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The Cytotoxic Effect of Estradiol Valerate, Progesterone, and Testosterone on Brain Glioblastoma (A172) , Colorectal Cancer (HT29) and Human Embryonic Kidney (HEK293) Cells and the Expression Levels of Bax, Bcl-2, and KAI-1/CD82 in A172 and HT29 cells

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Abstract: Although the anticancer effects of sex steroids have been widely reported, the results are still controversial. This study investigated the cytotoxic effects of testosterone (T), estradiol (E), and progesterone (P) on A172, HT29, and HEK293 cells and the expression levels of Bax, Bcl-2, and CD82/KAI-1 in A172 and HT29 cells. Cell lines were divided into control group and groups treated with 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml, and 10 mg/ml of T, P, and E valerate. The cytotoxic effect was measured using MTT assay. 0.1 mg/ml of T, 0.1 mg/ml of P, and 0.1 mg/ml of E were used to evaluate the expression levels of Bax, Bcl-2, and KAI-1 genes using real-time PCR. One-way analysis of variance was used to analyze the data. Treatment with higher concentrations of T, E, and P led to decreased cell viability in Hek293 and HT29 cells. Treatment of A172 cells with higher concentrations of T, and of P led to decreased cell viability. Bax expression level increased in HT29 and A172 cells treated with T, and P. The expression level of the Bcl-2

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decreased in HT29 cells treated with T, and P, and in A172 cells treated with P. Treatment of A172 cells with T, P, and E decreased KAI-1 expression level. Despite estradiol, testosterone and progesterone have cytotoxic effects on colon cancer and brain glioblastoma cells through the Bax gene-dependent apoptosis pathway. Estradiol and progesterone may have anti-metastatic effects on colon cancer cells.

Keywords: Estradiol; Testosterone; Progesterone; Bax; Bcl-2; KAI-1; HEK293; HT29; A172.

1 Introduction

Colon cancer is one of the fourth most common cancer after lung, breast, and prostate cancer. In terms of mortality, it is the second most common cancer after lung cancer (Pashapour et al., 2020). On the other hand, the current treatment for this cancer is mainly surgery and other methods are not very successful (Rezaianzadeh et al., 2015; Dolatkhah et al., 2015; Arnold et al., 2017; Siegel et al., 2020; Sung et al., 2021). Glioblastoma is one of the most common and deadly types of central nervous system (CNS) cancers. The average survival rate in this type of brain tumor is short. Although the prevalence of this cancer is lower than other types of cancer, it is associated with severe complications and is difficult to treat (Zhang et al., 2021; Caniglia et al., 2021). In recent years, several studies have been conducted on the apoptotic effects of sex hormones on cancer cells and this topic has been considered by many scientists, but this field still faces many challenges. The findings of some studies have indicated that beta-estradiol administration can inhibit the proliferation of colon cancer cells by an anti-apoptotic pathway (Bjørge et al., 2016). Steroid hormones have a capability to prevent certain cancers of central nervous system including astrocytoma brain tumor (Contreras and Cittelly, 2020; Costa et al., 2020; Bello-Alvarez and Camacho-Arroyo, 2021). In contrast, recently the female sex hormones have been reported to have a significant role in colon cancer and glioblastoma cells development (Lei et al., 2013; Deli et al., 2019; Bouras et al., 2021). Although experimental and clinical findings show that sex steroid hormones may inhibit colon cancer and brain glioblastoma cells proliferation (Chen et al., 2019; Contreras and Cittelly, 2020; Costa et al., 2020; Bouras et al., 2021; Bello-Alvarez and Camacho-Arroyo, 2021), association of sex steroids with colon cancer and glioblastoma development is still unclear (Zhang et al., 2021; Caniglia et al., 2021). Considering the roles played by sex hormones in colon cancer and glioblastoma cells proliferation (Chen et al., 2019; Contreras and Cittelly, 2020; Costa et al., 2020; Bello-Alvarez and Camacho-Arroyo, 2021; Bouras et al., 2021) which are unclear in many aspects, the present study aimed to investigate the cytotoxic effects of estradiol valerate, progesterone, and testosterone on brain glioblastoma (A172), colorectal cancer (HT29) and non-cancerous human embryonic kidney (HEK293) cells and the expression levels of Bax, Bcl-2, and KAI-1/CD82 in A172 and HT29 cells.

2 Materials and Methods

2.1. Hormones:

Progesterone, estradiol, and testosterone in pure powder form were purchased from Abu Reihan Pharmaceutical Company, Tehran, and kept at room temperature. 1 mg of pure powder of each

hormone was dissolved in 1 ml of DMSO and 1 ml of TWEEN 80 % and 7 ml of phosphate-buffered saline (PBS) (Sigma-Aldrich).

2.2. Cell culture:

Cerebral glioblastoma cells (A172), colorectal adenocarcinoma cells (HT29), and human embryonic kidney cells (HEK293) were obtained from Pasteur Institute cell bank, Tehran, Iran. The samples were transferred to the laboratory in a nitrogen tank and stored under standard conditions. The cells were cultured in DMEM supplemented with 10 % Foetal Bovine Serum (FBS) and 1 % antibiotics (penicillin/streptomycin). Cells were then preserved in incubator (37 °C, 5 % CO2 atmosphere).

2.3. MTT assay:

MTT assay was performed for assessing viability of A172, HT29 and HEK293 cells treated with 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml of progesterone, estradiol, and testosterone 24 hours after incubation. Cells were seeded in 96-well plates with 1×0^4 cells/well and incubated. The complete growth medium was removed, and the cells were serum-starved for 24 h prior to treatment. Cells were divided into control (untreated) group and groups incubated in culture medium alone (untreated cells) served as control group. In experimental groups, groups treated with 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml of progesterone, estradiol, and testosterone. 24 h after treatment, the medium was removed and 100 µL of MTT solution was added to each well, and the plates were incubated for 4 h. The MTT solution was removed and 100 µL aliquots of dimethyl sulfoxide (DMSO) were added to each well to dissolve the formazan crystals followed by incubation for 20 min. Treatments were performed in eight replicates. The absorbance of the resultant solution was calculated by a microplate reader (Bio-Rad, Hercules, CA) at wavelength of 570 nm. Cell viability was calculated as follows:

[Optical density (OD) of the sample/OD of the control] × 100.

2.4. Reverse transcription polymerase chain reaction (RT-PCR):

To study the expression levels of the gene by RT-PCR, the primers were designed using Analysis Primer Analysis 7 software and the NCBI database (Table 1). The cells were then cultured in 6-cell plates (500,000 cells per cell) and the cytotoxic effect of hormones on cell lines was determined using the MTT assay. The cells were then incubated for 24 h and total RNA was extracted. In the next step, total RNA was reverse-transcribed to cDNA. Finally, the AB Applied Biosystem PCR machine was used to evaluate the expression levels of the genes. The $2^{\Delta\Delta Ct}$ method was used to calculate the relative gene expression (Changizi et al., 2020; Aborehab et al., 2020).

Table 1. Primer sequences of the GAPDH, BCL-2, Bax and KAI-1 genes.

Genes	Directions	Primers
GAPDH	F	5'-CCCACTCCTCCACCTTTGAC-3'
=	R	5'-CATACCAGGAAATGAGCTTGACAA-3
BCL-2	F	5'-TGTGGATGACTGAGTACCTGAACC-3'

Continued Table 1.

Genes	Directions	Primers
=	R	5'-CAGCCAGGAGAAATCAAACAGAG-3'
BAX	F	5'-TTGCTTCAGGGTTTCATCCAG-3'
=	R	5'-AGCTTCTTGGTGGACGCATC-3'
KAI-1	F	5' CTCAGCCTGTATCAAAGTCA-3'
=	R	5' CCCACGCCGATGAAGACATA-3'

2.5. Statistical analysis:

Data were analyzed using SPSS (version 21.0; SPSS, Chicago, IL, USA). Kolmogorov-Smirnov test was used to examine the normal distribution of data. Differences between cell viability in groups were tested using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test. All data are expressed as the mean±standard deviation. Value differences were considered significant if $P< 0.05$.

3 Results and Discussions

3.1. The effect of testosterone, estradiol valerate, and progesterone on viability of HT29 cells:

Treatment of HT29 cells with 1 mg/ml of testosterone, progesterone, and estradiol valerate significantly reduced the cell viability compared to control group ($P<0.001$) (Figures: 1, 2 and 3). Likewise, estradiol valerate at 0.1 mg/ml and 0.01 mg/ml and progesterone at 0.1 mg/ml concentration caused a significant decrease in HT29 cells viability compared with control group ($P<0.05$ and $P<0.001$, respectively) (Figures: 2 and 3). In contrast, Treatment of HT29 with testosterone (0.01 mg/ml and 0.1 mg/ml) significantly increased the cell viability compared to control group ($P<0.01$ and $P<0.001$, respectively) (Figure 1). Similarly, 0.001 mg/ml and 0.01 mg/ml of progesterone significantly increased the viability of HT29 cells compared with control group ($P<0.01$ and $P<0.05$, respectively) (Figure 3). However, 0.001 mg/ml of testosterone and estradiol valerate did not have a significant effect on HT29 cell viability compared with control group (Figures: 1 and 2).

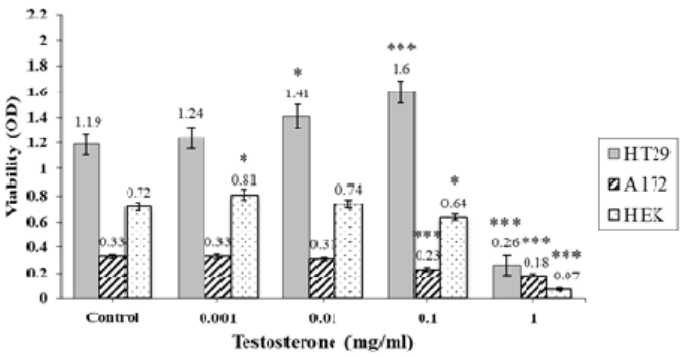

Figure 1: The effect of testosterone on the viability of HT29, A172 and HEK cells. * indicates significant difference compared with control (*: $P<0.05$; ***: $P<0.001$).

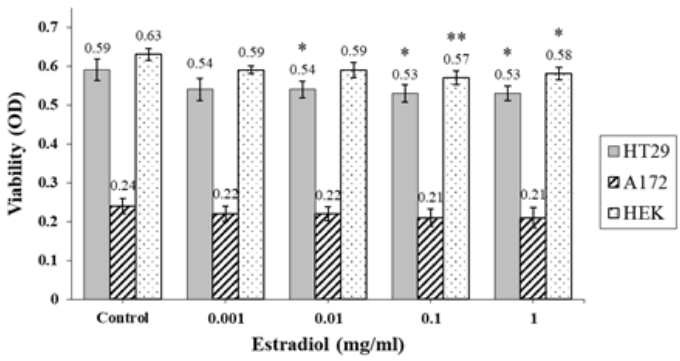

Figure 2: The effect of different concentrations of estradiol valerate on viability of HT29, A172 and HEK cells. * indicates significant difference compared with control group (*: $P<0.05$; **: $P<0.01$).

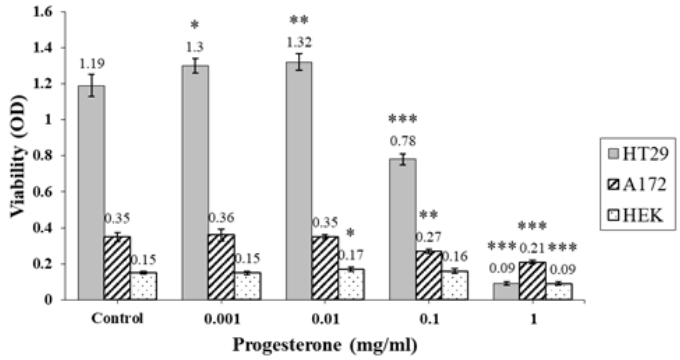

Figure 3: The effect of different concentrations of progesterone on viability of HT29, A172 and HEK cells. * indicates significant difference compared with control group (*: $P<0.05$; **: $P<0.01$; and ***: $P<0.001$).

3.2. The effect of testosterone, estradiol valerate, and progesterone on viability of A172 cells:

The results showed that treatment of testosterone and progesterone with 1 mg/ml of testosterone and progesterone significantly reduced the viability of A172 cells compared to control group ($P<0.001$). 0.1 mg/ml of testosterone and progesterone also significantly reduced the viability of A172 cells compared to control group ($P<0.001$ and $P<0.01$, respectively). However, lower concentrations of testosterone and progesterone (0.001 and 0.01) mg/ml did not significantly change the viability of A172 cells compared to control group (Figures: 1 and 3). Moreover, none of the concentrations of estradiol valerate used in this study showed a significant effect on viability of A172 cells compared to control group (Figure 2).

3.3. The effect of testosterone, estradiol valerate, and progesterone on viability of EK 293 cells:

The results demonstrated that 1 mg/ml of testosterone, progesterone, and estradiol valerate significantly reduced the viability of HEK 293 cells compared with control group ($P<0.001$, $P<0.001$, and $P<0.05$, respectively) (Figures: 1, 2 and 3). Besides, estradiol valerate and testosterone (0.1 mg/ml) significantly decreased the viability of HEK 293 cells compared to control group ($P<0.05$ and $P<0.01$, respectively) (Figures: 1 and 2). However, 0.001 mg/ml of testosterone and 0.01 mg/ml of progesterone caused a significant rise in HEK293 cell viability compared with control group ($P<0.05$) (Figures: 1 and 3). Estradiol valerate and progesterone with the concentration of 0.001 mg/ml, 0.01 mg/ml of testosterone and estradiol valerate, and 0.1 mg/ml progesterone had no significant effect on the viability of HEK 293 cells compared to control group (Figures: 1, 2 and 3).

3.4. The effect of testosterone, estradiol valerate, and progesterone on expression level of Bax, Bcl-2, and KAI-1/CD82 genes in HT29 cells:

The least concentrations of testosterone (1 mg/ml), progesterone (0.1 mg/ml) and estradiol valerate (0.1 mg/ml), which did not show the significant cytotoxic effects on noncancerous HEK293 cells but had significant cytotoxic effects on both HT29 and A172 cell lines were used to evaluate the gene expression level. The results revealed that 1 mg/ml of testosterone and 0.1 mg/ml of progesterone significantly increased the expression level of the Bax apoptotic gene in HT29 cells compared with control group ($P<0.001$ and $P<0.01$, respectively), while the cells receiving 0.1 mg/ml of estradiol valerate showed a significant decrease in Bax expression level ($P<0.001$) (Figure 4). The expression level of Bcl-2 anti-apoptotic gene in HT29 cells treated with 1 mg/ml of testosterone and 0.1 mg/ml of progesterone significantly decreased compared with control group ($P<0.001$ and $P<0.05$, respectively), but Bcl-2 expression level increased significantly in response to 0.1 mg/ml of estradiol valerate ($P<0.001$) (Figure 5). The expression level of the KAI-1/CD82 anti-metastatic gene decreased significantly in HT29 cells treated with 1 mg/ml of testosterone and increased significantly in the groups receiving 0.1 mg/ml of progesterone and 0.1 mg/ml of estradiol valerate compared with control group ($P<0.001$ and $P<0.01$, respectively) (Figure 6).

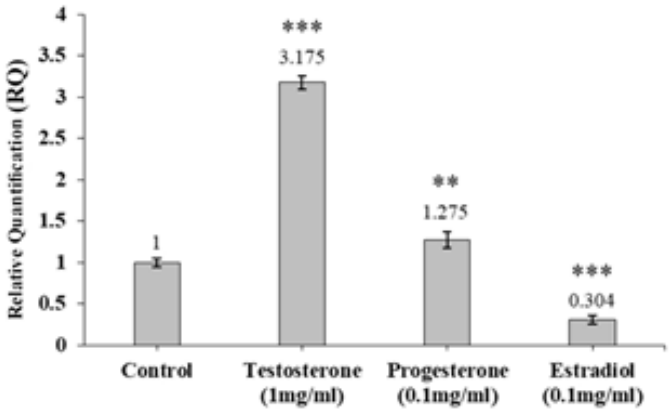

Figure 4: The effect of testosterone, progesterone, and estradiol valerateon on expression level of BAX gene in HT29 cells. * indicates significant difference compared with control (**: $P<0.01$; ***: $P<0.001$).

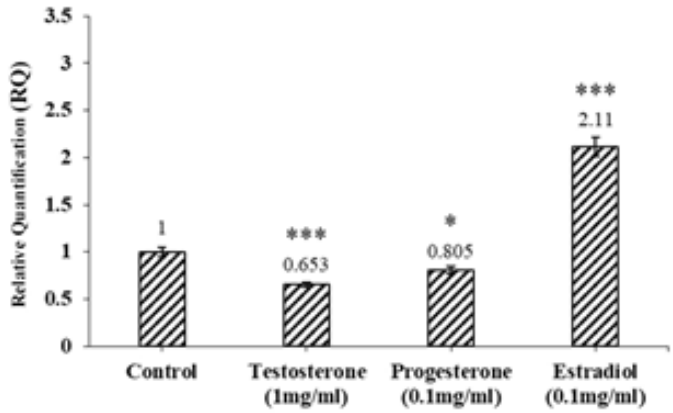

Figure 5: The effect of testosterone, progesterone, and estradiol valerate on expression level of Bcl-2 gene in HT29 cells. * indicates significant difference compared with control group (*: $P<0.05$; ***: $P<0.001$).

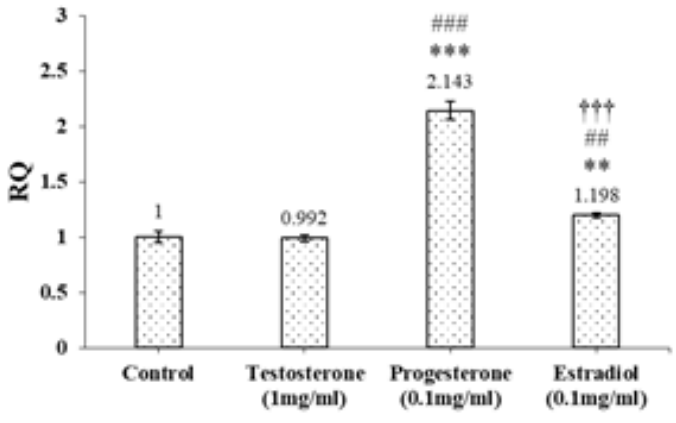

Figure 6: The effect of testosterone, progesterone, and estradiol valerate on expression level of the KAI-1 /CD82 gene in HT29 cells. * indicates significant difference compared with control (**: $P<0.01$; ***: $P<0.001$).

3.5. The effect of testosterone, estradiol valerate and progesterone on expression level of BAX, Bcl-2 and KAI1/CD82 genes in A172 cells :

The expression level of Bax gene increased significantly in A172 cells treated with testosterone (0.1 mg/ml) and progesterone (1 mg/ml) compared to control group ($P<0.01$ and $P<0.001$, respectively). However, Bax expression level did not change significantly in A172 cells treated with estradiol valerate (1 mg/ml) (Figure 7). The expression level of Bcl-2 gene did not change significantly in A172 cells after treatment with 0.1 mg/ml of testosterone compared to control group, whereas progesterone (0.1 mg/ml) significantly decreased Bcl-2 expression level ($P<0.001$). Also, 1 mg/ml of estradiol valerate significantly increased Bcl-2 expression level in A172 cells compared with control group ($P<0.001$) (Figure 8). The expression level of KAI-1/CD82 anti-metastatic gene significantly reduced in A172 cells in response to progesterone (0.1 mg/ml) and testosterone (0.1 mg/ml) and estradiol valerate (1 mg/ml) compared with control group ($P<0.001$, $P<0.05$, and $P<0.001$, respectively)(Figure 9).

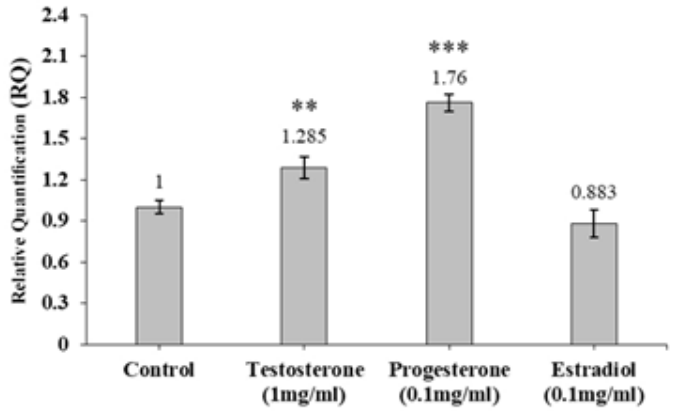

Figure 7: The effect of testosterone, progesterone, and estradiol valerate on the expression levels of the Bax gene in A172 cells. * indicates significant difference compared with control (**: $P<0.01$; ***: $P<0.001$).

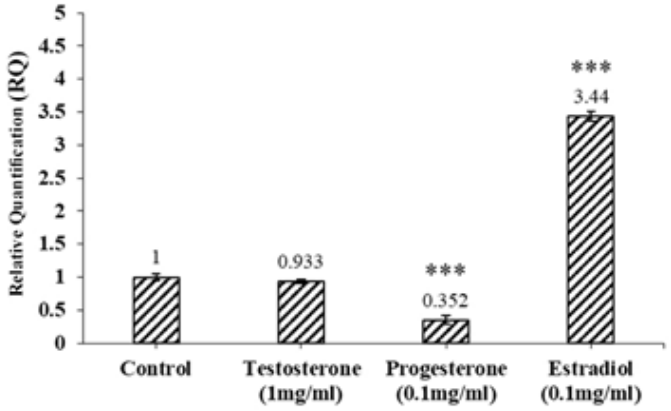

Figure 8: The effect of testosterone, progesterone, and estradiol valerate on expression level of Bcl-2 gene in A172 cells. * indicates significant difference compared with control group (***: $P<0.001$).

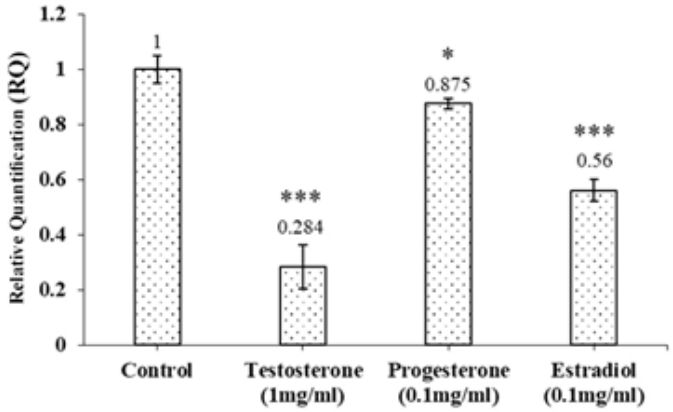

Figure 9: The effect of testosterone, progesterone, and estradiol valerate on expression level of the KAI-1/CD82 gene in A172 cells. * indicates significant difference compared with control (*: $P<0.05$; ***: $P<0.001$).

Although it has been widely reported that male and female sex steroid hormones have inhibitory effects on certain types of cancer cells (Chen et al., 2019; Contreras and Cittelly, 2020; Costa et al., 2020; Bello-Alvarez and Camacho-Arroyo, 2021; Bouras et al., 2021), their role in inducing the apoptosis and metastasis in colorectal cancer and brain glioblastoma cells is one of the most challenging issues. Therefore, in the present study, we used the MTT assay and RT-PCR to investigate the effects of estradiol valerate, progesterone, and testosterone on proliferation of cerebral glioblastoma (A172) and colon adenocarcinoma (HT29) cells and the expression levels of Bax, Bcl-2, and KAI-1/CD genes to reveal the possible cytotoxic and apoptotic effects of testosterone, progesterone and estradiol valerate on A172 and HT29 cells.

3.6. The effect of progesterone, testosterone, and estradiol valerate on viability of HT29, A172, and HEK293 cells:

The results of this study showed that testosterone, progesterone and estradiol have a potentially anti-proliferative effect on HT29 and A172 cells. In accordance with the findings of this study, Fourkala et al., (2012) assessed the cytotoxic effects of the combination of nickel and testosterone on HCT116 cancer cells using the MTT technique and showed that this combination can inhibit the growth of cancer cells by inhibiting topoisomerase 1. Motylewska and Mełeń-Mucha, (2009) in addition, investigated the direct effects of progesterone and estrone after 24 and 72 h of exposure on the prevention of the growth of MC38 colon cancer cells and showed that progesterone inhibited MC38 cancer cells proliferation dose-dependently. Furthermore, Gao et al.,(2009) assessed the cytotoxic effects of sex steroid derivatives using the MTT assay on colon cancer cells and concluded

that titanocenyls containing androgen and progesterone derivatives had high anti-proliferative activity on cancer cells. The effect of sex steroids was assessed on the prevention of ovarian cancer and the results showed that progesterone prevented the growth of human ovarian cancer cells and metastasis (Jeon et al., 2016). Medroxyprogesterone acetate also showed an inhibitory effect on human colon cancer cell lines (HCT116 and HT29). Medroxyprogesterone acetate exerts its anti-proliferative effect by modulating the expression levels of cell cycle proteins and cyclin-dependent kinase-2 activity. These results may help elucidate the protective effect of medroxyprogesterone acetate on the risk of colon cancer (Tanaka et al., 2008). Farahmandlou et al., (2017) demonstrated that different doses of testosterone have enhancing or suppressive effects on non-cancerous human embryonic kidney, colon cancer, and brain glioblastoma cell proliferation *in vitro*. It has also been shown that progesterone inhibits the growth of human brain glioblastoma cells both in vitro and in vivo and also increases the survival time in mice without side effects. Progesterone at high concentrations (20, 40, and 80) µM significantly reduced tumor cell viability and tumor growth during five weeks of treatment, and also increased the survival time of tumor-bearing mice without the symptoms of systemic toxicity by 60 % (Atif et al., 2015). Altinoz and others showed that the low and high doses of progesterone had stimulant and inhibitory effects on the growth of human cerebral glioblastoma U87 and A172 cell lines, respectively. They evaluated changes in the growth and proteomic profiles at high doses of progesterone (100 and 300) µM and it was found that increasing the dose of progesterone inhibited the growth of cerebral glioblastoma cells (Altinoz et al., 2020). In contrast to these findings, research on the stimulatory effects of testosterone, progesterone, and estradiol on brain glioblastoma cells has shown that these hormones can stimulate the progression of brain glioblastomas by increasing the proliferation, migration and invasion of brain glioblastoma cells (Gierisch et al., 2013; Sun et al., 2015; Elmaciet al., 2019; Bello-Alvarez and Camacho-Arroyo, 2021). The risk of cerebral glioblastoma is lower in young females and its incidence enhances after menopause suggesting likely protective roles of sex hormones (Sun et al., 2015). This is confirmed by research of Elmaci and others, who have proven that medroxyprogesterone acetate reduces rat C6 glioma growth in vitro (Elmaciet al., 2019). Furthermore, there is some evidence that oral contraceptives have a beneficial impact on the risk of colorectal cancer (Gierisch et al., 2013). This is consistent with the results we obtained.

3.7. The effect of progesterone, testosterone, and estradiol on the expression levels of Bax and Bcl-2, and KAI-1 /CD82 genes HT29 and A172 cells:

Our findings showed that 0.1 mg/ml of testosterone and progesterone dramatically increased the Bax/Bcl-2 gene expression levels in the brain glioblastoma and colon cancer cells indicating that testosterone and progesterone can induce apoptosis in A172 and HT29 cells. On the other hand, estradiol increased Bcl-2 gene expression level and decreased Bax gene expression level in both A172 and HT29 cells. In line with the findings of this study, Sasso et al., (2019) assessed the apoptotic effects of progesterone and estradiol on colorectal cancer cells by real-time PCR in mice. The results of this study showed that progesterone and estradiol significantly reduced the proliferation of cancer cells through the expression of apoptotic genes. Another study on the apoptotic and metastatic effect of estrogen on lung cancer cells using real-time PCR showed that the cytotoxic effect of estrogen on lung cancer cells is mediated by apoptotic pathway (Keyan et al., 2021). Behl et al. investigated the apoptotic effects of 17β-estradiol on colon cancer cells in mice using cell counting and reported that this hormone induced apoptosis through DNA damage (Behl et al., 1995). Moreover, the cytotoxic effects of 17β-estradiol and testosterone were evaluated on leukemia cells by flow cytometry and the results demonstrated that these hormones could inhibit the growth

of cancer cells through apoptosis in a dose-dependent manner (Mossuz et al., 1998). Regarding the induction of apoptosis by sex hormones in reproductive cancer cells, it has been indicated that sex steroid hormones kill apoptotic cells through DNA fragmentation (Kiess and Gallaher, 1998). The effect of estradiol at a cytotoxic concentration was investigated on the expression levels of Bax and Bcl-2genes in HT29 cells using real-time PCR and it was found that estradiol significantly decreased and increased Bax and Bcl-2 expression levels, respectively. Estradiol exerted its effect on colorectal adenocarcinoma cells through Bax-independent cell death (Farahmandlou and Afrasiab, 2019). According to a study by Farahmandlou et al., (2019a) the expression levels of Bax genes in A172 cells receiving testosterone at cytotoxic concentration increased significantly, but the expression levels of the Bcl-2 gene did not change significantly. Qin et al. investigated the apoptotic effects of estradiol on gastric cancer cells and concluded that estradiol could induce tumor attenuation by inducing apoptosis and reducing cell viability directly in these cells. The results of cell viability test also demonstrated that estradiol reduced cell viability through the expression of the caspase-3 gene in both SGC7901 and BGC823 cell lines. In addition, the expression levels of Bcl-2 and Bcl-xL reduced in both cell lines (Qin et al., 2014).

Our findings showed that testosterone and progesterone increased and decreased the expression levels of the KAI-1/CD82 gene in brain glioblastoma and colorectal adenocarcinoma cells, respectively. Estradiol also increased and decreased the expression level of the KAI-1/CD82 gene in A172 and HT29 cells, respectively. Consistent with these findings, Christgen and others assessed the anti-metastatic effect of sex hormones on breast cancer cells using real-time PCR and the results showed that estradiol, similar to progesterone, can inhibit the CD82/KAI-1 anti-metastatic gene (Christgen et al., 2009). However, in contrast to this finding, the results of a study on the cytotoxic effects of testosterone, progesterone, and estradiol using real-time PCR showed that these hormones can cause metastasis in cancer cells by reducing the expression levels of CD82/KAI-1 anti-metastatic gene in brain glioblastoma cells (Farahmandlou et al., 2019b).

Regarding the mechanism of action of steroid hormones in brain glioblastoma and colorectal adenocarcinoma cells, it seems that these hormones can increase the expression levels of apoptotic genes and decrease the expression levels of CD82/KAI-1 anti-metastatic gene in cells after passing through the cell membrane by diffusion (Bao et al., 2007; Vasconsuelo et al., 2011; Parsons et al., 2018). This is in agreement with the fact that a rise in the expression levels of apoptotic genes leads to a reduction in cell viability (Sasso et al., 2019). Therefore, in the present study, steroid hormones reduced the proliferation of brain glioblastoma and colorectal adenocarcinoma cells by affecting the expression levels of Bax, Bcl-2, and CD82/KAI-1 genes.

This research faced some limitations regarding the assessment of the effect of sex steroid hormones on the expression levels of tumor suppressor genes and caspases, as well as the measurement of free radicals and cell cycle in brain glioblastoma and colon cancer cells. More in vivo and in vitro research are needed to determine whether sex steroid hormones inhibitory effects on HT29 and A172 cells proliferation are mediated apoptotic pathways.

4 Conclusion

The results of this study showed that testosterone and progesterone have both proliferative and anti-proliferative effect on non-cancerous HEK293 and colorectal cancer cells, while estradiol

had anti-proliferative effect. In addition, while estradiol had no significant proliferative or anti-proliferative effect, testosterone and progesterone had anti-proliferative effect on brain glioblastoma cells. The cytotoxic effect of testosterone and progesterone on colon cancer and brain glioblastoma cells is mediated by Bax-dependent apoptosis. Estradiol prevents glioblastoma cancer cells by anti-apoptotic pathway. Furthermore, the cytotoxic dose of testosterone and progesterone did not alter anti-metastatic CD82/KAI-1 expression level in brain glioblastoma cells. Although cytotoxic dose of progesterone increased anti-metastatic CD82/ KAI-1 expression level in colon cancer cells, the cytotoxic dose of testosterone did not significantly change the CD82/KAI-1 expression level.

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6 Conflict of interests

The authors declare that there are no competing interests.

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