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# Evaluation of cytotoxic effects of amitriptyline on ovarian cancer cells

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**Abstract:** Many studies have shown that antidepressants can have anticancer effects; however, the anti-cancer effects of the antidepressant amitriptyline on ovarian cancer cells are unclear. Accordingly, the present study investigated the cytotoxic effects of amitriptyline on ovarian cancer cells *in vitro*. In this experimental-laboratory study, ovarian cancer (A2780) cells were divided into control (untreated) group and groups treated with 78.125, 156.25, 312.5, 625, 1250 and 2500 µg/ml of amitriptyline. Cell viability was measured using MTT assay method 24 and 48 hours after treatment. Data was analyzed using one-way analysis of variance. Ovarian cancer cell viability significantly reduced after treatment with 78.125, 156.25, 312.5, 625, 1250, and 2500 µg/ml after 24 and 48 hours ($p < 0.001$). IC50 the drug was 1144 and 1071 for 24 and 48 hours, respectively. The findings of this study indicate that amitriptyline has cytotoxic effects on ovarian cancer cells.

**Keywords:** Amitriptyline, A2780 cell line, Viability

## 1 Introduction

Ovarian cancer, which is caused by the abnormal growth of ovarian cells, is the fifth most common and deadly cancer in women, starting in the ovary. Overall, 4% of all cancers and 23% of female genital cancers are ovarian cancers. The prevalence of this type of cancer also

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increases with age. According to the Annual Cancer Registry Center in Iran, ovarian cancer is the eighth most common cancer in Iranian women and the age of onset is 40-60 years. It is also more common in North America and Europe than in Africa and Asia. The risk of ovarian cancer is higher in people who have never had children. People who start ovulating at a younger age or menopause at an older age are also at risk. Other factors that can cause this cancer include postmenopausal hormone therapy, fertility drugs, a family history (especially mutations in the BRCA1 and BRCA2 genes), and obesity. Early pregnancy at an early age, premature menopause, and the use of hormonal contraceptives, fallopian tubes, and breastfeeding are some of the factors that reduce the risk of developing the disease. Women with endometriosis have a 30% higher risk of ovarian cancer than other women. In the early stages of cancer, there may be no symptoms, but as the disease progresses, its symptoms include pain in the pelvis, lower abdomen, indigestion and heartburn, frequent urination, pain during intercourse, digestive problems, nausea, extreme tiredness and loss of appetite. The diagnosis is usually confirmed by a biopsy, which is removed during surgery (Stewart et al., 2019).

Studies show that some antidepressants, such as amitriptyline, can affect reproductive cancers (Steingart and Cotterchio, 1995). Amitriptyline is a brand name of Elavil that is used to treat a number of psychiatric disorders. The mechanism of action of tricyclic antidepressants in the brain is inhibition of serotonin and norepinephrine reuptake by blocking the function of serotonin and norepinephrine transporters. Therefore, they increase brain levels of serotonin and norepinephrine. The main use of this drug is to treat depression and anxiety, but it is also used to treat neuropathic and neurogenic pain, nocturia, and migraine prophylaxis, and in rare cases to treat Attention Deficit Hyperactivity Disorder (ADHD), bipolar disorder, and sleep disorders. It has a variety of side effects including dizziness, headache, weight gain, mood disorders such as anxiety and restlessness, hypotension, loss of libido and impotence, delirium, and confusion. These complications occur in more than 1% of people (Huo et al., 2018).

Studies have shown that antidepressants have anticancer effects on lung and bowel cancers (Toh et al., 2007). Findings show that regular use of selective serotonin reuptake inhibitors is associated with a reduced risk of colorectal cancer through its anti-promoter or direct cytotoxic effect (Coogan et al., 2009). Antidepressants are not only able to induce apoptosis in tumor cells, but can also prevent apoptosis. Thus, there is strong evidence that antidepressants have a direct inhibitory effect on tumor cells (Frick and Rapanelli, 2013). Oxidative therapy is an anti-cancer strategy based on the induction of high levels of oxidative stress, which is achieved by increasing intracellular reactive oxygen species (ROS). Research on the antitumor potential of amitriptyline in three human tumor cell lines shows that in lung cancer, cervical cancer, and hepatoma, compared to the cytotoxic effect of amitriptyline with conventional chemotherapy, amitriptyline has the highest cellular damage and induces high levels of ROS, followed by serious irreversible mitochondrial damage. As a result, based on ROS production capacity and inhibition of antioxidants in tumor cells, amitriptyline is being introduced as a promising new drug to be tested for anticancer therapy (Cordero et al., 2010). Antidepressants such as amitriptyline can not only induce apoptosis in tumor cells but can also stop the cell cycle. The results also show that amitriptyline increases dose-dependent apoptosis in human cancer cells more effectively than other conventional chemotherapy drugs (Bartholomä et al., 2002; Cordero et al., 2009). Contrary to research findings that indicate the anticancer effects of antidepressants,

some research has shown that antidepressants have no effect on cancer prevention or causing cancer, in this regard, research on the relationship between antidepressants and cancer has shown that the use of antidepressants may be associated with the risk of breast cancer. The results suggest that the use of tricyclics for more than 2 years may be associated with a doubling of breast cancer (Cotterchio et al., 2000; Kelly et al., 1998). There is evidence that taking antidepressants is associated with an increased risk of genital and gastrointestinal cancers (Wu et al., 2015). It is hypothesized that antidepressants may have growth-promoting effects on cancer, including melanoma, fibrosarcoma, breast tumors (Kelly et al., 1998), and colon cancer (Iishi et al., 1993). It has also been suggested that some antidepressants suppress immune function and increase the risk of cancer (Steingart and Cotterchio, 1995). *In vitro* and *in vivo* experiments have shown that antidepressants may cause genetic and carcinogenic toxicity in animal models. Antidepressants can affect dopamine or norepinephrine levels, which can increase gonadotropin levels and increase the risk of ovarian cancer (Harlow et al., 1998). Harlow and Kramer were the first to report the association between the use of antidepressants and the risk of ovarian cancer, especially in people under 50 years of age (Harlow and Cramer, 1995). A meta-analysis study reported that the risk of breast and ovarian cancer may be increased by taking antidepressants, especially selective serotonin reuptake inhibitors including amitriptyline (Cosgrove et al., 2011). Human studies have shown a positive and statistically significant association between amitriptyline and liver cancer and a negative association with pancreatic cancer. These studies also showed that amitriptyline enhances tumor growth (Steingart and Cotterchio, 1995).

Given the widespread prevalence of ovarian cancer in the world, and since ovarian cancer is the second most prevalent female reproductive cancer and causes more deaths in women than any other cancer, the present study investigated the cytotoxic effect of amitriptyline on ovarian cancer cells and the results of this study are of particular importance regarding the relationship between amitriptyline and ovarian cancer.

## 2 Materials and Methods

In this experimental-laboratory study, ovarian cancer (A2780) cells were purchased from the Pasteur Institute of Iran cell bank and stored in a nitrogen tank at -196 ° C. A2780 cell lines were divided into control groups (non-treated) and cells treated with 78.125, 156.25, 312.5, 625, 1250 and 2500 µg/mL of amitriptyline. Amitriptyline was provided as a pure powder from Abu Reihan Pharmaceutical Company and subsequently dissolved in PBS solvent. Then, the obtained solution was sterilized using a syringe filter and the desired concentrations were prepared serially. MTT assay was used to evaluate the cytotoxic effect of the drug on the target cells. In this regard, considering the sufficient culture medium for the cells, as well as considering at least 6 repetitions, different doses of drug were added to the wells containing the cells and the plates were kept in the incubator for 24 hours. During the desired time, the liquid from the drain plate and MTT dye were added. 4 hours after adding the dye, the MTT solution was drained and DMSO was added. After complete dissolution, the optical absorption of the solutions was read at 570 nm. Subsequently, based on the light absorption of the samples, the

percentage of cell viability in each group was calculated (Stockert et al., 2012; Gholamhosseyni et al., 2018).

SPSS18 statistical software was used to analyze the data. Data were analyzed using one-way analysis of variance (ANOVA). Differences between groups were considered significant at the level of ($p < 0.05$).

# 3 Results and Discussions

A2780 cells viability did not significantly change in groups treated with 39.0625 and78.125 µg/mL of amitriptyline compared with control group 24 hours after treatment. However, cell viability significantly decreased in A2780 cells treated with 156.25, 312.5, 625, 1250 and 2500 µg/mL compared to control group 24 hours after treatment (Figure 1).

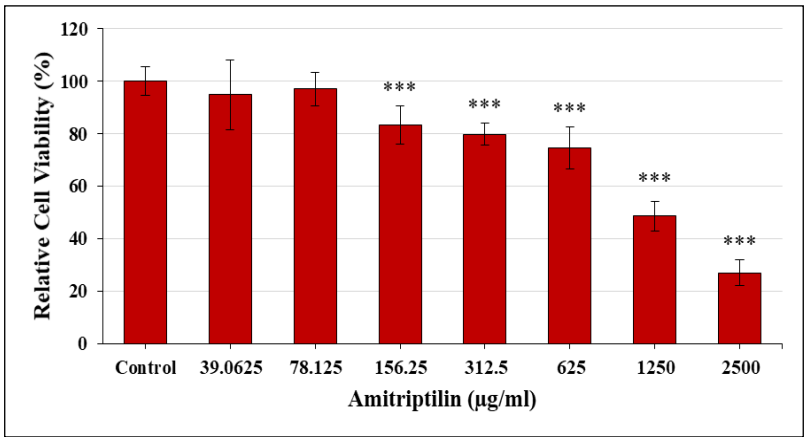


**Figure 1:** Viability of A2780 cells treated with different concentrations of amitriptyline 24 hours after treatment. * indicates a significant difference compared to the control group (***: $p < 0.001$).

The results showed that the IC50 value was 1071 µg/ml for amitriptyline against ovarian cancer cells for 24 hours (Figure 2).

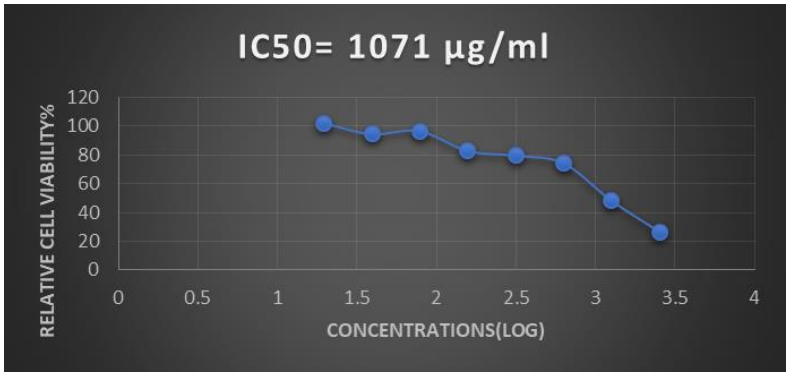


**Figure 2:** IC50 curve of amitriptyline 1071 µg/mL against A2780 cells after 24 hours.

The results of ovarian cancer cells viability determined by MTT showed that cell viability in

A2780 cells treated with 39.0625 µg/mL of amitriptyline did not significantly change compared with control group 48 hours after treatment. However, cell viability significantly decreased in A2780 cells treated with 78.125, 156.25, 312.5, 625, 1250, and 2500 µg/mL compared to control group 48 hours after treatment (Figure 3).

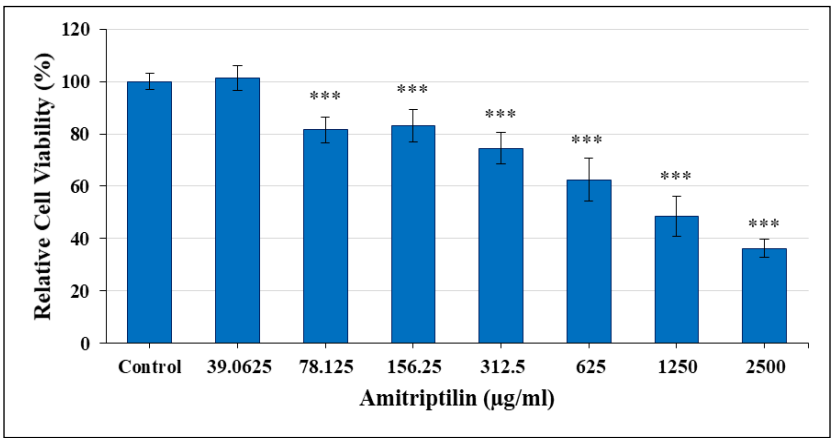


**Figure 3:** Viability of A2780 cells treated with different concentrations of amitriptyline 48 hours after treatment. * indicates a significant difference compared to the control group (***: $p < 0.001$).

The half-maximal inhibitory concentration (IC50) of amitriptyline was calculated by linear approximation regression of the percentage viability versus the amitriptyline concentration. The results showed that the IC50 value was 1144 µg/mL for amitriptyline against ovarian cancer cells for 24 hours (Figure 4).

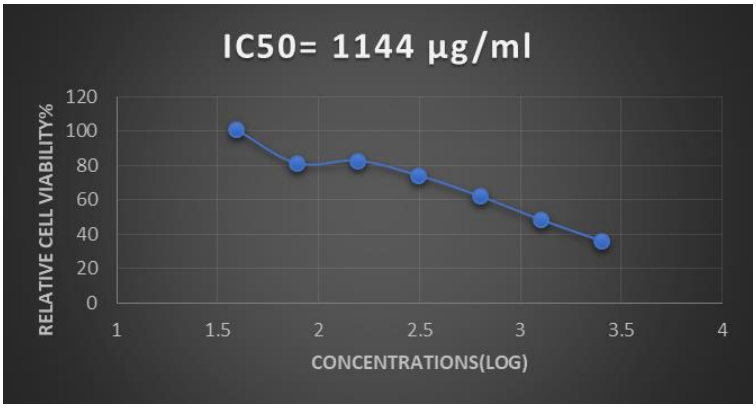


**Figure 4:** IC50 of amitriptyline calculated from curve constructed by plotting A2780 cell viability (%) versus amitriptyline 24 hours after treatment.

Some studies have shown that antidepressants are particularly important in inhibiting the proliferation of cancer cells (Toh et al., 2007; Coogan et al., 2009; Frick and Rapanelli, 2013). In this regard, the results of the present study show that the antidepressant drug, amitriptyline, has cytotoxic effects on ovarian cancer cells. Consistent with this finding, other studies have shown that antidepressants have inhibitory effects on cancer cells. The results of a study on acute oxidative damage to cancer cells by amitriptyline in comparison with some conventional

chemotherapy drugs revealed that amitriptyline has a potential for the treatment of cancer, however, it is commonly prescribed for depression and the treatment of several neurological and inflammatory diseases, such as fibromyalgia, chronic fatigue syndrome, migraine, irritable bowel syndrome, and unusual pain (Watson, 1994; Gruber et al., 1996). The addition of amitriptyline to cell cultures has been shown to cause several symptoms of toxicity. In addition, several reports have shown that the toxicity of this drug is due to increased oxidative stress with the production of high amounts of ROS (Slamon and Pentreath, 2000). Amitriptyline disrupts mitochondrial function and oxidative stress in human fibroblasts and produces a dose-dependent inhibition of growth (Moreno-Fernández et al., 2008). Thus, amitriptyline increases ROS levels and decreases antioxidant defense, the lack of adequate antioxidant defense makes tumor cells very vulnerable to oxidative stress. As a result, manipulation of ROS levels with redox modulation is a way to selectively kill cancer cells without causing significant toxicity to normal cells (Schumacker, 2006; Trachootham et al., 2009). It has also been reported that amitriptyline taking is not associated with an increased risk of ovarian cancer (Moorman et al., 2005; Dublin et al., 2002; Coogan et al., 2000; Stapel et al., 2021).

In contrast, some research findings have shown that antidepressants do not have a significant effect on cancer cells. In this regard, the results of a study on the effect of antidepressants on breast cancer indicates that amitriptyline has not a significant effect on the prevention and control of cancer, even in some cases, it plays a role in cancer development (Sharpe et al., 2002; Steingart et al., 2003; Hilakivi-Clarke et al., 1993; Harlow et al., 1998; Cotterchio et al., 2000; Harlow and Cramer, 1995). Amitriptyline has been shown that can bind to the membrane of cancer cells (Cordero et al., 2010) and cause apoptosis in cancer cells (Mao et al., 2011), by which induces its cytotoxic effects on cancer cells (Lee et al., 2010). However, further studies are needed to determine the exact mechanism of action of amitriptyline on ovarian cancer cells.

## 4 Conclusion

Overall, the findings of this study showed that amitriptyline has cytotoxic effects on ovarian cancer cells *in vitro*.

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