Quantitation of the antitumor Activity of Thymoquinone (TQ) against Thymoquinone (TQ)

Afrah Fatthi Salama1, Saad Mohamed El-Gendy2, Ahmad Ahmad ABarbary3, Shabaan El-Sayed Abdel-Azez1**

1Division of Biochemistry, Chemistry Department, Faculty of Science, Tanta University, Egypt 2Department of Cancer Biology, National Cancer Institute, Cairo university, Egypt, and 3Chemistry Department, Faculty of Science, Tanta University, Egypt

*Corresponding to: Shabaan El-Sayed Abdel-Azez1**

(Biomil2010@yahoo.com)

ABSTRACT

Background: There is a growing interest in investigating the anti-tumor effects of thymoquinThe aim of the present study was first to examine whether Thymoquinone Has anti-neoplastic, cytotoxic or apoptotic effect on hepatoma cells (Hep-G2) and lung cancer (H460) cells compared with cisplatin. Secondly, to clarify any relationship between antineoplatic effect of thymoquinone with cytotoxicity and apoptosis via estimation of the proliferation rat, using the SRP, MTT and flow cytometry assays. one (TQ), the bioactive constituent of the volatile oil of black seed. It is not known, however, whether their anti-tumor effects are due to apoptotic or necrotic effects. Aim: The goal of this study was to investigate the anti-neoplastic, cytotoxic or apoptotic effects using the hepatoma (Hep-G2 cells) and lung cancer (H460) cell lines. Methods: The anti-tumor effects of TQ was measured using sulphorodamine B (SRB) cytotoxicity, MTT, and flow cytometry assays. Results: Treatment of Hep-G2 and H460 cells with TQ (50, 100, 200, 300 and 400ug/ml) resulted in significant decreases in the proliferation and survival rates of Hep-G2 and H460 cells after 48 hours and completely inhibited the cell growth after 72 h of treatment as compared to the effect of cisplatin. Interestingly, the anti-tumor effects of these agents were found to be mediated though induction of potent apoptotic effects as compared to cisplatin, which induced both cell apoptosis and necrosis. Conclusion: Supplementation of TQ and garlic could be used at least in part as adjuvant therapy during anticancer treatments.

Keywords: Thymoquinone; Apoptosis; Cell cycle; Anti-cancer; Hep-G2 cells; H460 cells

Introduction

Cancer is one of the major human diseases and causes considerable suffering and economic loss Worldwide. Although considerable progress has been made in treating cancer, the incidence and Mortality rate for most forms of cancers still remain very high. Therefore, further research is needed for the development of safe products for the prevention and treatment of all human cancers (Jayaprakasam *et al*., 2003).

There has been growing interest in naturally occurring phytochemical compounds with anti-cancer potential, because they are relatively nontoxic, inexpensive and available in an ingestive form. More than 25% of drugs used during the last decades are directly derived from plants such as The black seed, Turmeric (*Curcumin longa*), Green tea and garlic , while the other 25% are chemically altered natural products *(Vuorelaa et al., 2004).*

Aim

The aim of the present study was first to examine whether Thymoquinone Has anti-neoplastic, cytotoxic or apoptotic effect on hepatoma cells (Hep-G2) and lung cancer (H460) cells compared with cisplatin. Secondly, to clarify any relationship between antineoplatic effect of thymoquinone with cytotoxicity and apoptosis via estimation of the proliferation rat, using the SRP, MTT and flow cytometry assays.

Materials and Methods

Materials

Reagents and drugs

Thymoquinone was purchased from Sigma Company. Cisplatin was purchased from Bristol-Myers Squibb Company, 96 well plates was purchased from Greiner Bio-One Bioscience, Germany. Trypan blue were obtained from Sigma Chemical Co. The tumor cell line were maintained in National cancer institute in liquid nitrogen (-180Oc) using RPMI-1640 (supplemented with 1 % glutamic acid, 10 % fetal bovine serum, 100 u/ml penicillin and and 100 ug/ml streptomycin obtained from Gibco-BRL (Gaithersburgm, MD, USA)

Cell culture:

Human hepatoma (Hep-G2) and lung cancer (H460) cell lines were cultured according to standard protocol of cell culture (Lee, 1991 and Freshney, 1993).

Methods

The anti-tumor effects of TQ was measured using sulphorodamine B (SRB) cytotoxicity, MTT, and flow cytometry assays.

Results

antiproliferative effects

(Table 1). Cell density of hepatoma cells treated with different concentrations of Thymoquinone (μg/ml), compared with that of Cisplatin (μg/ml)

Parameter	Cell density (cell x104/ml)			
	Day-1	Day-2	Day-3	Day-4
Control				
Mean SD	50 2	200 5	300 6.2	500 8.2
Thymoquinone (μg/ml)				
25	49.3 2†	196.7 2.1†	294.3 3.2†	489.3 2.3†
50	48.3 1†	191.3 1.53*	286 2.1*	305 4.2**
100	46.3 1*	184 4.9**	242 5.7**	223 1.3**
200	35 2**	120 5**	90 5**	41 3.1**
300	28.3 2**	100 1.9**	50 5**	19 2.5**
400	10.3 1.0**	30 5.0**	20 2.0**	2.3 0.5**
Cisplatin(μg/ml)				
0.5	45 2.0†	120 5.04*	69.3 4.0**	47 2.5**
2	16.7 2.0**	46.0 1.2**	18.7 3.0**	6.0 3.1**
4	13.0 2.7**	16.0 3.0**	3.33 2.8**	1.0 0.55**
16	9.70 1.5**	9.33 3.1**	1.66 1.1**	0.93 0.4**
24	6.0 1.0**	5.33 1.7**	1.33 0.7**	0.33 0.1**

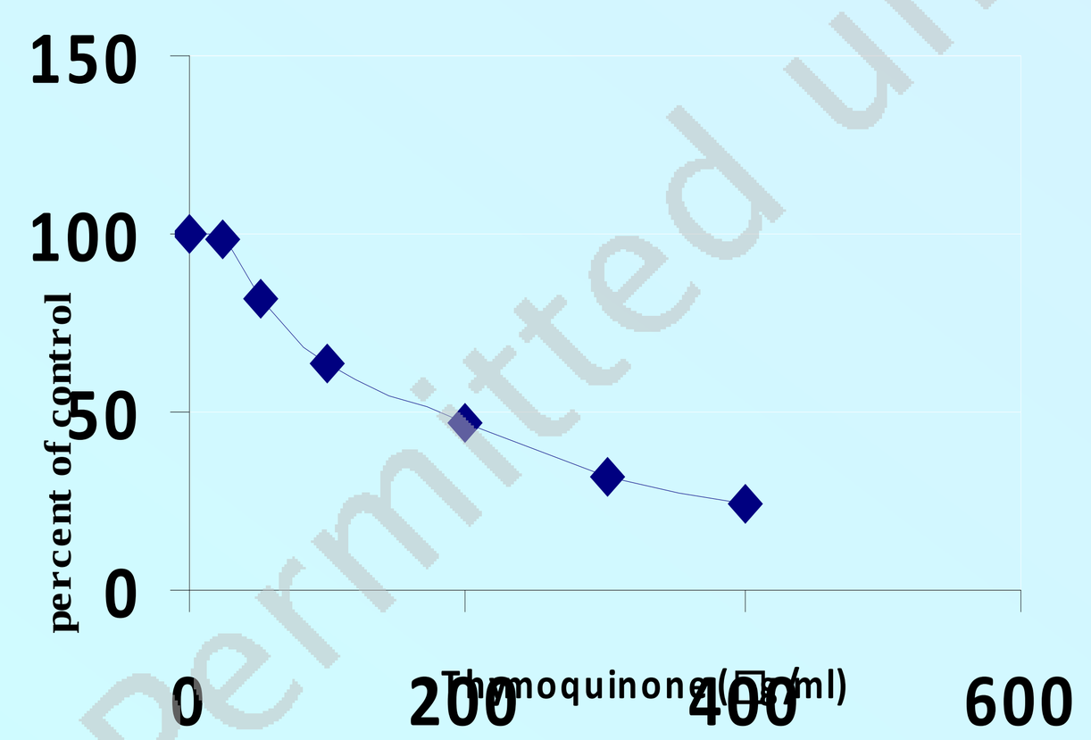
Treatment of hepatoma cells with higher concentrations of cisplatin (2, 4, 16 and 24 μg/ml) were significantly decreased the cell proliferation after 48 hours and completely inhibit the cell growth after 72 and 96 hours specially with concentrations 16 and 24 μg/ml.

(Table 2). Cell density of H460 cells treated with different concentrations of Thymoquinone (μg/ml), compared with that of Cisplatin (μg/ml)

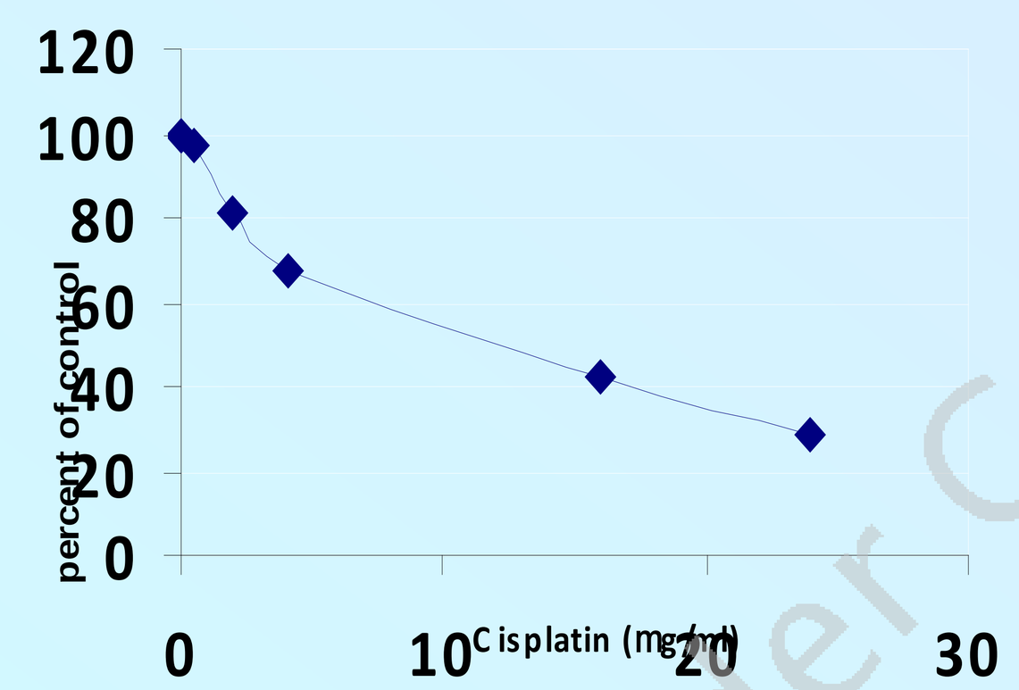
Parameter	Cell density [cell x104/ml]			
	Day-1	Day-2	Day-3	Day-4
Control				
Mean SD	10 0.89	50 2.1	80 3.04	200 3.9
Thymoquinone (μg/ml)				
25	9.7 0.6†	48 1.5†	76.7 1.5†	191 3.6†
50	9 0.57†	44 1.5*	68 0.71**	126 4.2**
100	7.7 1*	37.7 1.2**	57.3 2.2**	102 2.1**
200	6.3 1.1**	27.7 1.5**	39 1**	59 2.1**
300	4.3 0.9**	15.3 1.6**	19 1.4**	9 0.9**
400	2 0.57**	6.7 1.5**	4 1.0**	1 0.8**
Cisplatin (μg/ml)				
0.5	9.7 1.4†	45.3 2.5†	35 2.6**	27.7 2.1**
2	9.3 1.5†	30.7 1.5*	16.3 1.5**	7 0.58**
4	8.7 0.6†	16.3 1.5*	3.3 0.57**	0.7 0.25**
16	7.0 1.5*	10.3 1.47**	1.7 0.18**	0.33 1.1**
24	3**	4**	0**	0**

Higher concentrations of thymoquinone 100, 200, 300 and 400 mg/ml showed highly significant decrease (P<0.001) in the proliferation rates of H460 cells Also, cisplatin with concentrations 16 and 24 μg/ml significantly decreased the proliferation rate of H460 cells after 48 hours and completely killed the cells after 72 h of treatment.

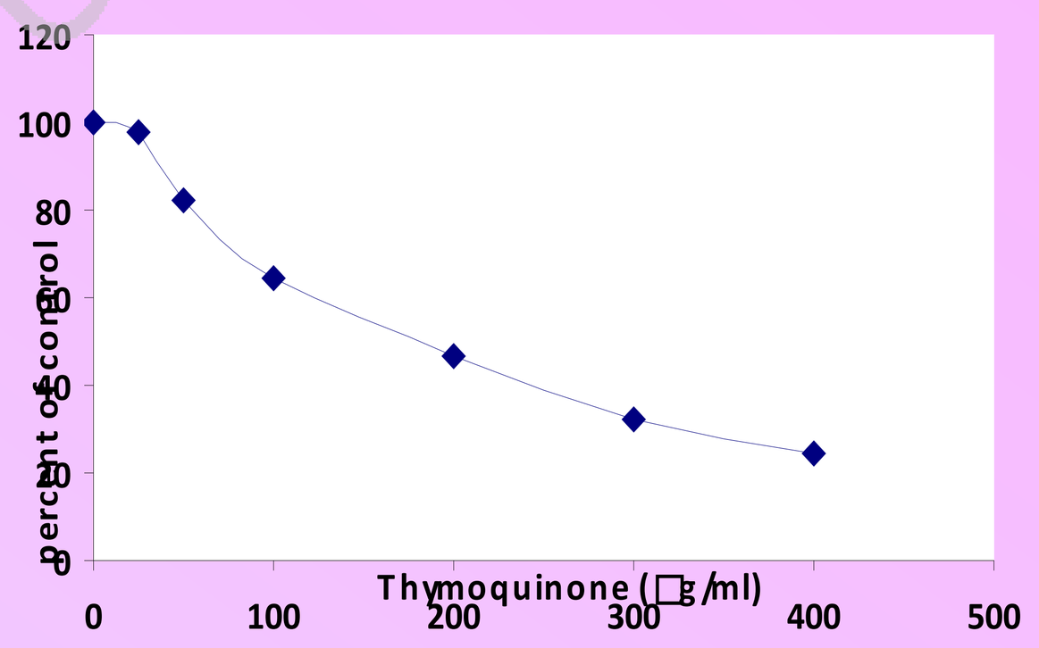
Cell cytotoxicity studies (SRP assay)



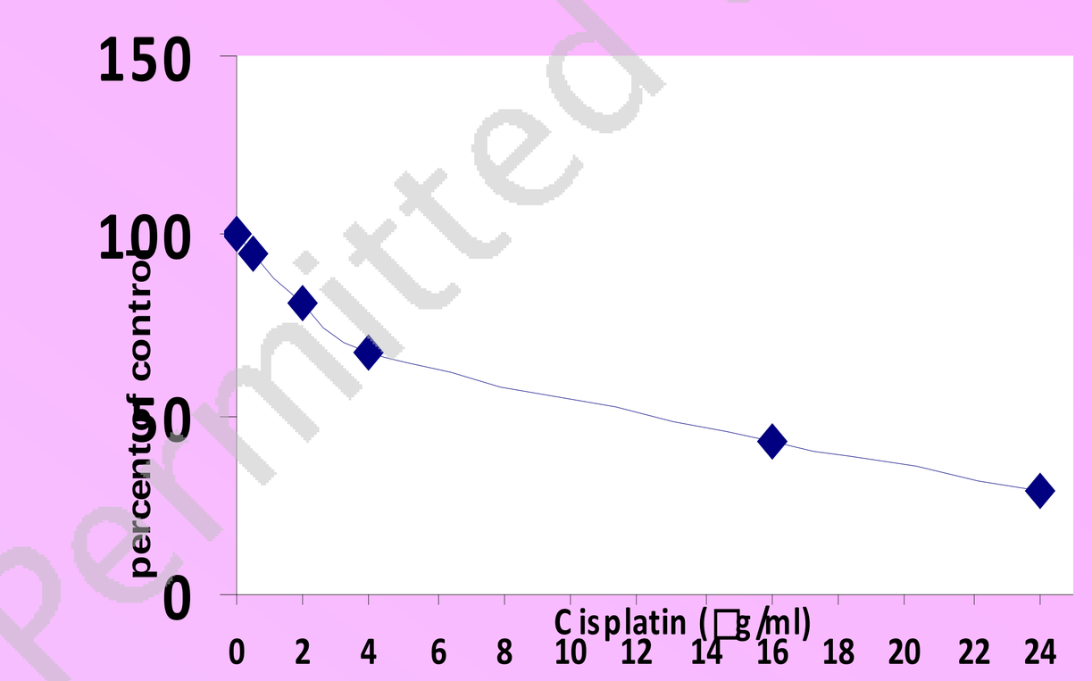
Figures 5



Figures 6



Figures 7



Figures8

Concentrations of thymoquinone (50, 100, 200, 300 and 400 mg/ml) and cisplatin (2, 4, 16 and 24 mg/ml) showed highly significant inhibition (P<0.001) of the survival of hep-G2 cells by (17.7%, 35.8%, 52.8%, 67.6% and 76.1% respectively for thymoquinone and (18.5%, 32.8%, 57.5% and 71% for cisplatin in comparison to untreated cells (Figures 1-2).

Concentrations of thymoquinone (50, 100, 200, 300 and 400 mg/ml) and cisplatin (2, 4, 16 and 24 mg/ml) showed highly significant inhibition (P<0.001) of the survival of H460 cells by (22%, 46.3% 68.4%, 81.7% and 92% respectively for thymoquinone) and (28.3%, 47.7%, 69% and 85%respectively for cisplatin) in comparison to the untreated cells (Figures 3-4).

Flow cytometric analysis

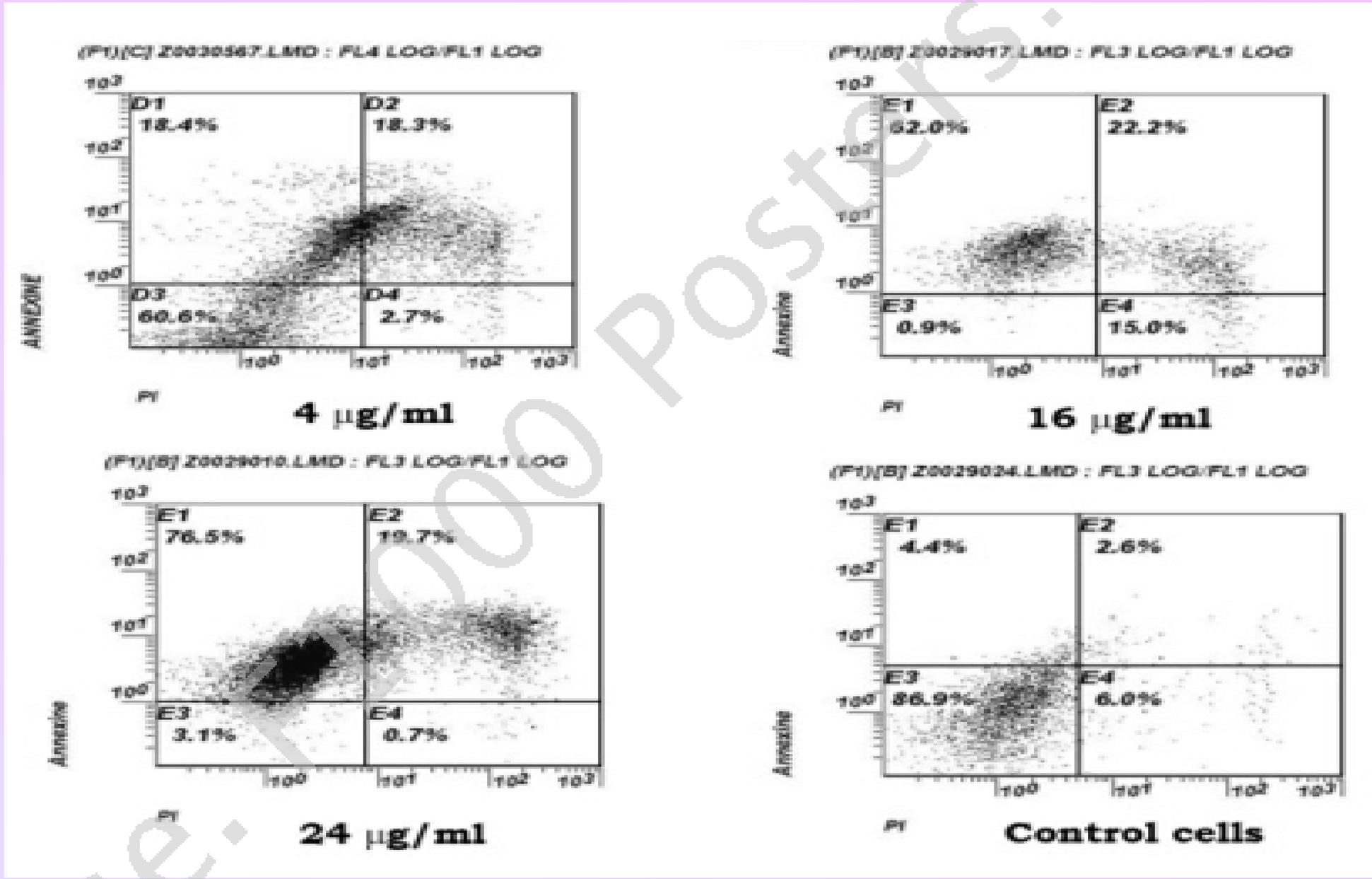


Figure 9

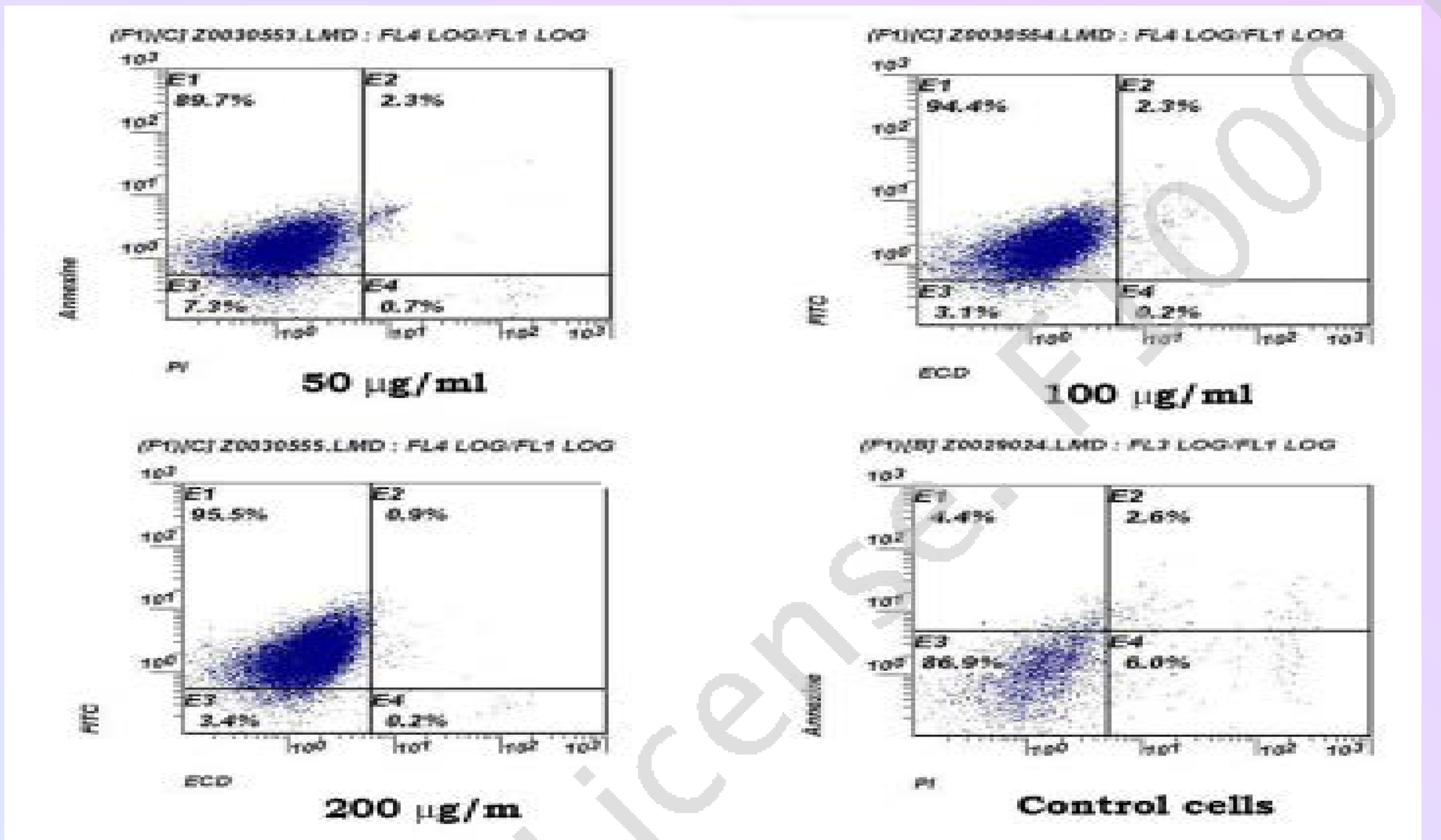


Figure 10

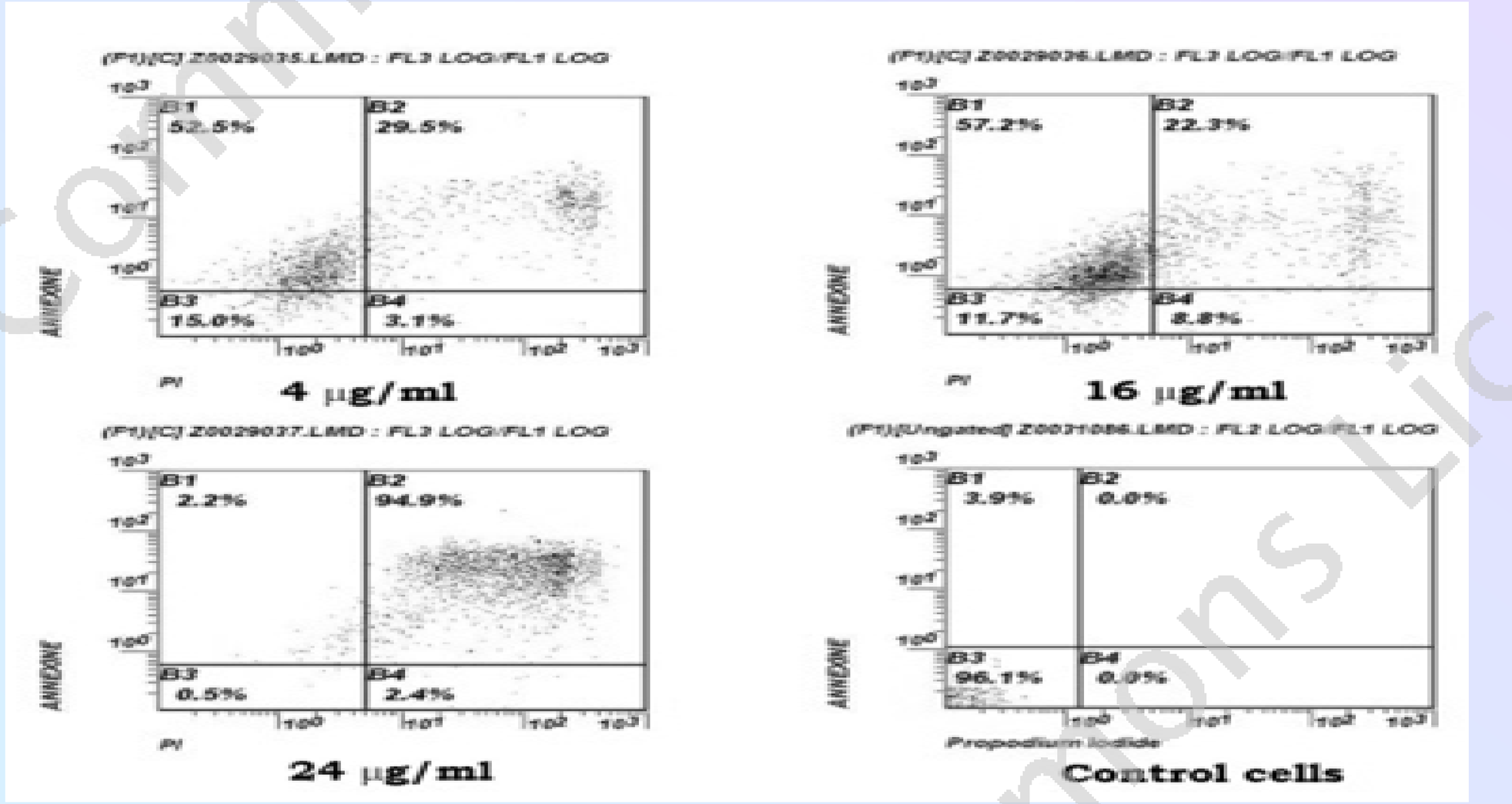


Figure 10

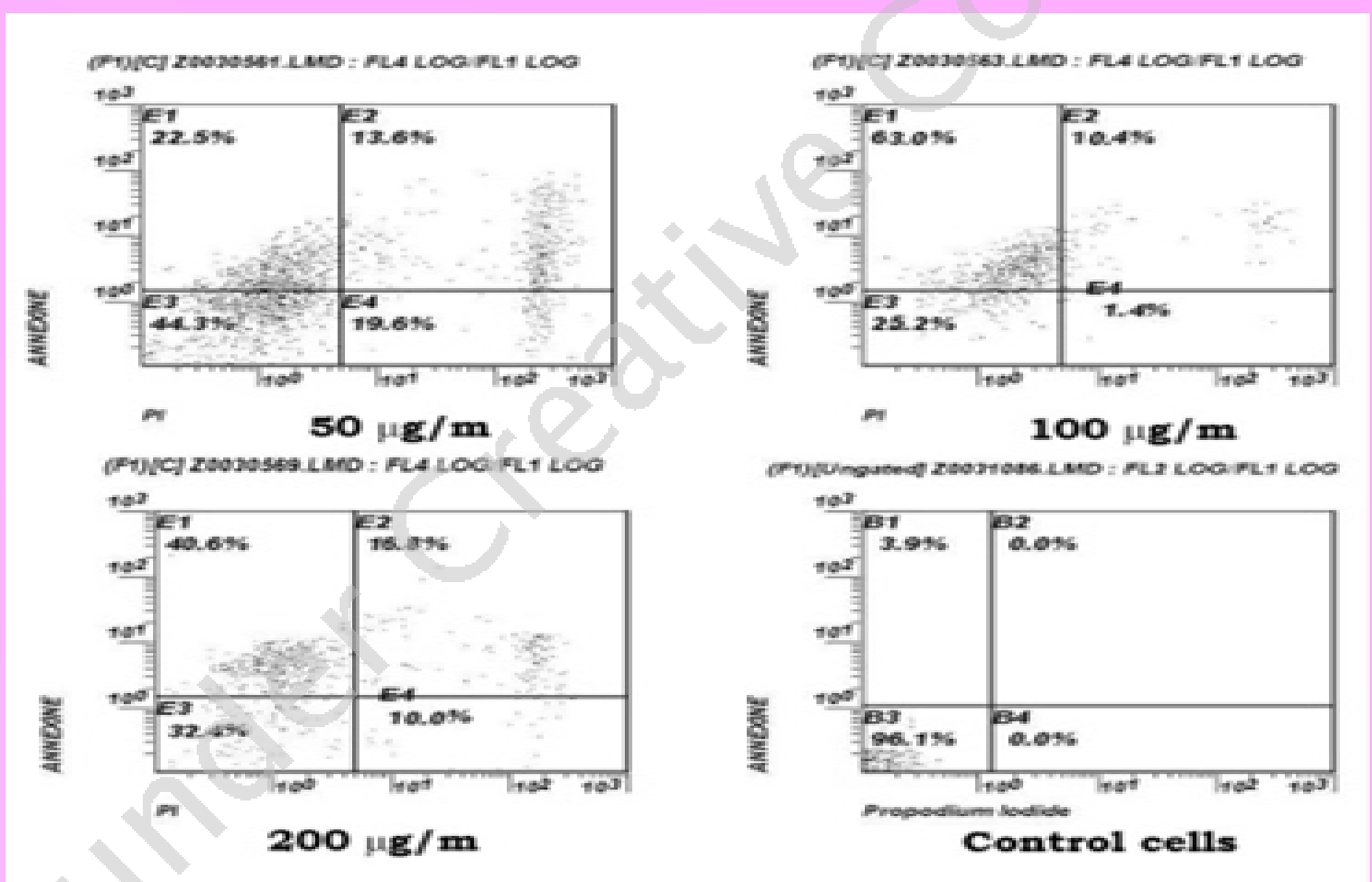


Figure 11

Incubation of HepG2 cells with 50 µg/ml of thymoquinone resulted in 89.7% of cells being in early apoptosis. In cells incubated with 100 and 200 µg/ml of thymoquinone, the proportions of apoptosis were 94.4 % and 95.5 % respectively. These results indicated that, with increasing the concentration of thymoquinone, there was a gradual increase in the intensity of cell staining with annexin V-FITC reflecting increasing in apoptosis. Incubation of HepG2 cells with 4 µg/ml of cisplatin resulted in 18.4% of cells being in early apoptosis. In cells incubated with 16 and 24 µg/ml of cisplatin, the proportions of apoptosis were 62% and 76.5% respectively. Meanwhile, with increasing concentration of cisplatin, there was a gradual increase in the intensity of cell staining with annexin V-FITC and propidium iodide reflecting the increase of necrosis (Figures 9 - 10).

Incubation of H460 cells with 50 mg/ml of thymoquinone for 48 hours, followed by flow cytometric analysis resulted in 22.5% of cells being in early apoptosis. Whereas, cells treated with 100 and 200 mg/ml of thymoquinone, the proportions of apoptosis were 20.6 % and 65.4 % respectively. In addition, there was increase in the proportion of necrotic cells.Flow cytometric analysis of H460 cells treated with 4 mg/ml of Cisplatin for 48 hours, showed that, 52.5% of cells being in early apoptosis. Whereas cells treated with higher concentrations of cisplatin 16 mg/ml resulted in increasing the proportion of apoptosis to 57.2% and whil 24 mg/ml resulted in decresing the proportion of apoptosis to %2.2%. In the same time, the percent of necrotic cells were dramatically increased to 22.3% and 94.9% respectively (Figures 10 - 11).

CONCLUSION:

From aforementioned results, it could be concluded that:

Thymoquinone has ant proliferative effects on both Hep-G2 and H460 cells and induce their action through apoptosis and cell cytotoxicity.

TQ can be promising anti-cancer therapeutic agents for hepatocellular carcinoma and lung cell cancer and a potential candidate to be further evaluated.

We advise for supplementation of thymoquinone (or black seeds) as a protective and preventive mechanisms against cancer.

Also, co-treatment with the chemotherapy by these natural products to attenuate the toxic side effect of the chemotherapy.

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