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The apoptotic effects of progesterone and testosterone on colon cancer (SW480) cells

Azita Tishehyar[1*], Rahim Ahmadi[1], Minoo Mahmoudi[1], Abdolreza Mohamadnia[2]

[1]. Department of Biology, Hamedan Branch, Islamic Azad University, Hamedan, Iran
2. Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

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*Corresponding Author:

Azita Tishehyar

Address: Department of Biology, Hamedan Branch, Islamic Azad University, Hamedan, Iran

E-mail address:

azita.tishehyar@gmail.com

ABSTRACT

Introduction: The association of sex steroids with cancer cell proliferation has been reported in recent studies; however, the findings are still controversial. The present study aimed to determine the cytotoxic effects of progesterone and testosterone on colon cancer (SW480) cells and to evaluate the expression levels of Bcl-2 and Bax genes in SW480 cells.

Materials and Methods: The SW480 cell line was divided into a control group (untreated) and groups treated with 125, 250, 500, and 1000 µg/mL of testosterone and progesterone. Cell viability was quantified by the MTT assay. qRT-PCR was performed to evaluate gene expression levels. Flow cytometry was used to assess the apoptosis in cancer cells. Data were analyzed using the student's t-test and ANOVA.

Results: The expression level of the Bax gene significantly decreased in SW480 cells exposed to a cytotoxic dose of progesterone. Moreover, the expression level of the Bax gene significantly increased in the SW480 cells exposed to a cytotoxic dose of testosterone.

Discussion: The results of the present study showed that testosterone might affect the apoptosis of colon cancer cells at low concentrations. Studies have shown that progesterone can induce tumor cell death in cancer cells. Accordingly, the clinical use of testosterone and progesterone therapy for cancer treatment is highly controversial.

Keywords: Progesterone, Testosterone, SW480, Apoptosis

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1. Introduction

Studies have shown that sex hormones play an important role in the development of many types of cancer (1); however, they have been reported to have anticancer effects on colon cancer (1-2). Among colon cancers, colorectal adenocarcinoma accounts for more than 95% of colon and rectal cancers. Besides, colon cancer is one of the most common cancers, accounting for 100,000 deaths annually. Colorectal cancer is recognized as the second most commonly diagnosed cancer in women and the third most common cancer in men worldwide (3).

Although testosterone exerts an inhibitory effect on the growth and development of some types of cancer cells (4), there is evidence suggesting that testosterone can be a contributing factor in the formation of colon tumors (5). It has also been reported that men tend to have a higher incidence of colorectal cancer than women of similar age (6). In vitro experimental findings have shown that male steroid hormones can cause adenocarcinomas and stimulate the proliferation of adenocarcinoma cells (7).
Various studies have demonstrated that progesterone interacts with different types of cancer cells. While progesterone has anti-proliferative effects on colon cells (8), synthetic progestins have been reported to show anticancer effects against colorectal cancer (9). Combined use of estrogen and progesterone leads to breast, cervical, and liver cancer in women (10). Sex steroid hormones, especially testosterone, can stimulate the proliferation of cancer cells or inhibit their growth and proliferation. Sex steroids can also play a role in preventing metastasis or stimulating metastasis in cancer cells (2). The association between female sex steroids and lung cancer has been reported in women receiving hormone therapy (11). Moreover, laboratory studies on the role of estrogen and progesterone in lung cancer have revealed that progesterone has a major role in the development of lung cancer cells in vitro and in vivo (12).

Although previous studies have investigated the effects of testosterone and progesterone on colon cancer cells, the findings are highly controversial (4, 6, 12, 13). There are few studies focusing on the cytotoxicity mechanisms of testosterone and progesterone on the expression of clusters of differentiation B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) genes. Considering the effects of testosterone and progesterone on cell proliferation as well as metastasis in colorectal cancer cells (4–14), this study aimed to determine the apoptotic effects of progesterone and testosterone on SW480 cancer cells.

2. Materials and Methods

This research was carried out at Javid Biotechnology Institute (Tehran, Iran) after obtaining approval from the Institutional Ethics Review Committee of Biomedical Research of Hamedan University of Medical Sciences (Hamedan, Iran) (ID number: IR.UMSHA.REC.1399.342).

2-1. Cell lines

SW480 cells were obtained from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran).

2-2. Hormone preparation

Progesterone and testosterone were obtained from Aburaihan Pharmaceutical Company (Tehran, Iran) and dissolved in dimethyl sulfoxide (DMSO), Dulbecco's Modified Eagle's Medium (DMEM), and phosphate-buffered saline (PBS) and finally prepared serially (125, 250, 500, and 1000 μg/mL) (15).

2-3. Cell cultures

SW480 cells were cultured in DMEM, supplemented with 10% fetal bovine serum (FBS) and 1% gentamicin, and kept in a humidified atmosphere with 5% CO2 in a 37°C incubator. The cultured cell with 70–80% confluency was washed with PBS and detached from the flask using trypsin-EDTA with incubation at 37 oC for 3–4 minutes, followed by the addition of the culture medium containing 10% FBS to neutralize the excess trypsin-EDTA activity. The cell suspension was finally centrifuged, and the cell pellet was re-suspended in a fresh culture medium for further experiments.

2-4. Cytotoxicity assay

The effects of progesterone and testosterone on cell viability were determined using the MTT assay. The cells (1×104) were seeded in each well of a 96-well plate for 24 hours. They were treated with different concentrations of progesterone and testosterone (125, 250, 500, and 1000 μg/mL) for 24 hours and added to wells in eight replicates. The MTT solution (5 mg/mL) (DoBio Biotech, Shanghai, China), diluted in PBS, was then added to the culture medium (100 λ) and incubated with the cells for three hours at 37˚C in darkness. During this incubation period, water-insoluble formazan crystals were formed and dissolved by adding 100 λ/well of DMSO (Sigma). The optical density (OD) of each culture well was measured at 570 nm using a microplate reader. The wells containing the culture medium and MTT without the cells were considered blanks. The OD570 of cells without progesterone treatment was considered to be 100% viable.

Table 1. The characteristics of the primers used in the real-time RT-PCR reaction

Gene		Primer sequences
Bax	forward	5' CGGCAACTTCAACTGGGG 3'
	Reverse	5' TCCAGCCCAACAGCCG 3'
Bcl2	forward	5' GGTGCCGGTTCAGGTACTCA 3'
	Reverse	5'TTGTGGCCTTCTTTGAGTTCG 3'
GAPDH	forward	5' CCCACTCCTCCACCTTTGAC 3'
	Reverse	5' CATACCAGGAAATGAGCTTGACAA 3'

The percentage of cell viability (%) was measured as follows (16): Cell viability (%) = (OD570-630 of treated cells/OD570-630 of control cells) × 100%

2-5. Real-time PCR assay

A SYBR Green real-time quantitative PCR assay was employed to quantify the expression levels of Bax and Bcl-2 genes after the treatment of SW480 cells. Briefly, the cells were seeded into six-well plates (5×105 cells/well) and incubated for 24 hours. They were then exposed to progesterone and testosterone at the final half maximal inhibitory concentrations (IC50) (progesterone: 1350 µg/mL, testosterone: 740 µg/mL) and incubated for an additional 24 hours. The total mRNA was extracted from the cells and converted to complementary DNA using the RNA Isolation Kit (RNeasy Plus Mini Kit 50, Qiagen, Valencia, CA, USA) and the PrimeScriptTM First-Strand cDNA Synthesis Kit (Takara, Tokyo, Japan), respectively, according to the manufacturers' protocols. The primers used for real-time PCR are presented in Table 1. The expression level of target genes was studied using an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

GAPDH was used as an internal control. Each amplification reaction was performed in a 20-µL reaction mixture containing 10 µL of PowerUp 2X SYBR Green PCR Master Mix, 1 µL of each primer (2 µM), 2 µL of cDNA (100 ng), and 6 µL of double-distilled water. After denaturation at 95°C for 15 seconds and 60°C for one minute, amplification was followed by a melting step at 95°C for 20 seconds, at 60°C for 60 seconds, and at 95°C for 20 seconds (12). The expression level of genes was calculated based on the 2-CT method and normalized to the loading control, GAPDH (15–17).

2-6. Flow cytometric analysis of cell death

Differential counting of apoptotic, necrotic, and viable cells was carried out using a fluorescein isothiocyanate (FITC)-Annexin-V/propidium iodide (PI) staining kit (Hoffmann-La Roche Ltd., Basel, Switzerland). This assay involved simultaneous staining with both annexin-V and PI as DNA stains. Three subpopulations of cells were discriminated: (a) PI-negative and FITC-negative viable cells (PI-/FITC-) that maintain the typical asymmetry of plasma membrane lipids; (b) PI-negative and FITC-positive early apoptotic cells (PI-/FITC+) capable of transferring PI outside the cell; and (c) PI-positive and FITC-positive late apoptotic or necrotic cells (PI+/FITC+) with a loss of plasma membrane integrity. Briefly, the SW480 cell (3×105 cells/well) was incubated for 24 hours with 200 µg/mL of IC50 concentrations of progesterone and testosterone. The trypsinized cells were washed and suspended in an Annexin-V binding buffer. 5 and 10 µL of FITC Annexin-V and PI solutions were added to 100 µL of the cell suspension. Finally, apoptotic, necrotic, and viable cells were analyzed by a flow cytometer (15).

2-7. Statistical analysis

Statistical analyses were performed in SPSS version 21.0 (SPSS, Chicago, IL, USA). Differences in the cell viability of the groups were tested using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. An independent sample t-test was used to determine differences in gene expression. All experiments were performed with at least three replicates. All data are expressed as mean±standard deviation (SD), and a P-value <0.05 was considered statistically significant.

3. Results and Discussions

3-1. Cytotoxic effects of progesterone and testosterone on the proliferation of SW480 cells

The results of the MTT assay showed that the viability of SW480 cells significantly decreased only in groups exposed to 1000 µg/mL of progesterone compared to the control group ($P<0.05$, $P<0.01$, and $P<0.001$, respectively) (Figure 1). However, SW480 cell viability was significantly reduced in groups exposed to 125, 250, 500, and 1000 µg/mL of testosterone compared to the control group ($P<0.001$) (Fig. 2).

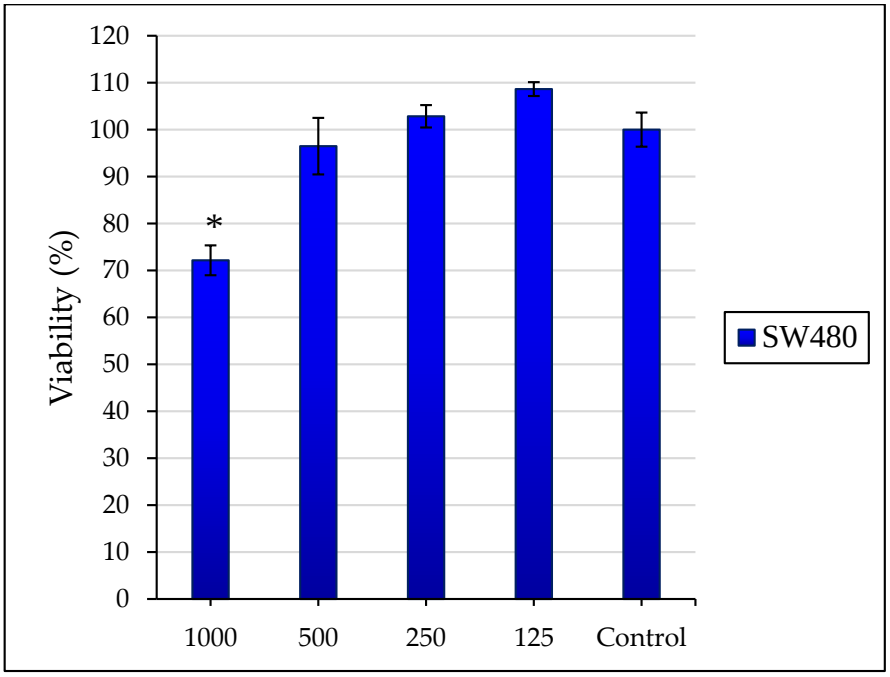


Fig. 1. Viability of SW480 cell after 24h incubation with progesterone. * and ** represent significant difference compared to control group (*: P<0.05, **: P<0.01).

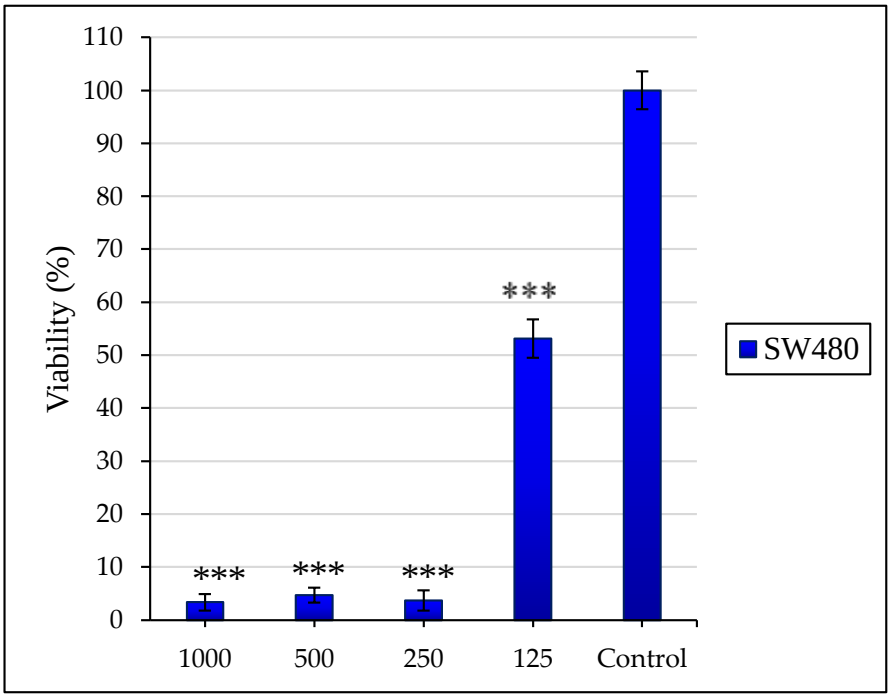


Fig. 2. Viability of SW480 cells after 24h incubation with testosterone. *** represent significant difference compared to control group (***: P<0.001).

3-2. Effects of progesterone and testosterone on the expression level of Bax and Bcl-2 genes in SW480 cells

IC50 dose of progesterone Compared to the control group, the relative expression level of Bax significantly increased ($P<0.001$) and Bcl-2 significantly decreased ($P<0.05$) (Fig. 3).

3-3. Evaluation of SW480 cell apoptosis by flow cytometry

Flow cytometry was used to discriminate between early apoptotic cells and late apoptotic and necrotic cells. Figure 5a1 represents the control SW480 cells, in which there are almost no apoptotic cells. In progesterone- and testosterone-treated SW480 cells (Fig 5a2, 5a3), a significant increase in early and late apoptotic cells and a significant decrease in live cells were found, and analysis of cell populations indicated distinct sets of populations. Annexin V-positive and PI-negative cells increased significantly via treatment of SW480 cells with IC50s of progesterone and testosterone compared to the control group, indicating the translocation of PS as an early event in the apoptotic process. Although studies have shown that steroid hormones have apoptotic effects on cancer cells (Simoes et al., 2015), the apoptotic effects of testosterone and progesterone on colon cancer cells still remain one of the challenging issues. Although studies have shown that steroid hormones have apoptotic effects on cancer cells (Simoes et al., 2015), the apoptotic effects of testosterone and progesterone on colon cancer cells still remain one of the most challenging issues.

3-4. Cytotoxic effects of progesterone and testosterone on SW480 cells

The present results showed that the survival of SW480 cells significantly decreased under the IC50 of progesterone compared to the control group. An interesting finding related to the SW480 cells was that at all dilutions, even the lowest dilution, there was a significant reduction in cell survival as compared to the control group. A study on the effects of testosterone on the growth and development of some cancer cells showed its inhibitory effect on cancer cells (4). Studies regarding the effects of progesterone on cancer cells have shown that progesterone stimulates the apoptotic pathway through cytosolic receptors and inhibits cell proliferation. In a study examining the effects of progesterone on colon cell proliferation, the results showed that progesterone had anti-proliferative effects on these cells (8). In line with the present results, some studies have shown that sex steroid hormones can play a role in inhibiting some cancers, such as breast cancer (1).

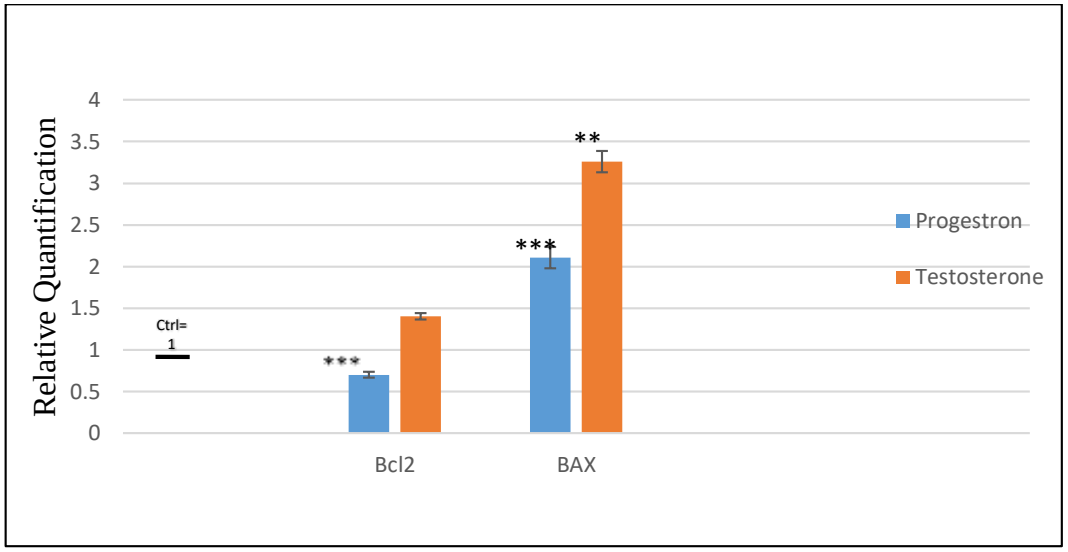
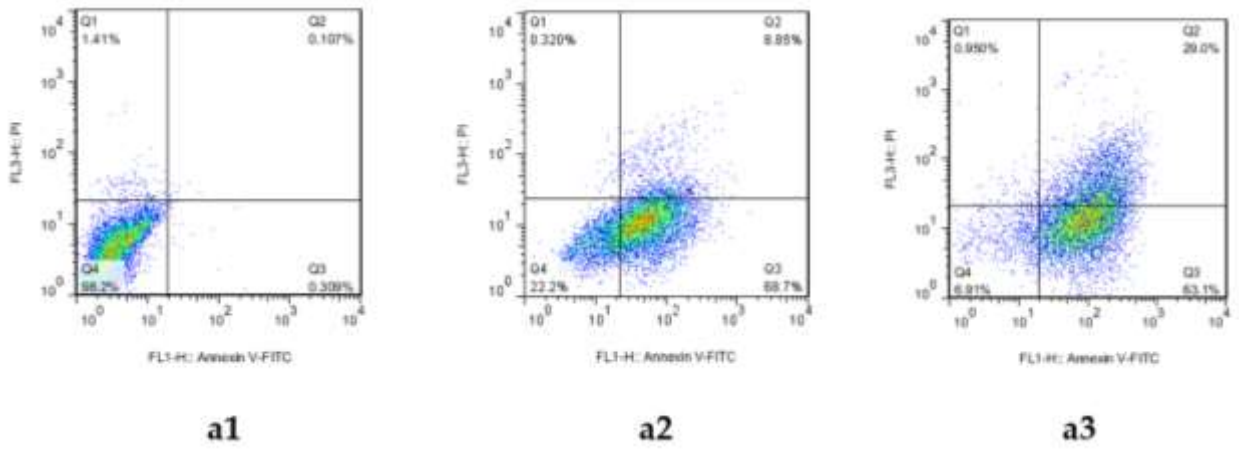


Fig. 3. Partial expression level (RQ) of Bcl2 and Bax genes in SW480 cell exposed to IC50 of progesterone and testosterone compared with control group. Ctrl indicates control group=1. * Indicates significant difference compared to control group (*: P<0.05, **: P<0.01 and ***: P<0.001).

In contrast to the findings of this study on the cytotoxic effects of progesterone and testosterone on the survival of SW480 cells, studies on testosterone have shown that it can be an effective factor in the development of colon tumors (5). In line with these findings, another study reported that men are more likely to develop colorectal cancer than women of the same age in the United States (6). Besides, the results of some studies have shown that male steroid hormones cause adenoma cancers and stimulate the proliferation of adenoma cancer cells (7). On the other hand, studies on women receiving hormone therapy have reported a higher rate of lung cancer (11).

3-5. Effects of progesterone and testosterone on expression levels of Bax and Bcl-2 genes in SW480 cells

The results of studies on apoptotic Bax and anti-apoptotic Bcl-2 genes showed that both testosterone and progesterone significantly increased the Bax gene expression in the SW480 cells, and inactive testosterone and progesterone significantly decreased the Bcl2 gene expression. Testosterone had no effect on the Bax gene expression, while it significantly decreased the expression of the Bcl-2 gene. Based on the results, it can be inferred that the cytotoxic effect of progesterone on colon cancer cells is dependent on the Bax gene apoptosis pathway.



a1 a2 a3

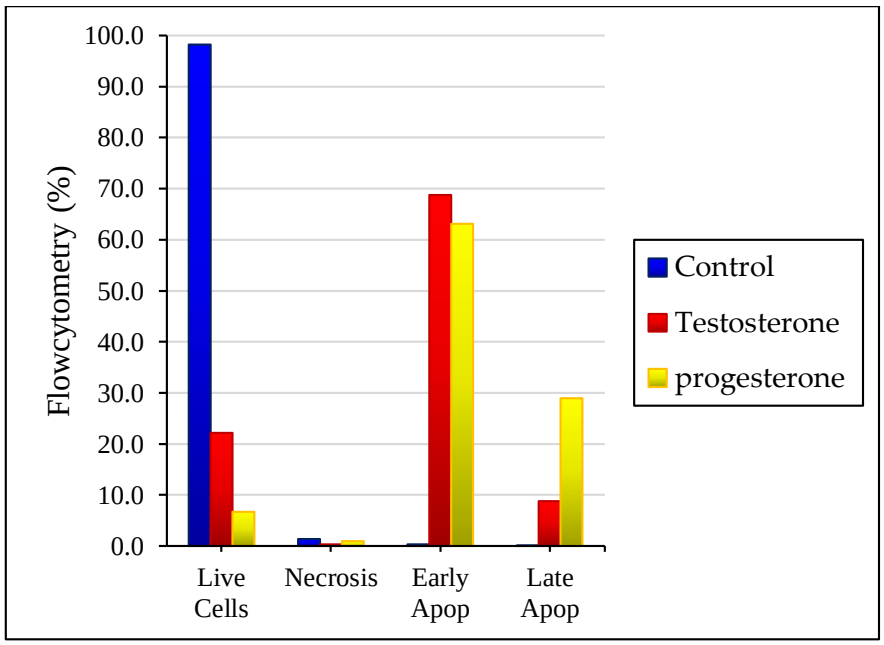


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Fig. 4. Apoptosis in SW480 cell line induced by progesterone and testosterone: Q1: Necrosis; Q2: Late Apoptosis; Q3: Early Apoptosis; Q4: Viable cells. (a1) Control SW480 cells; (a2) SW480 cells treated with IC50 of progesterone, (a3) SW480 cells treated with IC50 of testosterone. Percentage of apoptotic increased in treated cells compared with control groups. The analysis was done by FACSDiva Version 6.1.3. * and *** represent significant difference compared to control group (*: P<0.05 and ***: P<0.001).

Consistent with the present findings, the results of a study on testosterone showed that it could play an effective role in the apoptosis of colon cancer cells (18). Another study showed that the effects of testosterone on cancer cells could be related to the genomic pathway and affect the apoptotic pathway, thereby influencing the expression of apoptotic Bax and anti-apoptotic Bcl-2 genes (7). Studies on

progesterone have shown that in prostate and breast cancers, exposure to progesterone inhibits the growth of cancer cells. However, the cytotoxic effects of progesterone derivatives on the studied cancer cells significantly differ (8).

In contrast to the findings of the present study regarding the cytotoxic effects of progesterone and testosterone on the expression of Bax and Bcl-2 genes, some studies have shown that steroid hormones do not cause apoptosis in cancer cells but stimulate cancer cells. In this regard, research shows that male steroid hormones can cause adenocarcinoma and stimulate the proliferation of adenocarcinoma cells (7). Also, some studies have revealed that treatment of glioblastoma cells with dihydrotestosterone prevents apoptosis in these cells (19). On the other hand, some studies have reported that the effect of testosterone on T98G glioblastoma cells is applied in a non-genomic manner (20). Also, androgens, especially at low concentrations, have no effect on the growth and development of glioblastoma cells (14).

4. Conclusion

The results of this study showed that the cytotoxic effect of testosterone on colon cancer cells is mediated by the Bax-dependent apoptosis pathway. These findings are of great importance, as they revealed that progesterone and testosterone hormones, especially testosterone, could be used as anti-cancer agents in colon cells. Although this study had some limitations in the research phase, especially in the study of other genes, proteins, and enzymes associated with apoptosis, the present findings can be used in the treatment of colon cancers. However, further cellular, molecular, and clinical research is needed to determine whether testosterone and progesterone can be effective in treating colon tumors in animal and human models without side effects.

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Conflict of interests

The authors state that there are no conflicts of interest regarding the publication of this article.

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