The Effects of Cytotoxic Dose of *Estradiol valerate* on Caspase 9 Activity level in Colon Cancer (HT29) Cells

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***Abstract*—**Anticancer effects of estrogens have been reported in recent in vitro studies. We exerted this laboratory experimental research to assess the effects of cytotoxic dose of *Estradiol valerate* on caspase 9 activity in colon cancer (HT29 cells) in cell culture. Cytotoxic concentrations (1mg/ml) of *Estradiol valerate* was used in our study. HT29 cells were purchased from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were grown and incubated in standard situation. Then, cells were sub-cultured into 75cm2 flasks, 96-well plates or 6-well plates. Activity level of caspase 9 was evaluated using calorimetric assay (405nm) through microplate reader. Analyses were conducted using the SPSS 20 and ANOVA. Our results indicated that exposure to 1mg/ml of *Estradiol valerate* led to significant increase in caspase 9 activity compared to control cells.

***Keyword*---** *Estradiol valerate*, Caspase-9, HT29 Cells.

I. Introduction

The HT-29 cell line is the most commonly used colorectal adenocarcinoma cell line with epithelial morphology, which is used in many cellular studies. These cells were first produced in 1964 for 44-year-old Caucasian women with colon adenocarcinoma. HT29 cells under the standard conditions have an unpolarized growth, and they form undifferentiated cell lines that provide a good, To investigate the effects of various factors on these cells. By creating mutations in the intestinal tract, carcinogenic signaling pathways are activated in the cells, some of which play an important role in the development of cancer. [1-2]

Sex steroid hormones, in particular estradiol, progesterone and testosterone, are among the most famous hormones in the reproductive system, and their study of the development, proliferation, or inhibition of cancer cell growth has a prominent place of research. In this regard, steroid hormones can stimulate cancer cells or inhibit their growth and replication. Estradiol hormones can play a role in preventing metastases or stimulating metastases in cancer cells. Studies have shown that estradiol hormone can inhibit or stimulate the development of gastrointestinal tumors, in particular colorectal tumors, and proliferation of tumor cells in the digestive tract. [3.4]

Caspases are a family of endoproteases that provide critical links in cell regulatory networks controlling inflammation and cell death. The activation of these enzymes is tightly controlled by their production as inactive zymogens that gain catalytic activity following signaling events promoting their aggregation into dimers or macromolecular complexes. Activation of apoptotic caspases results in inactivation or activation of substrates, and the generation of a cascade of signaling events permitting the controlled demolition of cellular components. Activation of inflammatory caspases results in the production of active proinflammatory cytokines and the promotion of innate immune responses to various internal and external insults. Dysregulation of caspases underlies human diseases including cancer and inflammatory disorders, and major efforts to design better therapies for these diseases seek to understand how these enzymes work and how they can be controlled. [5,6]

Caspase-9 is a member of the cysteine proteases involved in cytokine and apoptosis processing. When cells receive the apoptotic stimulus, the mitochondria release cytochrome c, which binds to APAF-1, which is homologous to Ced-1 mammals with dATP, is released. The resulting complex regenerates Caspase-9, which activates it. Caspase-9 activates downstream caspases, such as Caspase 3, -6, and -7, which initiate caspase cascades.[5,7]

II. Material And Methods

Cytotoxic concentrations (1mg/ml) of *Estradiol valerate* was used in our study. HT29 cells were purchased from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were grown and incubated in standard situation. Then, cells were sub-cultured into 75cm2 flasks, 96-well plates or 6-well plates. Activity level of caspase 9 was evaluated using calorimetric assay (405nm) through microplate reader. Analyses were conducted using the SPSS 20 and ANOVA.

III. Results

Our results indicated that exposure to 1mg/ml of *Estradiol valerate* led to significant increase in caspase 9 activity compared to control cells.

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IV. Discussion

In our study, we found that exposure of colon cancer cells to cytotoxic dose of *Estradiol valerate* led to increased activity of caspase 9 enzyme indicating that apoptosis occurred through internal pathway in which caspase 9 is activated.

In line with our findings, studies have shown that sex steroid hormones have anticancer effects on various types of cancer cells, inducing apoptosis in cancer cell lines in vitro resulting in decreased proliferation rate in cells. In research conducted by Honda et al in 2015 about the effects of male and female sex hormones on the proliferation of cancer cells in the prostate and the cells in the culture medium, the results showed that sex hormones play a vital role in the development and proliferation of cells. Based on the findings of this study, steroid hormones, by binding to their receptors, induce the expression of specific tRNAs, which, likewise, play an important role in the proliferation of cancerous cells. [8]

In a study conducted by Levinteir et al. In 1992 on the study of the effects of steroid hormones on the proliferation of human colon cancer cells in cell culture media, the results showed that the analogue of estrogen and ethinyl progesterone could inhibit the growth of colon carcinoma cells. [9]

The research also has shown that expression of estrogenic and androgenic receptor genes plays an important role in the development of colon and rectal cancer. In this regard, the findings have shown that the β-estrogen receptor gene plays a more important role in the development of colorectal cancer than the alpha estrogen receptor gene. [10]

V. Conclusion

In our study, we found that exposure of colon cancer cells to cytotoxic dose of *Estradiol valerate* led to increased activity of caspase 9 enzyme indicating that apoptosis occurred through internal pathway in which caspase 9 is activated.

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