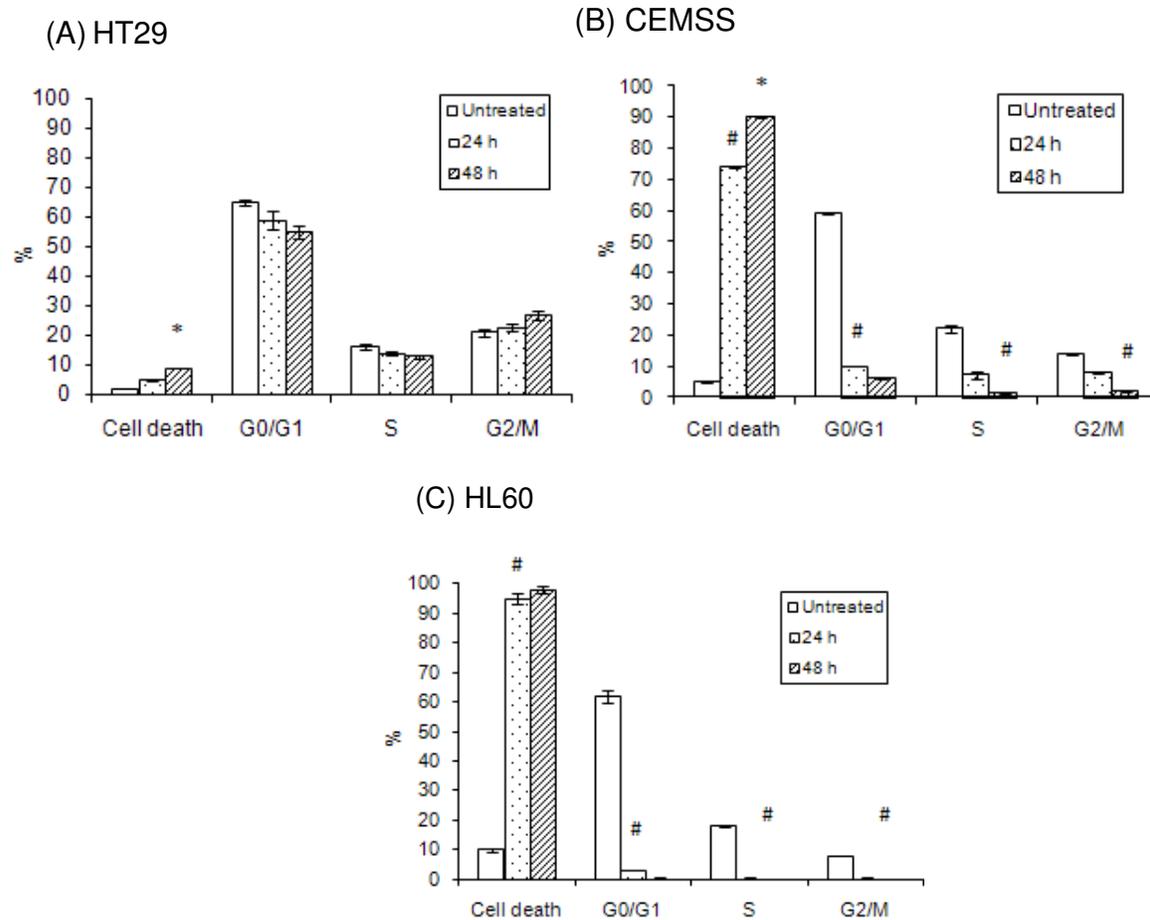


Figure 4. Effects of thymoquinone (TQ) on cell cycle in (A) HT29 (B) CEMSS and (C) HL-60 cells determined by flow cytometric analysis. The cells were treated with IC₅₀ value of MTS assay for 24 h and 48 h. Percentage of cell population in each cell cycle phases were determined using the Summit V4.3 analysis software. * $p < 0.01$ was considered significant compared to 24 h treatment. # $p < 0.01$ was considered significant compared to untreated cells.

REFERENCES

- Aggarwal BB, Kunnumakkara AB, Harikumar KB, Tharakan ST, Sung B, Anand P (2008). Potential of spice-derived phytochemicals for cancer prevention. *Planta Med.*, 74(13): 1560-1569.
- Ali BH, Blunden G (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytother. Res.*, 17: 299-305.
- Allahghadri T, Rasooli I, Owlia P, Nadooshan MJ, Ghazanfari T, Taghizadeh M, Astaneh SDA (2010). Antimicrobial property, antioxidant capacity, and cytotoxicity of essential oil from cumin produced in Iran. *J. Food Sci.*, 75(2): H54-H61.
- Al NG, Ismail M (2009). Regulation of apolipoprotein A-1 and apolipoprotein B100 genes by thymoquinone rich fraction and thymoquinone in Hepg2 cells. *J. Food Lipids*, 16: 245-258.
- Al NG, Ismail M, Latifah SY (2009). Effects of thymoquinone rich fraction and thymoquinone on plasma lipoprotein levels and hepatic low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase genes expression. *J. Funct. Foods*, 1: 298-303.
- Arayne MS, Sultana N, Mirza AZ, Zuberi MH, Siddiqui FA (2007). *In vitro* hypoglycemic activity of methanolic extract of some indigenous plants. *Pak. J. Pharm. Sci.*, 20(4): 261-268.
- Arslan SO, Gelir E, Armutcu F, Coskun O, Gurel A, Sayan H, Celik IL (2005). The protective effect of thymoquinone on ethanol-induced acute gastric damage in the rat. *Nutr. Res.*, 25: 673-680.
- Banerjee S, Kaseb AO, Wang Z, Kong D, Mohammad M, Padhye S, Sarkar FH, Mohammad RM (2009). Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. *Cancer Res.*, 69(13): 5575-5583.
- Boskabady MH, Mohsenpoor N, Takaloo L (2010). Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. *Phytomed.*, 17(10): 707-713.
- Effenberger NK, Schobert R (2010). Combinatorial effects of thymoquinone on the anti-cancer activity of doxorubicin. *Cancer Chemother. Pharmacol.* DOI 10.1007/s00280-010-1386-x.
- EI M MA, Zhu Q, Wang QE, Wani G, Wani AA (2005). Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. *Int. J. Cancer*, 117: 409-417.
- EI NN, Chatila M, Moukadem H, Vuorela H, Ocker M, Gandesiri M, Schneider SR, Gali MH (2010). Reactive oxygen species mediate thymoquinone induced apoptosis and activate ERK and JNK signaling. *Apoptosis*, 15: 183-195.
- Gali MH, Kuester D, Mawrin C (2008a). Thymoquinone triggers inactivation of the stress response pathway sensor CHEK1 and contributes to apoptosis in colorectal cancer cells. *Cancer Res.*, 68: 5609-5618.
- Gali MH, Ocker M, Kuester D (2008b). Thymoquinone reduces mouse colon tumor cell invasion and inhibits tumor growth in murine colon cancer models. *J. Cell Mol. Med.*, 12: 330-342.

- Gerlier D, Thomasset N (1986). Use of MTT colorimetric assay to measure cell activation. *J. Immunol. Methods*, 94: 57-63.
- Hassan SA, Ahmed WA, Galeb FM, El-Taweel MA, Abu-Bedair FA (2008). *In vitro* challenge using thymoquinone on hepatocellular carcinoma (HepG2) cell line. *Iranian J. Pharm. Res.*, 7(4): 283-290.
- Ho KL, Yazan LS, Ismail N, Ismail M (2009). Apoptosis and cell cycle arrest of human colorectal cancer cell line HT-29 induced by vanillin. *Cancer Epidemiol.*, 33: 155-160.
- Ismail N, Ismail M, A. Latiff L, Mazlan M, Mariod AA (2008). Black cummin seed (*Nigella sativa* Linn.) oil and its fractions protect against beta amyloid peptide-induced toxicity in primary cerebellar granule neurons. *J. Food Lipids*, 15: 519-533.
- Kwon KB, Park BH, Ryu DG (2007). Chemotherapy through mitochondrial apoptosis using nutritional supplements and herbs: A brief overview. *J. Bioenerg. Biomembr.*, 39: 31-34.
- Machmudah S, Shiramizu Y, Goto M, Sasaki M, Hirose T (2005). Extraction of *Nigella sativa* L. using Supercritical CO₂: A study of antioxidant activity of the extract. *Separation Sci. Tech.*, 40: 1267-1275.
- Mariod AA, Ibrahim RM, Ismail M, Ismail N (2009). Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cummin (*Nigella sativa*) seedcake. *Food Chem.*, 116(1): 306-312.
- Mbarek LA, Mouse HA, Elabbadi N, Bensalah M, Gamouh A, Aboufatima R, Benharref A, Chait A, Kamal M, Dalal A, Ziad A (2007). Anti-tumor effect of blackseed (*Nigella sativa* L.) extracts. *Braz. J. Med. Biol. Res.*, 40: 839-847.
- Mousavi SH, Tayarani-Najaran Z, Asghari M, Sadeghnia HR (2010). Protective effect of *Nigella sativa* extract and thymoquinone on serum/glucose deprivation-induced PC12 cells death. *Cell. Mol. Neurobiol.*, 30(4): 591-598.
- Musa D, Dilsiz N, Gumushan H, Ulakoglu G, Bitiren M (2004). Antitumor activities of an ethanol extract of *Nigella sativa* seeds. *Biologia Bratislava*, 59(6): 735-740.
- Norwood AA, Tan M, May M, Tucci M, Benghuzzi H (2006). Comparison of potential chemotherapeutic agents, 5-fluoruracil, green tea, and thymoquinone on colon cancer cells. *Biomed. Sci. Instrum.*, 42: 350-356.
- Pourmortazavi SM, Hajimirsadeghi SS (2007). Supercritical fluid extraction in plant essential and volatile oil analysis. *J. Chromatogr.*, A 1163: 2-24.
- Ragheb A, Attia A, Eldin WS, Elbarbry F, Gazarin S, Shoker A (2009). The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: A review. *Saudi J. Kidney Dis. Transpl.*, 20(5): 741-752.
- Salomi NJ, Nair SC, Jayawardhanan KK, Verghese CD, Panikkar KR (1992). Antitumor principles from *Nigella sativa* seeds. *Cancer Lett.*, 63: 41-46.
- Sethi G, Ahn KS, Aggarwal BB (2008). Targeting nuclear factor- κ B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. *Mol. Cancer Res.*, 6(6): 1059-1070.
- Swamy SMK, Tan BKH (2001). Extraction, isolation and characterization of anti-tumor principle, alpha-hederin, from the seeds of *Nigella sativa*. *Planta Medica*, 67: 29-32.
- Terzi A, Coban S, Yildiz F, Ates M, Bitiren M, Taskin A, Aksoy N (2010). Protective effects of *Nigella sativa* on intestinal ischemia-reperfusion injury in rats. *J. Investig. Surg.*, 23(1): 21-27.
- Thabrew MI, Ragai RM, Mohammed AM, Robin DH (2005). Cytotoxic effects of a decoction of *Nigella sativa*, *Hemidesmus indicus* and *Smilax glabra* on human hepatoma HepG2 cells. *J. Life Sci.*, 77: 1319-1330.
- Torres MP, Ponnusamy MP, Chakraborty S, Smith LM, Das S, Arafat HA, Batra SK (2010). Effects of thymoquinone in the expression of mucin 4 in pancreatic cancer cells: implications for the development of novel cancer therapies. *Mol. Cancer Ther.*, pp. 1535-7163.
- Vagi E, Simándi B, Daood HG, Deak A, Sawinsky J (2002). Recovery of pigments from *Origanum majorana* L. by extraction with supercritical carbon dioxide. *J. Agric. Food Chem.*, 50: 2297-2301.
- Wang YY, Zheng XX (2002). A flow cytometry-based assay for quantitative analysis of cellular proliferation and cytotoxicity *in vitro*. *J. Immunol. Methods*, 268: 179-188.
- Yaman I, Balıkcı E (2010). Protective effects of *Nigella sativa* against gentamicin induced nephrotoxicity in rats. *Exp. Toxicologic Pathol.*, 62(2): 183-190.
- Yazan LS, Ng WK, Al-Naqeeb G, Ismail M (2009). Cytotoxicity of thymoquinone (TQ) from *Nigella sativa* towards human cervical carcinoma cells (HeLa). *J. Pharm. Res.*, 2(4): 585-589.