RESEARCH ARTICLE

Editorial Process: Submission:05/15/2018 Acceptance:07/26/2018

Comparative Analysis of the Effects of 17-Beta Estradiol on Proliferation, and Apoptosis in Hepatocellular Carcinoma Hep G2 and LCL-PI 11 Cell Lines

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Abstract

Background: Phytoestrogens are a group of natural compounds with estrogen-like activity and similar structure to estradiol that structurally mimic the mammalian estrogen 17-β estradiol (E2). They have a biphasic effect and exert pleiotropic effects which induce or inhibit estrogen action by activation/inhibition of the estrogen receptors (ERs). These compounds can induce apoptosis at high concentrations. The previous finding indicated that E2 inhibited cell growth and induced apoptosis in hepatocellular carcinoma (HCC) PLC/PRF/5 cell line. The aim of the present study was to investigate the apoptotic and proliferative effects of E2 on hepatocellular carcinoma HepG 2 and LCL-PI 11 cells. **Methods:** The Hep G2 and LCL-PI 11 cells were cultured and treated with E2 for different time periods and then MTT [3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl -2H- tetrazolium bromide] assay and flow cytometry assay were done to determine cell viability and cell apoptosis respectively. **Results:** E2 had inhibitory and apoptotic effects on Hep G2 cell line, whereas it indicated a biphasic effect on LCL-PI 11 cell line. The half-maximum inhibitory concentration (IC50) value was 3 μM. The inhibitory effect of E2 on Hep G2 cells was observed with all concentrations of E2 ($P < 0.087$), whereas E2 showed a biphasic effect on LCL-PI 11. This compound induced significant apoptosis in Hep G2 cell line at the all treatment times versus control groups, whereas, in the LCL-PI 11 cell, significant apoptotic cells were observed after 72 and 96h ($P < 0.001$). **Conclusion:** E2 can inhibit cell growth and induce apoptosis in hepatocellular carcinoma HepG 2 and LCL-PI 11 cell lines.

Keywords: Estradiol- viability- apoptosis- hepatocellular carcinoma

Asian Pac J Cancer Prev, **19 (9)**, 2637-2641

Introduction

Phytoestrogens are a group of natural compounds, polyphenolic non-steroidal plant compounds, with estrogen-like activity and similar structure to estradiol that structurally mimic the mammalian estrogen 17-β estradiol (E2) by binding to estrogen receptors (ERs). The source of these compounds includes legumes, soybeans, fruits, vegetables, whole rye and flax seeds and whole grains (Oseni et al., 2008). This group can be classified into four main subgroups based on their chemical structure, including flavonoids, isoflavonoids, stilbenes, and lignans (Lephart., 2015). Several recent epidemiological studies reported that diets rich in phytoestrogens, particularly soy and unrefined grain products, was associated with low risk of some cancers (Cotterchio et al., 2006; Zaineddin et al., 2012). The mechanism of the phytoestrogens may be the possible binding to ERs because of their structural similarity to E2. They have a biphasic effect, estrogenic and antiestrogenic activity, and exert pleiotropic effects which induce or inhibit estrogen action by activation/inhibition of the ERs (Oseni et al., 2008). Based on a prospective cohort study, isoflavone consumption is associated with a reduced risk of breast cancer (Dong, 2011).

A similar study has shown that soy food intake plays a protective effect against premenopausal breast cancer (Rossi et al., 2008; Wu et al., 2008). The association between isoflavones and flavonols consumption and ovarian cancer risk reduction has been reported. Experimental studies have demonstrated that phytoestrogens induce apoptosis at high concentrations in ER-positive breast cancer cells (Wu et al., 2008; Thompson et al., 2005). Furthermore, it has indicated that phytoestrogens ingestion is associated with the less aggressive breast tumors in rodent (Velentzis et al., 2008). In other cancers such as lung and endometrial cancer, consumption of soy products is associated with a decreased risk of these cancers (Yang et al., 2011; Bandera et al., 2009).

The previous finding indicated that E2 inhibited cell growth and induced apoptosis in hepatocellular carcinoma (HCC) PLC/PRF/5 cell line (Bandera et al., 2009). With regard to the previous result, the present study was

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assigned to investigate comparative analysis of the effects of E2 on proliferation, and apoptosis in hepatocellular carcinoma Hep G2 and LCL-PI 11 cell lines.

Materials and Methods

Human hepatocellular carcinoma Hep G2 and LCL-PI 11 cells were purchased from the National Cell Bank of Iran-Pasteur Institute and maintained in Dulbecco minimal essential medium (DMEM) containing 100 mL/L fetal bovine serum (FBS), 100 U/mL streptomycin, and 100U/mL penicillin at 37 ºC in a humidified atmosphere containing 5% CO_2. E2 was obtained from Sigma and dissolved in dimethyl sulfoxide (DMSO) to make a stock solution; DMSO was present at 0.01–0.3% in the medium based on the IC50 (half-maximum inhibitory concentration) index. The stock solution was further diluted with cell culture medium to yield final E2 concentrations. Phosphate-buffered saline (PBS) and MTT [3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl -2H- tetrazolium bromide] were purchased from Sigma (Sigma, St. Louis, MO). All other chemicals were obtained from the best sources available.

Cell culture and cell viability assay

The Hep G2 and LCL-PI 11 cells were cultured with DMEM (pH 7.2–7.4) supplemented with 1% sodium pyruvate (Sigma), 1.5 g/L sodium bicarbonate, 10% fetal bovine serum and 1% antibiotics, including 1% penicillin/streptomycin and 25 ug/ml amphotericin B (Sigma) at 37 °C in 5% CO_2 to promote attachment. When the cells reached > 80% confluence, 5×10^5 cells were seeded into 96-well plates, allowed to adhere for 24 h and subsequently treated with medium containing different doses of E2 (0.01, 0.1, 1, 5 and 10 µM/lit) to determine the viability and IC50 values. After 24, 48, 72 and 96 h of the treatment, the effect of E2 was assessed by MTT assay according to standard protocols. In this regard, the Hep G2 and LCL-PI 11 cells were washed twice with PBS, and a fresh medium containing MTT (0.5 mg/mL) was added and finally, after 4-hour incubation, the formazan crystals were dissolved in DMSO and the absorbance was measured at 570 nm. All experiments were repeated three times, with at least three measurements (triplicates).

Cell apoptosis assay

To assess the apoptosis by flow cytometry assay, the Hep G2 and LCL-PI 11 cells were seeded at a density of 5×10^5 cells/well in 24-well plates and treated with E2 (3µM based on IC50 values) for 24, 48, 72 and 96 h. After treatment times, all the adherent cells were harvested by trypsinization, washed with PBS and resuspended in Binding buffer (1x). Then, Annexin-V-(FITC) and propidium iodide (PI, Becton-Dickinson, San Diego, CA) were used for staining according to the manufacturer's instructions. Finally, the apoptotic cells were counted by FACScan flow cytometry (Becton Dickinson, Heidelberg, Germany).

Results

Result of determination of cell viability by MTT assay

The effects of the E2 on the Hep G2 and LCL-PI 11 cell viability were assessed by MTT assay after treatment with various doses of E2 (as mentioned above). As shown in Figure 1, the inhibitory effect of E2 on Hep G2 cells was observed with all concentrations of E2 at different time periods ($P < 0.087$), whereas E2 showed a biphasic effect on LCL-PI 11 (Figure 2), it significantly increased the number of LCL-PI 11viable cells with 0.01 and 0.1 µM concentration and decreased the number of viable cells with 1, 5 and 10 µM concentrations as shown in Figure 2 ($P < 0.001$). Reduction of cell viability by 50% (IC50) required 3 µm E2. Each experiment was repeated three times for consistency of the result.

Result of determination of the cell apoptosis by flow cytometry assay

To determine cell apoptosis, the Hep G2 and LCL-PI 11 cells were seeded at a density of 5 × 105 cells/well

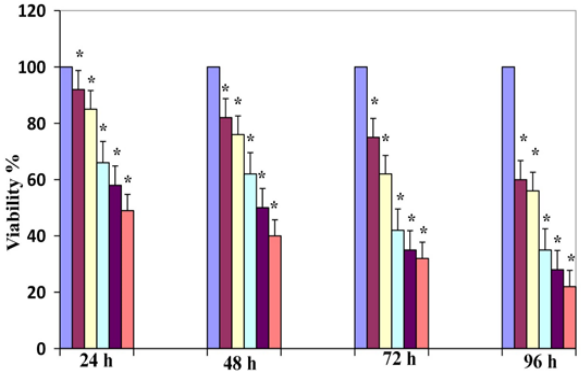

Figure1. Inhibitory Effect of E2 on Hep G2 Cell Line. E2 had a significant inhibitory effect on Hep G2 cell at all concentrations and time periods. Mean values from the three experiments ± standard error of mean are shown. Asterisks (*) indicate significant differences between treated cells and the control group (*$P < 0.087$). The first column of each group presents viability of the control group.

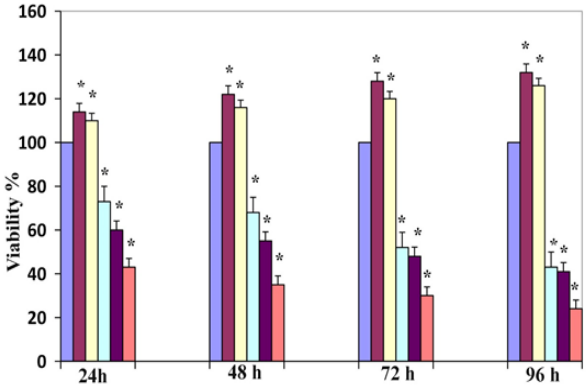

Figure 2. Inhibitory Effect of E2 on LCL-PI 11 Cell Line. E2 exerted a biphasic effect on LCL-PI 11 cell. Mean values from the three experiments ± standard error of mean are shown. Asterisks (*) indicate significant differences between treated cells and the control group (*$P < 0.001$).

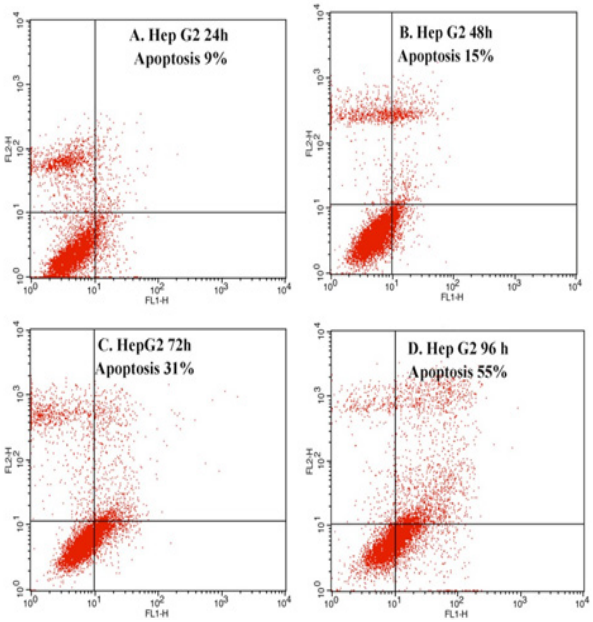

Figure 3. Apoptotic Effect of E2 on Hep G2 Cell Line. The result of flow cytometry indicated that E2 (3 µM) can induce significant apoptosis in Hep G2 cell at all treatment times.

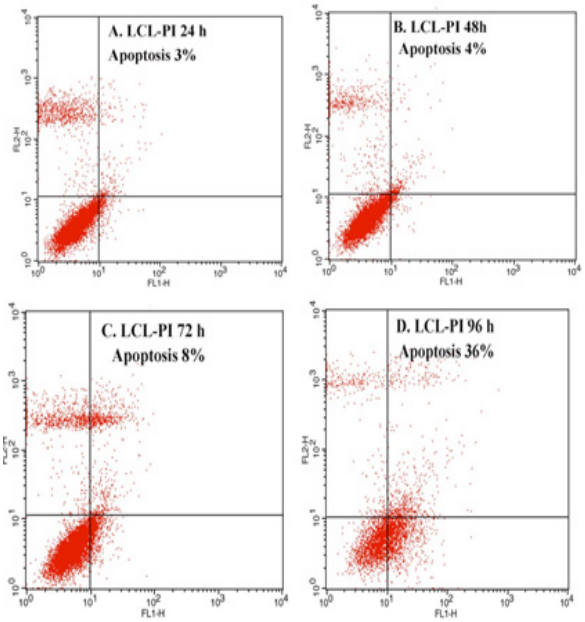

Figure 4. Apoptotic Effect of E2 on LCL-PI 11 Cell Lines. The result of flow cytometry indicated that E2 (3 µM) can induce significant apoptotic after 72 and 96 h of treatment.

Table 1. The Percentage of Apoptotic Cells after Treatment with E2 (3 µM) for 24, 48, 72 and 96 h

Cell lines	Drug	Dose/ µm	Duration/ h	Apoptosis%	P-value
Hep G2	E2	3	24	9	0.001
			48	15	
			72	31	
			96	55	
LCL-PI 11	E2	3	24	3	1
			48	4	0.738
			72	8	0.001
			96	36	

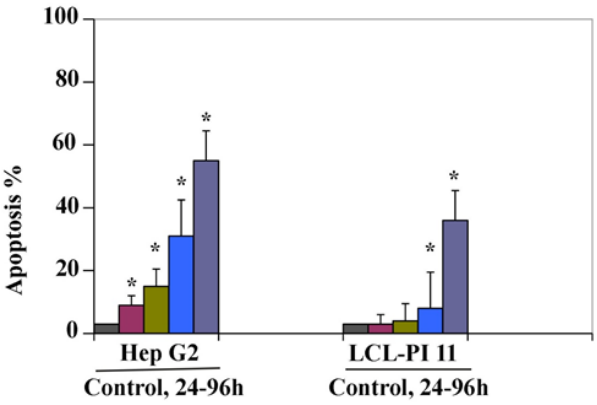

Figure 5. Effects of E2 (3 µM) on Hep G2 and LCL-PI 11 Cell Apoptosis. Asterisks (*) indicate significant differences between treated cells and the control group. Results were obtained from three independent experiments and were expressed as a mean ± standard error of the mean.

in 24-well cluster plates and treated with E2 (3 µM) for different time periods (24, 48, 72 and 96 h) and flow cytometry was performed to determine the apoptotic cells. As shown in fig. 3, E2 induced significant apoptosis in Hep G2 cell line at the all treatment times versus control groups, whereas, in the LCL-PI 11 cell, significant apoptotic cells were observed after 72 and 96h (Figure 4). Maximal apoptotic cells were obtained in HepG2 cell line after 96 h of treatment (fig. 5). The percentage of apoptotic cells in all experimental groups are shown in table 1.

Discussion

In the present study, the effect of E2 on cell viability and apoptosis in hepatocellular carcinoma Hep G2 and LCL-PI 11 cell lines were investigated. Previous findings indicated that E2 can inhibit cell growth and induce apoptosis in hepatocellular carcinoma PLC/PRF/5 cell line (Kavoosi et al., 2016). In the current study, E2 demonstrated an inhibitory and apoptotic effect in hepatocellular carcinoma Hep G2 cell line, whereas it had a biphasic effect on LCL-PI 11 cell line. Consistent with present findings, the apoptotic and inhibitory effects of E2 have been reported in hepatocellular carcinoma and breast cancer MCF-7 (Yang et al., 2012; Liu et al., 2003; Lewis et al., 2005) and MDA-MB-231 cell lines (Moggs et al., 2005). Similar to the current results, it has been reported that E2 plays a significant stimulatory effect on human LNCaP prostate cancer cells (Arnold et al., 2005), and prostate stromal cells (Zhang et al., 2008). The possible mechanisms by which E2 may exert its apoptotic effect is through G2/M-phase arrest, decreased NF- κB activity (Liu et al., 2003), activation of the FasR/FasL death-signaling pathway (Lewis et al., 2005; Dodwell et al., 2005), activation of the mitochondrial apoptotic

pathway, induction of Bax, Bak, Bim, and p53 (Lewis et al., 2005; Lewis-Wambi et al., 2009).

E2 exerts its proliferative effect through different mechanisms. This agent bound to ERα by which activates the protein kinase B (survival factor Akt) (Szegő et al., 2006; Levin, 2009) as well as stimulating growth factor receptor activity (Heldring et al., 2007). In the prostate cancer PrSCs and WPMY-1 cells, it has reported that E2 stimulates cell proliferation by activation of ERK and inhibition of PD98059, a selective ERK inhibitor (Zhang et al., 2008). The proliferative effect of E2 on hepatocellular carcinoma Hep G2 cell is mediated by activation of the MAPK/ERK pathway which increases the cyclin D1 gene expression through activation of transcription factor AP-1 transcription factor (Marino et al., 2002). Furthermore, it has been demonstrated that E2 exerts its stimulatory effect through a cross-talk between G-protein-coupled receptor (GPR30) and ER, and activation of the rapid epidermal growth factor receptor (EGFR)/ERK/fos pathway, which, in turn, stimulates mouse spermatogonial GC-1 cell proliferation (Sirianni R et al., 2008). In the current study, the mechanisms of antiproliferative and apoptotic effects of E2 were not evaluated. It will be a subject of a separate research.

In conclusion, the current study shows the potent inhibitory and apoptotic effects of E2 against hepatocellular carcinoma in vitro, strongly supporting E2 as a candidate cancer therapy in hepatocellular carcinoma.

Conflicts of interest

The authors report no conflicts of interest in this work.

Acknowledgements

This article was supported by adjutancy of research of Jahrom medical University-Iran. This article has been extracted from Ms Faeze dehghani's thesis.

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