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The cytotoxic effect of antidepressants sertraline on human cervical cancer (HeLa) cells

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Abstract

It has been reported that antidepressants drugs may have anticancer effects, though the findings on anticancer effects of antidepressant sertraline are controversial. The present study was carried out to determine the cytotoxic effects of sertraline on cervical cancer (HeLa) cells. Hela cells were divided into control group and groups treated with 1.5625, 3.125, 6.25, 12.5, 25, 50 and 100 μg/ml of sertraline. MTT assay method was used to measure the cytotoxic effects of sertraline on HeLa cells 24 and 48 hours after treatment. Data were analyzed using ANOVA. Treatment with sertraline (6.25, 12.5, 25, 50 and 100 μg/ml) led to decreased HeLa cells viability compared to control group 24 and 48 hours after treatment. The IC50 value was 16.5 and 4.3 μg/ml for 24, 48 hours after treatment, respectively. Our findings indicated that sertraline has cytotoxic effects on cervical cancer cells *in vitro*, however, further research are required to elucidate the anticancer effects of sertraline on cervical tumors *in vivo*.

Keywords: Sertraline, HeLa, Viability

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1 Introduction

Cancer is currently one of the major public health problems, accounting for 12% of all deaths worldwide (Fan et al., 2014). The resulting death toll in 2020 is estimated at approximately 10 million. This number is projected to reach 28.4 million by 2040 (Sung et al., 2021). The most common psychiatric disorder in cancer patients is depressive disorder (Hopko et al., 2008). The prevalence of depression among people with cancer is 20 to 25% (Wicks et al., 2007). Most patients develop mood disorders, anxiety or depression after being diagnosed with cancer (Lu, et al., 2016). Depression is often undiagnosed and untreated in ccancer patients, leading to increased morbidity and mortality (Hopko et al., 2008). Also, untreated depression in the presence of comorbid conditions may lead to frequent clinic visits, increased costs, long-term hospitalization, and decreased adaptation and quality of life (Valente and Saunders, 1997). Early diagnosis of depression in cancer patients is therefore important. Interventions used to treat depression can include psychotherapy, social therapy, or medication (Dennis, 2014). Several studies suggest that antidepressants such as sertraline play significant role in depression treatment in patients with cancer (O'Connor et al., 2010). Sertraline is a selective antidepressant and central serotonin reuptake inhibitor (Wagner et al., 2003; Gil-Ad et al., 2008). In addition to its antidepressant effect, sertraline can play a supportive role in the effectiveness of anticancer drugs (Saletu et al., 1986). Sertraline appears to be also a promising new drug for anticancer therapy that bypasses the multi drug resistance (MDR) mechanism (Gil-Ad et al., 2008). Most studies have examined the effect of sertraline on cancer exacerbation and its complications. Few studies have been carried out regarding sertraline effects on cancer cells *in vitro* (Fisch, 2004). In this study, we investigated the cytotoxic effect of antidepressants sertraline on human cervical cancer (HeLa) cells *in vitro.*

2 Materials and Methods

2. 1. Drug and cell line

HeLa cancer cells were purchased from Pasteur Institute of Iran. These cells were delivered frozen in vials placed by an expert in a nitrogen tank to protect the cells. Sertraline was purchased from a Abu-Reyhan Pharmaceutical company (Tehran, Iran) and stored at room temperature away from moisture, heat and light.

2. 2. Cell culture

Hela cells were placed in a Falcon tube containing 10 ml of DMEM medium and were centrifuged at 300 rpm for 5 min. After centrifugation, the supernatant was discarded. 10 ml of fresh medium was added to cell pellet and mixed. The solution was poured in a flask and placed in incubator. Proliferating cells were attached to the bottom of the flask. Supernatant was discarded and cells were washed with PBS to remove the waste. PBS was then removed. To separate cells from each other and from the bottom of the flask, 0.5 ml of trypsin was used. Cells were incubated at 37 °C for 3 to 5 minutes. Reveres microscope was used to examine if the cells

were detaching from each other and from the bottom of the flask. DMEM was added to stop trypsinization. The solution was aspirated and poured to a 15 ml sterile Falcon tube and centrifuged at 1500 rpm for 5 min. After centrifugation, the cells were placed on the bottom of the flask. The medium was discarded, add fresh medium was poured into culture flask. After cell proliferation and reaching the confluencey 80 to 90%, they were used for treatment (Chinnapaka et al., 2020).

2. 3. Treatment

The proliferated cells were divided into control (untreated) group and groups treated with 1250, 625, 312.5, 156.25, 78.125 and 39.0625 μg/ml of sertraline for 24 and 48 hours.

2. 4. MTT assay

After 24 and 48 hours of treatment, MTT assay was used to evaluate the cytotoxic effect of sertraline on Hela cells. In this regard, considering sufficient culture medium for the cells, as well as considering at least 6 repetitions, different doses of the drug were added to the wells containing the cells and the plates were kept in the incubator for 24 hours. After the desired time had elapsed, the liquid was drained from the plate and MTT dye was added. Subsequently, 4-6 hours after dye addition, the MTT solution was drained and DMSO was added. After complete dissolution, the amount of light absorption of the solutions was read using 570 nm wavelengths and cell viability was calculated (Santos et al., 2016). The half-maximal inhibitory concentration (IC50) of sertraline was calculated by linear approximation regression of the percentage survival versus the sertraline concentration.

2. 5. Statistical analysis

The data were analyzed using SPSS software, Kolmogorov-Smirnov test (for normal data distribution), one-way analysis of variance (for group comparison) followed by Tukey's post hoc test. The significance level of the difference between groups was assumed to be $p < 0.05$.

3 Results and Discussions

Treatment with sertraline (6.25, 12.5, 25, 50 and 100 μg/ml) led to decreased HeLa cells viability compared to control group 24 and 48 hours after treatment (Figures 1 and 2). 3.125 μg/ml of sertraline significantly decreased the HeLa cells viability compared with control group 48 hours after treatment, however, did not significantly change the viability of HeLa cells 24 hours after treatment.

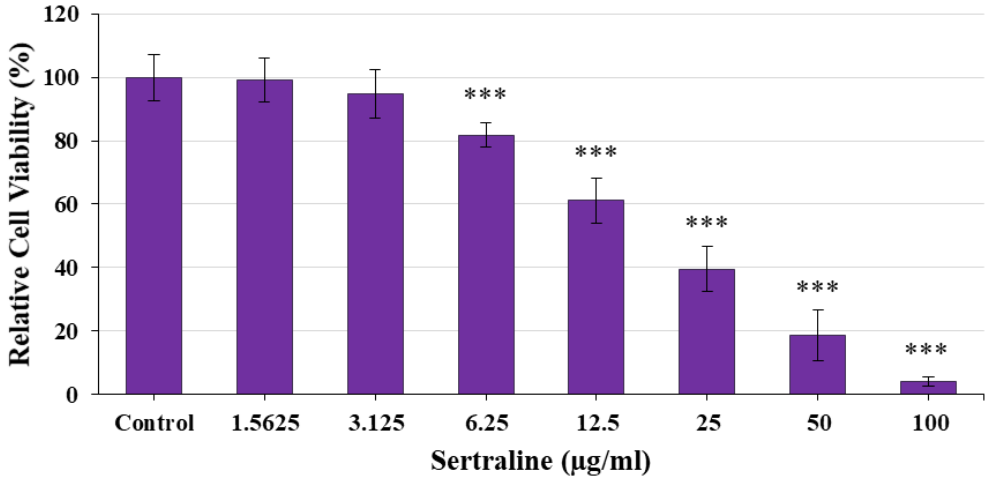

Figure 1. Viability of HeLa cells treated with sertraline 24 hours after treatment. * indicates significant difference compared with control group (***:p<0.001).

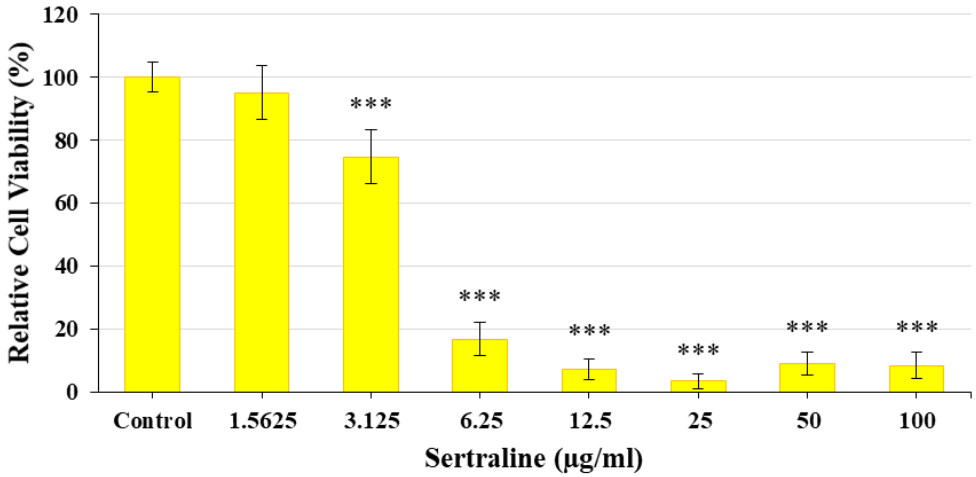

Figure 2. Viability of HeLa cells treated with sertraline 48 hours after treatment. * indicates significant difference compared with control group (***:p<0.001).

The half-maximal inhibitory concentration (IC50) of sertraline was calculated by linear approximation regression of the percentage survival versus the sertraline concentration. The IC50 value was 16.5 µg/ml for 24 hours after treatment (Figure 3).

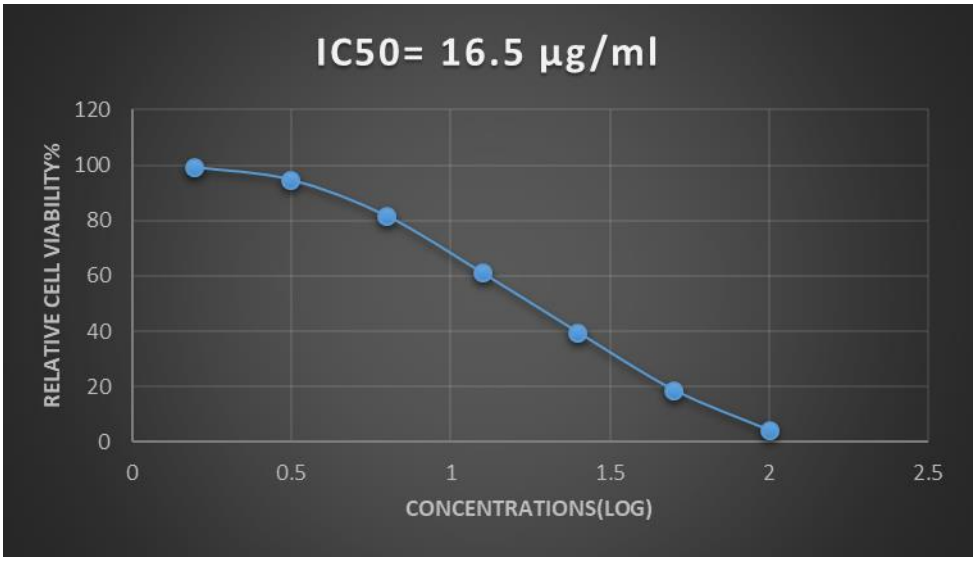

Figure 3. IC50 of sertraline 24 hours after treatment

The IC50 value was 4.3 µg/ml 48 hours after treatment showing that the duration of treatment plays a significant role in increasing of sertraline cytotoxicity on HeLa cells (Figure 4).

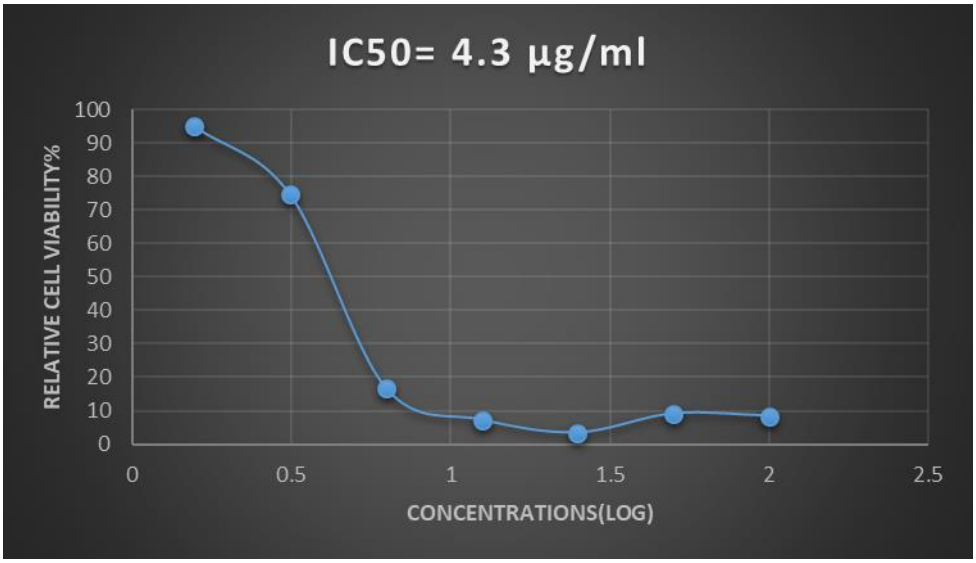

Figure 4. IC50 value of sertraline 48 hours after treatment

The results of this study show that sertraline can significantly reduce the viability of HeLa cancer cells, while few previous studies have shown the anticancer effects of sertraline against cervical cancer cell *in vitro* or *in vivo*. In line with our findings, it has been shown that sertraline was able to inhibit breast tumor growth (Geeraerts et al., 2021). The effect of tricyclic antidepressants on the inhibition of lung cancer and other neurological tumors were also examined and revealed that tricyclic antidepressants strongly induce apoptosis in lung and neurological tumor cancer cells in mice and humans. By activating stress pathways, they induce cell death induction in SCLC cells. The tricyclic antidepressants were also able to inhibit the growth of other endocrine neuronal tumors, including pancreatic neuroendocrine tumors and

Merkel cell carcinoma (Jahchan et al., 2013). Another study found that some antidepressants target the selective serotonin transporter (SSRI) and the norepinephrine transporter (NSRI), so they have the potential to act as anticancer agents (Cloonan et al., 2010). Antidepressants have been reported to induce apoptosis in glioma and neuroblastoma cell lines in mice (Levkovitz et al., 2005). Fluoxetine antidepressants also have inhibitory effects on B16F10 melanoma tumor growth mediated by increasing the mitogen-induced T cell proliferation, which may at least partially contribute to the antitumor mechanism of fluoxetine antidepressants (Grygier et al., 2013). The beneficial effects of fluoxetine and sertraline on tumor growth due to chronic stress and cell proliferation were investigated in a model of lymphoma mice revealing that the beneficial effects of fluoxetine and sertraline are mainly due to the recovery of the anti-tumor immune response (Di Rosso et al., 2018).

4 Conclusion

The results of this study showed that sertraline has significant cytotoxic effects on cervical cancer cells (HeLa) *in vitro* and the duration of treatment increases the cytotoxic effect of the drug on cervical cancer cells.

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Conflict of interests

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

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