Global Veterinaria 2 (5): 233-241, 2008
ISSN 1992-6197

The *In vitro* Promising Therapeutic Activity of Thymoquinone on Hepatocellular Carcinoma (HepG2) Cell Line

[1]Wafaa A. Ahmed, [2]Sohair A. Hassan, [3]Fayek M.Galeb, [4]Maha A. El -Taweel and [1]Farid A. Abu-Bedair

[1]Department of Cancer Biology, NCI, Cairo University, Cairo Egypt
[2]Department of Medicinal Chemistry, National Research Centre, Dokki, Giza, Egypt
[3]Department of Clinical Pathology Research Institute of Ophthalmology,Cairo University, Cairo Egypt
[4]Department of Clinical Pathology, NCI, Cairo University, Cairo Egypt

Abstract: Black seed *(Nigella sativa)* is considered as a biological response modifiers (B R M). Thymoquinone (TQ) is the bioactive and the most abundant constituent of the volatile oil of this seed which, has been shown to possess an anti-inflammatory, antioxidant and anti-neoplastic effects. In this study, the effect of TQ on HepG2 cell line was investigated in an attempt to identify its potential mechanism of action. Cell viability and proliferation were assessed against different concentrations of TQ and revealed a remarkable inhibition of HepG2 cells in a dose dependant manner. TQ ability-induced apoptosis was determined by Flowcytometry and by colorimetric measurement of Caspases 3 and 9. The apoptotic effect of TQ was much more dramatic after 12 hrs of treatment and the activity level of Caspases 3 and 9 was increased. Also, Flowcytometric analysis of cell cycle revealed an early G1/S arrest of cells which is characteristic of apoptosis. It could be concluded that Thymoquinone is a promising anti-cancer therapeutic agent for hepatocellular carcinoma.

Key words: Black seed (Thymoquinone) · Apoptosis · Cell cycle · Flowcytometry

INTRODUCTION

Cancer has become an important issue in medicine as it is a major cause of death in both the developed and developing countries and it is now well thought-out as a secondary to that of myocardial infarction [1]. A great majority of human cancers (about 80%-90%) are attributable to environmental factors [2]. However, it is not an easy task to eliminate carcinogenic causes from the environment. While modern surgery has significantly reduced the cancer mortality, the use of additional treatment such as radiotherapy and chemotherapy has resulted in no more than 5% reduction in the number of deaths [2]. Therefore, there is an ongoing search for better control and preventive methods in order to reduce cancer mortality and related side effects. Many investigations are now being carried out to discover naturally occurring compounds, which can suppress or prevent the process of carcinogensis [3,4]. Cancer treatments are a paradox, on one hand delivering powerful toxicity to a tumor, but on the other spreading toxicity to the rest of the body. The side effects on such treatments may causes death even before the cancer does. Biological response modifiers (B R Ms) are compounds that have a unique effect on physiological functions and can diminish the side effects of cancer treatments, while at the same time increasing their efficiency [5].

Herbal therapies are commonly used for the prevention and treatment of cancer despite the little understanding of their molecular and cellular basis of action. The black seed (*Nigella sativa*) herb grows in countries bordering the Mediterranean Sea and India and has been used in Middle East and Africa to promote health and fight diseases [6]. Nigella is considered as a BRM because studies show extracts from the seeds are toxic to cancer cells and in mice, prevents blood cell toxicity caused by anti-cancer drug Cisplatin [7]. Thymoquinone (TQ), the bioactive and the most abundant constituent of the volatile oil of black seed, has been shown to possess an anti-inflammatory and antioxidant effects [7- 9]. Also, TQ exerts an anti-neoplastic effect and is thought to be a promising dietary chemopreventive agent. Despite the promising antineoplastic activities of TQ, the molecular mechanism of its pharmacologic effects is poorly understood [10,11].

Corresponding Author: Dr. Sohair A. Hassan, Department of Medicinal Chemistry, National Research Centre, Postal code: 12622, Dokki, Giza, Egypt E-mail: sohaireslamaya@hotmail.com

Cell death can follow distinct pathways, apoptosis or necrosis. Necrosis appears to be the result of acute cellular dysfunction in response to severe stress condition or after exposure to toxic agents [12]. Apoptosis is a physiological form of cell death that occurs during development of multicellular organisms or during the development of the immune response. In addition, the apoptotic program can be activated in response to stress conditions, toxins chemicals, physical agents and others [13]. Several protease families are implicated in apoptosis, the most prominent being caspases, which are cesteine containing aspartic acid –specific proteases and all have similar site specific proteolytic activity [14,15].

The aim of this study was designed to investigate the possible beneficial effects of thymoquinone on hepatocellular carcinoma (HepG2) cell line in an attempt to identify its potential mechanism of action. This study was started on May (2006) by cooperation between National Cancer Institute and National Research Center, Cairo, Egypt.

MATERIALS AND METHODS

Reagents and Drugs: A purified preparation of TQ (>99% pure) was purchased from Fisher Scientific GmbH (Germany). Propidium iodide (PI) was purchased from Molecular Probes (Eugene, OR). RNase and Trypan blue were obtained from Sigma Chemical Co. Tumor cell line: HepG2 cell line was obtained frozen in liquid nitrogen (-180°C) from American Type Collection. The tumor cell line was maintained in National Cancer Institute, Cairo, Egypt, by serial sub-culturing in 75 cm^2 cell culture flasks (Fisher Scientific, Pittsburgh, PA) using 10 ml of RPMI-1640 [supplemented with 1% (2 mM) glutamic acid, 10% unheated fetal bovine serum (FBS), 100 µ/ml penicillin and 100µg/ml streptomycin] obtained from Gibco – BRL (Gaithersburg, MD). All cells were grown at 37ºC in atmosphere of 5% CO^2.

Cell Proliferation and Sulforhodamine B (SRB) cytotoxic test: The SRB colorimetric assay was Optimized by Papazisis *et al.* 1998[16]: Cells in exponential growth phase were washed, trypsinized and resuspended in RPMI medium. For experiments, cells seeded on 6- well plates at a density of 105 cells per well and left to grow. Cells were treated with defined concentration of TQ. Cell proliferation assay and cytotoxicity were determined using the cell Titer 96TM non–radioactive cell proliferation assay. Cells were plated on microtitre plate wells and incubated for 24hrs in a humidified atmosphere, during this period a partial monolayer was formed. The cells were then exposed to different concentrations of TQ dissolved in methanol (400, 200, 100, 50 and 25 µM/ml) prepared by serial two-fold dilution of the test drugs in triplicate using a constant volume of 20 µL and maintaining the total well volume of 200 µL. The methanol concentration in treated and control wells did not exceed 0.1% per well. The cells were incubated at the same pervious concentrations for a period of 48 hrs. Changes of the cell cultures were examined using an inverted microscope(Olympus IX2 model) and cellular viability was determined using light microscope employing Trypan blue dye exclusion technique at 12 and 24 hrs time intervals. Add 50 µL of cold (50% TCA) to the top of 200 µL culture medium in each well to produce a final TCA concentration of 10%. Microplate were left for 30 min at 4ºC and subsequently washed 5 times with deionized water. Microplate was then left to dry at room temperature for at least 24 hrs. Add 100 µL 0.4% (W/V) of Sulforhodamine B (SRB- Sigma) in 1% Acetic Acid to each well and left at room temperature for 20 min. SRB was removed and the plate washed 5 times with 1% Acetic Acid before air drying. Bound SRB was solubilized with 200 µL of 10mM unbuffered Tris-base solution (Sigma) and plate was left on a plate shaker for at least 10 min. Absorbance was read in a 96-well plate reader at 492 nm subtracting the background measurement at 620 nm.

Apoptosis assay: Apoptosis was detected by MoFlow Flowcytometer, Dako Cytomation using Phosphatediyl Serine DetectionTM Kit. Cells growing on 6-cm culture dishes were collected by trypsinization after treatment with TQ for 6 and 12hrs, washed with PBS. Dilute calcium buffer in deionized water (20X) and stored at 4°C. Wash the cells and readjust the cell concentration to 1.5x10-6 cells/ml in Calcium buffer. Add 10 µL Annexin V FITC to 100 µL cell suspension then incubate for 20min in ice in the dark. Wash the cells with Calcium buffer and Add 10 µL Propidium Iodide (PI). Keep the cells at 4°C until ready to be analysed by Flowcytometry. Viable cells are not stained; Apoptotic cells exclude PI and express Phosphatidyl stain by green colour while necrotic cells are permeable to PI, which associates with nuclear DNA and are visible as red fluorescence [17].

The caspase 3 and 9 colorimetric protease assay: (ApoTarget TM) Kit is used for *in vitro* determination of proteolytic activity of the enzymes in lysates of mammalian cells (BioSource International, Inc. 542 Flynn Road Camarillo, California 93012, USA).

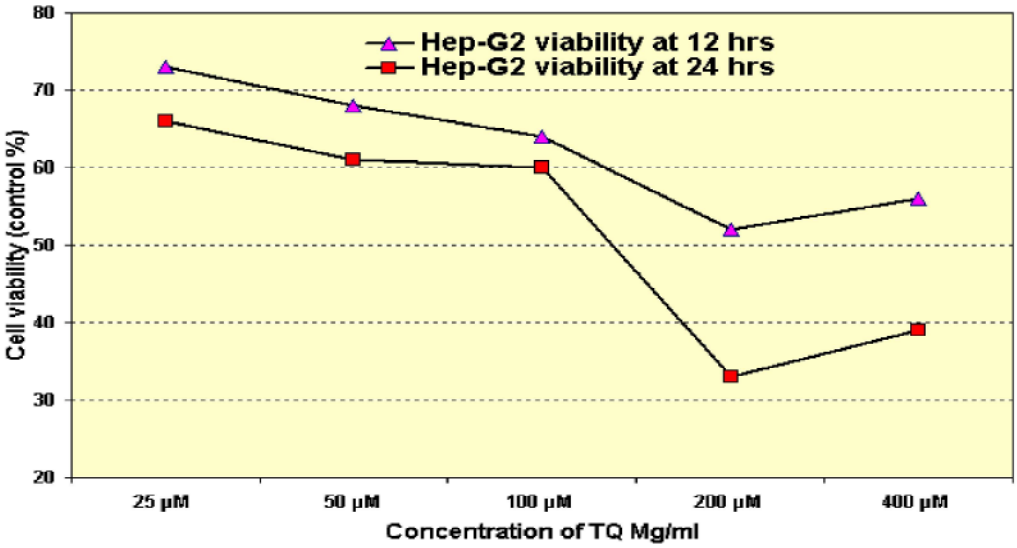


Fig. 1: Dose response curve on the effect of TQ on HepG2 viability at 12 hrs and 24hrs of exposure determined by Trypan Blue exclusion test

Induce apoptosis in cells by TQ in different concentration. Cells were counted and pellet of 3–5 Million cells per sample is adjusted. Resuspend cells in 50 µL of chilled Cell Lysis Buffer then cells are incubated on ice for 10 minutes. Centrifuge and assay the protein concentration by Bradford method (Fig. 1) in cytosol extract. Each cytosol was diluted to a concentration of 50 to 200 µg protein per 50 µL. Cell Lysis Buffer, Reaction Buffer (containing DDT) was added to each sample. Add 5 µL of the 4 mM DEVD-pNA substrate (200 uM final concentrations) and incubated at 37ºC for 2 hours in the dark. Then, the absorbance was recorded at 405 nm using spectrophotometer [18].

Cell cycle analysis by Flowcytometery: Cells were seeded in 6-well micoplate at a density of 5 x 10^5 cell per well. They were incubated and allowed to grow to 40-50 confluence after which they were treated with TQ and incubated for further 6-12 hrs. Cells were then harvested by trypsin release, washed twice with PBS (pH 7.4), permeabilized with 70% ethanol. Cells were washed twice with ice-cold PBS, treated with 1% RNase and incubated for 10 min at room temperature followed by addition of Propidium Iodide (100 µg/ml final concentration). Distribution of cells in G1, S and G2 phases with different DNA contents was determined using a MoFlow Flowcytometer, Dako Cytomation [19].

RESULTS

Growth and proliferation inhibition: Hepatpcellular carcinoma (HepG2) cells were treated with graded concentration (25-400 uM) of TQ for 12-24 hrs. The viability and cell proliferation test were monitored. The data indicated that treatment of cells with concentration < 200 µM resulted in significant inhibition of viability of the cells at 12 – 24 hrs as compared with control (Fig. 1). TQ was found to remarkably inhibit the proliferation of HepG2 cells in a dose depended manner with IC50 (50% of inhibitory concentration of 350 µM), (Fig. 2).

TQ induce apoptosis: Treatment of HepG2 cells with TQ resulted in no necrotic cells after 6 and 12 hrs. However the apoptotic effect by TQ was much more dramatic in that more than 57% of cells became apoptotic after 12 hrs of treatment compared with control (Fig. 3, 4).

The apoptotic effect of TQ was also examined by measuring the activity of caspase 3 and 9 enzymes. The enzyme activities were determined in relation to the different concentrations of protein content (50–250 µg/ sample) of cells treated with graded dose of TQ (100, 200, 400 µM). The result obtained showed increase of the activity of both enzymes with increase the dose of TQ (Fig. 5-6).

Cell cycle analysis: To dissect the mechanism for the anti-proliferative effects of TQ, it was important to

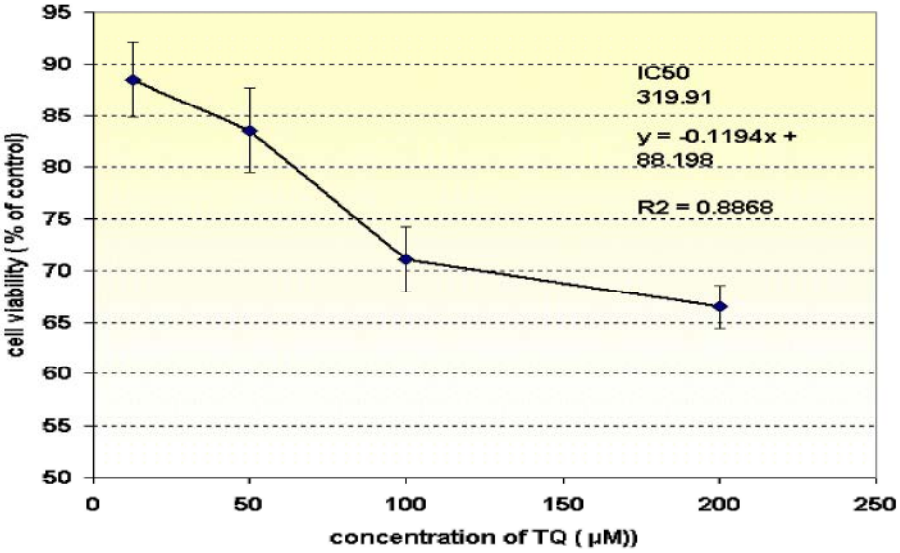
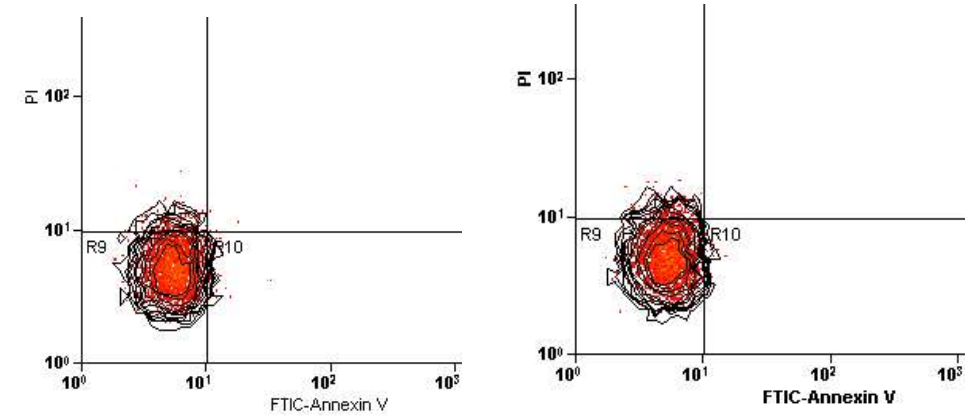
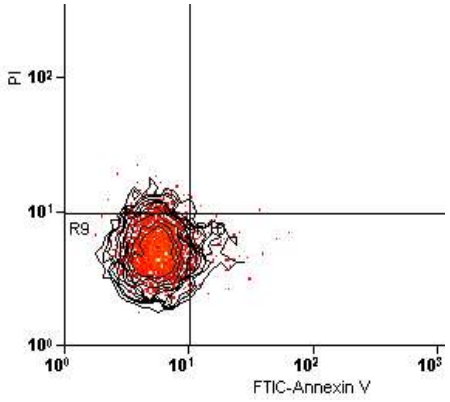


Fig. 2: SRB cytotoxicity assay for determination of mortality of HepG2 cell line under different concentration of TQ

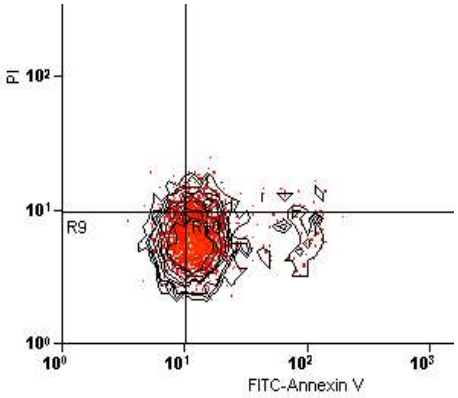


Control 6hrs

Control 12hrs



TQ 6hrs



TQ 12hrs

Fig. 3: Flowcytometric detection of Apoptosis in HepG2 cell line under the effect of TQ (IC50) concentration

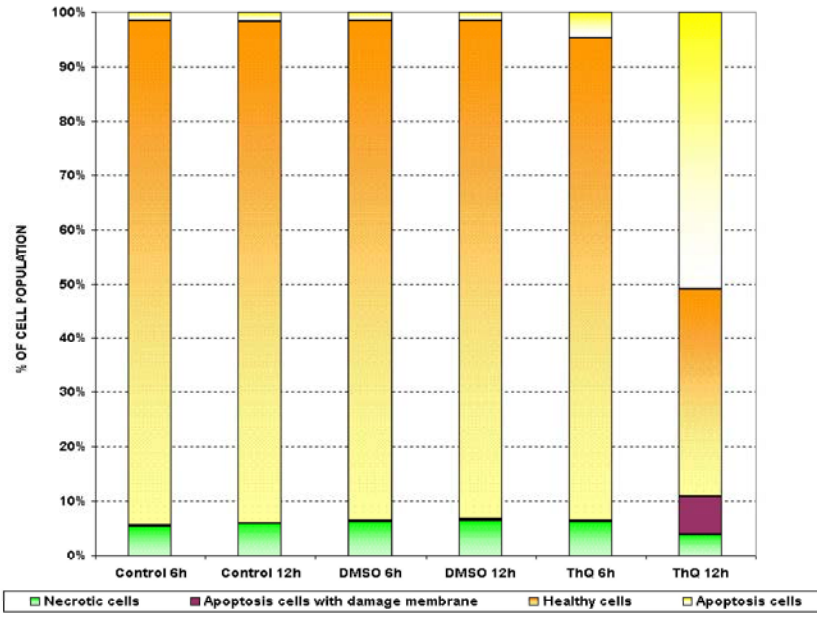


Fig. 4: Represented Apoptosis/Necrosis analysis of HepG2 cell line under the effect of TQ (IC50) concentration

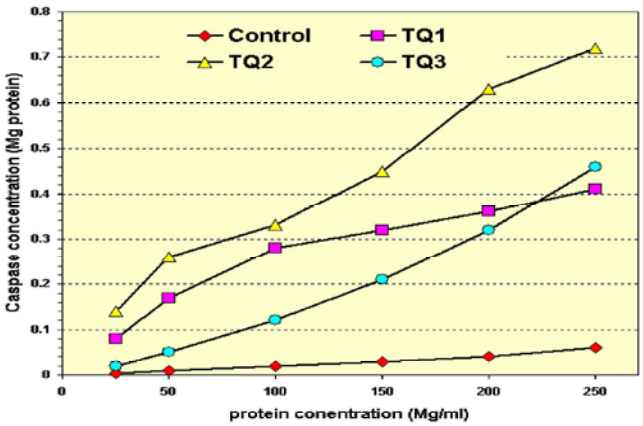


Fig. 5: Caspase 3 concentration (mg protein) in Hep-G2 cell line different concentration of TQ

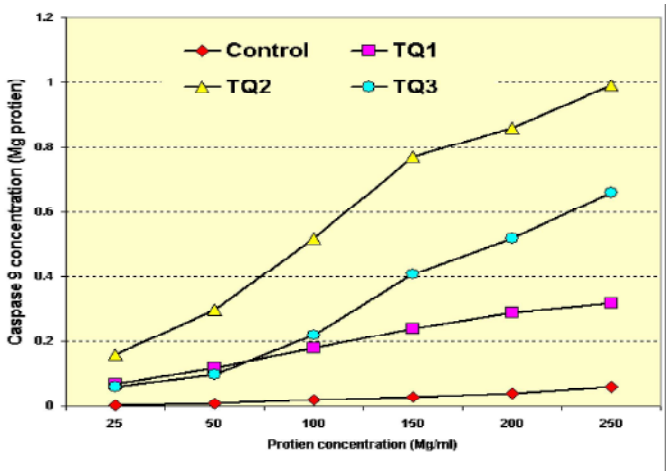


Fig. 6: Caspase 9 concentration (mg protein) in HepG2 cell line under different concentration of TQ

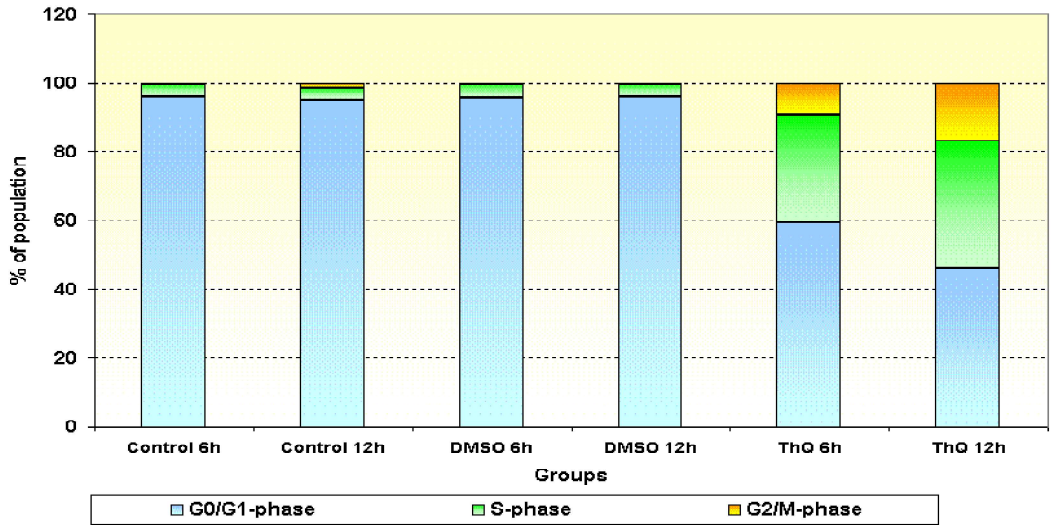


Fig. 7: Cell cycle analysis of HepG2 cell line under the effect of TQ (IC50) concentration

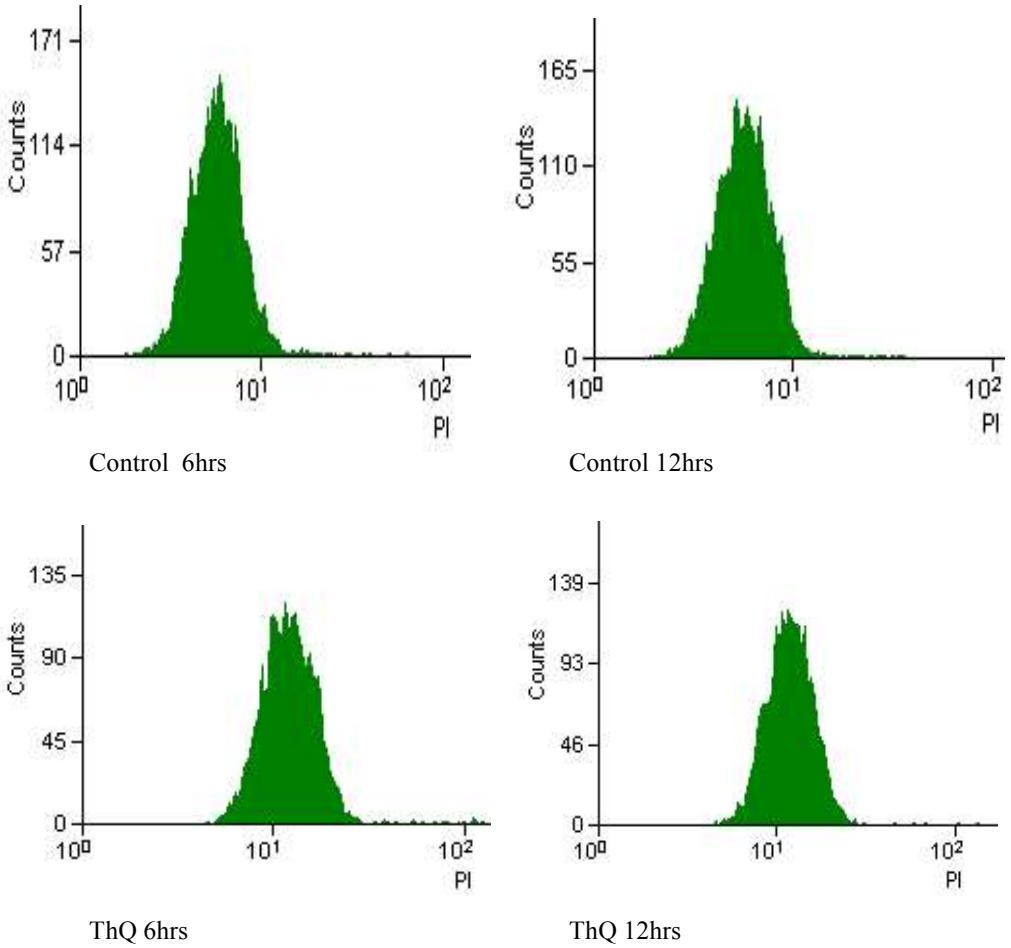


Fig. 8: Cell cycle analysis of Hep-G2 cell line under the effect of TQ (IC50) concentration by flowcytometry" inside defined squre

determine whether the growth inhibitory effect of TQ is associated with specific changes in cell cycle progression or not. HepG2 cells were treated with TQ for 6 or 12 hrs and harvested for Flowcytometeric analysis of cell cycle and DNA contents by PI staining. Treatment with 350 µM of TQ caused a concentration-dependent decrease in the number of HepG2 cells in the S-phase and an i. Providing evidence of G1 arrest (Fig. 7, 8).

DISCUSSION

Hepatocellular carcinoma (HCC) represents the fifth most common malignancy as regard the main cause of mortality in patients with chronic liver diseases. HCC is a tumor characterized by high local invasiveness and high metastatic efficiency. There is no doubt that certain herbal products contain chemically defined components that can protect the liver from oxidative injury, promote virus elimination, block fibrogenesis, or inhibit tumor growth [20]. In the present study, we investigated the effect of thymoquinone on Hep-G2 cell line and attempted to identify its mechanism of action.

Although the thymoquinone (TQ) has confirmed significant effect *in vitro* and *in vivo* antineoplastic activities against different cell lines, the mechanism and molecular pathways of TQ action have not been fully examined [21,22]. However, assumptions indicated that the anti-tumor effect of TQ may be mediated by one or more of the following mechanisms; Antioxidant activity, Immunomodulatary action or cytotoxicity [23].

The results of this study revealed that TQ inhibited the viability and proliferation of tumor cells by a mechanism that involves cytotoxicity. *In vivo* study previously revealed that TQ protects rat liver against induced hepatocarcenogenesis and showed that the protective effect of TQ is due to its antioxidant activity. Also, it has been revealed that TQ has the ability to kill several types of tumors without significant cytotoxicity to normal cells indicating that this compound may be a potentially effective chemotherapeutic agent [24].

Apoptosis is an active, energy–dependent mechanism in which cells participate in their own destruction whereas necrosis is a passive process of cellular metabolic collapse followed by cellular disintegration [25]. The predominant form of cell death is likely apoptosis since evidence of apoptotic cell death was seen initially. However, at longer times of incubation and at a higher concentration, necrotic cell death was observed. So, TQ causes both forms of death, Apoptosis and Necrosis and the incidence of these forms of death is a dose- and time- dependant. Our results are consistent with the findings of other studies which prove that TQ produces both apoptotic/necrotic effects in different cancer cell lines [19,26,27].

Caspases are proenzymes that contain an active site of cystein nucleophile [28] which is prone to oxidation or thiol alkylation [20, 21]. It is therefore not surprising that activity of caspases is optimal under reducing environments. Any dying cell could be detrimental to caspase and render them inactive [29-31]. Inhibition of caspase activity in cells that would otherwise undergo apoptosis has recently been shown to drive cells into necrosis [32,33]. The results of the present study have been shown that TQ is an initiator of apoptosis through increase activation of caspase protease enzymes (Fig. 3 & 4). The results of the present study suggest the ability of TQ to increase the activity of both Caspases 3 and 9 in a dose dependant manner.

Cell cycle checkpoints and apoptosis play a key role in developmental biology and represent a new set of potential targeting for chemotherapeutic agents. Quinones represent a class of drugs that induce both effects [32,34]. Quinones induce free radical–mediated DNA strand breaks and also alkylate and cross- link DNA [35, 36]. In this study, Flowcytometeric analysis points to a possibility of an early G1/S arrest at 350 µM concentration of TQ (Fig. 6). A reduction in the number of S-phase cells and an increase in the G1 peak are feature characteristic of the early stage of G1/S phase arrest. Other mechanisms of toxicity include interference with cell cycle with upregulation of p21 protein by mutant p53, an inhibitor of cyclin–dependent kinases and an important negative regulator involved in cell cycle control [37, 38].

Although, the result of our study revealed an increase in cytotoxic effect of TQ *in vitro*, previous *in vivo* studies reported that TQ is non–toxic and protect against quinine (Doxorubicin) induce cardiotoxicity and nepherotoxicity without compromising its anti-tumor activity [39,40].

In conclusion, TQ has shown a cytotoxic effect on HepG2 cells, triggers caspase activation and apoptotic cell death and produce cell cycle arrest. These results suggest that TQ can be a promising anti-cancer therapeutic agent for hepatocellular carcinoma and prevents non-tumor tissues from sustaining chemotherapy-induced damage.

REFERENCES

1. Gruddy, S.M., 1991. Recent Nutrition Research, implications for foods of the future. Annuals of Medicine, 23: 187-193.
2. Benjamin, H.S.L., P.T. Padma and M.T. Jeffrey, 1990. *Allium sativum* (garlic) and cancer prevention. Intl. J. Vitamin Nutr. Res., 10: 937-948.
3. Wargovich, M.J., C. Woods and V.W.S. Eng, 1988. Chemoprevention of N-Nitrosomethyl benzyllamine-induced oesophageal cancer in rats by the naturally cancer thiother, sulphide. Cancer Research, 48: 6872-6875.
4. Thapliyal, R., S.S. Deshpande and G.B. Maru, 2002. Mechanism(s) of turmeric-mediated protective effects against benzopyrene-derived adducts. Cancer Letters, 175: 79-88.
5. Samantha, S., W. Nalinie, T. Ira, R. Neelakanthi and G. Mayuri, 2003. Protection against diethylnitrosoamine-induced hepatocarcinogenesis by an indigenous medicine comprised of *Nigella sativa*, Hemidesmus indicus and smilax glabra: a preliminary study. Journal of Carcinogenesis, 2: 6-18.
6. Gali-Muhtasib, H., M. Diab-Assaf, C. Boltze, J. AL-Hmaira and R. Hartig, 2004. Thymoquinone extracted from black seed triggers apoptotic cell in human colorected cancer cells via a p53-depenelent mechanism. Intl. J. Oncol., 25: 857-66.
7. Houghton, P.J., R. Zarka, B. Heras and R.S. Hoult, 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation. Planta Medicine, 61: 23-29.
8. Keuk, I., T. Michalska, K. Lichszteld, A. Kaldna and H.Y. Aboul-Enein, 2000. The effect of Thymol and its derivatives on reaction generating reactive oxygen species. Chemosphere, 41: 1059-1064.
9. Gali-Muhtasib, H., A Roessner and R. Schneider-Stock, 2006. Thymoquinone: a promising anti-cancer drug from natural sources. Intl. J. Biochem. Cell Biol., 38: 1249-53.
10. Shoieb, A.M., M. Elgayyar, P.S. Dudrick, J.L. Bell and P.K. Tithof, 2004. *In vitro* inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. Intl. J. Oncol., 22: 107-113.
11. El-Mahdy, M.A., Q. Zhu, Q.E. Wang, G. Wani and A.A. Wani, 2005. Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. Intl. J. Cancer, 117: 409-417.
12. Afshin, S., N. Helena, Z. Boris, P. Elizabeth and O. Sten,1999. A comparative study of apoptosis and necrosis in HepG2 cells: Oxidant–Induced Caspase Inactivation Leads to Necrosis. Biochem. Biophysic Res. J., 255: 6-11.
13. Zhivotovisky, B., D.H. Burgess, D.M. Vanags and S. Orrenius, 1997. Induction of apoptosis by chemical and physical agents. Biochem. Biophysic Res. J., 230: 481-488.
14. Alnemri, E.S., D.J. Livingston, D.W. Nicholson, G. Salvesen, N.A. Thornberry, W.W. Wong and J. Yuan, 1996. Caspases are the most proteases family implicated in apoptosis. Cell, 87: 171-185.
15. Monte, D., G. Bellomo, H. Thor, P. Nicotera and S. Orrenius, 1984. The cytotoxic effects of mendolione through oxidative stress. Biochemistry and Biophysics, J., 235: 343-350.
16. Papazisis, K.T., G.D. Geromichalos, K.A. Dimitriadis and A.H. Kortsaris, 1998 Optimization of the sulforhodamine B colorimetric assay. J. Theagenion Cancer Institute, 86: 189-197.
17. Salomi, N.J., S.C. Nair, K.K. Jayawardhanan, C.D. Varghese and K.R.Panikkar,1992. Anti-tumor principles from *Nigella sativia* seeds.Cancer Letter, 63: 41-46.
18. Donald, L.E., B.G. Reid and J.F. Liliana, 2000. Methods of cell cycle analysis and detection of apoptosis of teleost cells. J. Metho. Cell Science, 22: 2-3.
19. Casciola-Rosen, L.A., 1996 Apopain / CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. J. Exper. Med., 138: 1957-1964.
20. Detlef, S., J.I.A. Dong, B. Brinkhaus and E.G. Hahnl, 1999. herbal Products for liver diseases: A therapeutic Challenge for the new Millennium. Hepatol., pp: 1-6.
21. Samantha, S., W. Nalinie, T. Ira, R. Neelakanthi and G. Mayuri, 2003. Protection against diethylnitrosamine induced hepatocarcinogenesis by an indigenous medicine comprised of *Nigella sativa*. J. Carcenogenesis, 2: 6-21.
22. Ahmed, M., E. Mona, D. Pauls, B. Johnl and K. Patricia, 2002. *In vitro* inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. Intl. J. Oncol., 22: 107-113.
23. Evan, G. and T. Littlewood, 1998. A matter of life and cell death. Sci., 281: 1317-1322.

24. Rooney, S. and M.F. Ryan, 2005. Modes of action of alpha-hederin and thymoquinone, active constituents of *Nigella sativa*, against HEp-2 cancer cells. Anticancer Res., 25: 4255-4259.
25. Rooney, S. and M.F. Ryan, 2005. Effects of alpha-hederin and thymoquinone, constituents of Nigella sativa, on human cancer cell lines. Anticancer Res., 25: 2199-2204.
26. Alnemri, E.S., D.G. Livingstone, D.W. Nicholson and G. Salvesen, 1996. Caspases are the most proteases family implicated in apoptosis. Cell Biol. J., 87: 171-185.
27. Nobil, C.S., D.H. Burgess, B. Zhivotovsky, M.J. Burkitt, S. Orrenius and A.F. Stater, 1997. Mechanism of menadion induced necrosis. Chemical Res. Toxicol. J., 10: 636-643.
28. Nicholson, D.W., A. Ali, N.A. Thornberry and I.P. Vaillancourt, 1995. The activity of caspases is optimal under reducing environments. Nature J., 376: 37-43.
29. Hampton, M.B. and S. Orrenius, 1997. Hydrogen peroxide suppresses both the activation and activity of caspases. Federation of Euoropean Biochemical Societies Letter J. Letter J., 414: 552-556.
30. Nobil, C.S., M. Kamland, D.W. Nicholson, S. Orrenius and A.F. Slater, 1997. The oxidation of dithiocarbamates to dithiocarmamate disulphates has been shown to inhibit apoptosis through binding to active site of the caspases and inhibition of their enzymatic activity. Chemical Research and Toxicology, 10: 1319-1324.
31. Lemaire, C., K. Andreau, V. Souvannavong and A. Adam, 1998. Caspases contain an active site cysteine nucleophile which is prone to oxidation or thiol alkylation Federation of Euoropean Biochemical Societies Letter J., 425: 266-270.
32. Hartwell, L.H. and M.B. Kastan, 1994. Cell cycle control and cancer. Science, 266: 1821-1828.
33. Karp, J.E. and S. Broder, 1995. Molecular foundation of cancer, new targets for intervention. Intl. Med. J., 1: 309-320.
34. Dusre, L., J.M. Covey, C. Collins and B.K. Sinha, 1989. DNA damage, cytotoxicity and free radical formation by mitomycin C. in human cells, Chem. Biol. Intl. J., 71: 63-78.
35. Begleiter, A. and M.K. Leith, 1990. Activity of quinone alkylating agents in quinine-resistant cells. Cancer Res., 50: 2872-2876.
36. Morgan, W.A., J.A. Hartley and G.M. Cohen, 1997. Quinone-induced DNA single strand breaks in rat hepatocytes and human chronic myelogenous leukemia K562 cells. Biochem. Pharmacol. Res. J., 44: 215-221.
37. Wu, R.C., A. Hohenstein, J.M. Park, S. Muller, E. Cadenas and A.H. Schonthal, 1998. Role of p53 in aziridinylbenzoquinone-induced p21waf1 expression. Oncogene, 17: 357-365.
38. Qiu, X., H.J. Forman and E. Cadenas, 1996. Induction of p21 mediated by reactive Oxygen species formed during the metabolism of aziridinylbenzoquinones by HCT116 cells. J. Biol. Chem., 271: 31915-31921.
39. Roepke, M., A. Diestel, K. Bajbouj, D. Walluscheck, P. Schonfeld, A. Roessner, R. Schneider-Stock and H. Gali-Muhtasib, 2007. Lack of p53 Augments Thymoquinone-Induced Apoptosis and Caspase Activation in Human Osteosarcoma Cells. Cancer Biol. Therapy, 6: 112-128.
40. Badary, O.A., A.B. Abdel-Naim, M.H. Abdel-Wahab and F.M. Hamada, 2000. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicol., 143: 219-226.

(Received: 28/04/2008; Accepted: 25/05/2008)