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The effects of amitriptyline on human embryonic kidney cells growth and proliferation *in vitro*

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Abstract: Many studies have shown that antidepressants, including amitriptyline, can cause side effects on normal human cells, although the exact effects of amitriptyline on normal human cells are unclear. Accordingly, the present study investigated the cytotoxic effects of amitriptyline on human embryonic kidney cell line. In this experimental-laboratory study, cells were divided into control group (no drug treated) and amitriptyline treatment groups (treated with 39.06, 12.78, 156.25, 312.5, 625, 1250 and 2500 µg/ml of amitriptyline). MTT method was used to assay the cytotoxic effects of amitriptyline at 24 and 48 hours. The data were analyzed by one-way analysis of variance. Treatment with 39.06 and 78.12 µg/ml of amitriptyline for 24 hours had no significant effect on the viability of Hek-293 cells, but treatment with 156.25 µg/ml and higher concentrations of amitriptyline significantly reduced the viability of Hek-293 cells. Treatment of cells for 48 hours showed a significant reduction in cell viability at all concentrations. Amitriptyline has cytotoxic effects on normal human embryonic kidney cells and these effects depend on the dose and duration of treatment. Findings of this study can be of significant importance in terms of side effects of amitriptyline consumption on normal cells of the human body.

Keywords: Amitriptyline, HEK293, Viability

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1 Introduction

Amitriptyline, sold in pharmacies under the brand name Elavil, is a chemical and antidepressant drug that is often used to treat major depressive disorders as well as a variety of pain syndromes, including neuropathic pain, fibromyalgia, migraine and tension headaches (McClure and Daniels, 2021). The drug, which has a tricyclic chemical structure and is formulated as C20 H23 N, was discovered by scientists in the late 1950s. Amitriptyline has antidepressant effects by inhibiting the reabsorption of serotonin and norepinephrine in the brain, as well as by blocking the function of serotonin and norepinephrine transporters (Kruk et al., 2018).

Studies have shown that some antidepressants can have more cytotoxicity due to increased damage to mitochondria and oxidative stress (Han and Lee, 2009). Experiments show that antidepressants cause cytotoxic effects on normal human cells (Eisen et al., 1989). Antidepressants can cause metabolic changes in cells, even in low doses (Slamon and Pentreath, 1998). Antidepressants such as amitriptyline, fluoxetine, carbamazepine cause cellular respiration disorders by inhibiting complexes I and Π in the mitochondria (Cikánková et al., 2020). Amitriptyline can also increase neuronal death by increasing cAMP concentrations (Bartholomä et al., 2002). Studies have shown that amitriptyline can affect the metabolic processes of human cells (Nau et al., 2000). By contrast, it has been reported that amitriptyline has no significant influence on some types on normal human cells (Sim and North, 2010; Lu et al., 2018). On the other hand, HEK293 cells are normal human cells that were first cultured in 1973 by Frank Graham (Abaandou et al., 2021) and were widely used in biological studies in particular to assess the cytotoxic effects of drugs on human body cells (Eisen et al., 1989; Lu et al., 2018).

Considering the increasing use of amitriptyline antidepressant in the world (Bendtsen, 2003) and also the possibility of clinical complications due to excessive use of amitriptyline (Kruk et al., 2018), especially the side effects on various tissues of the human body and also considering that previous studies on the effects of amitriptyline have focused on the effects of the drug on cancer cells, and few research has been carried out investigating the effects of amitriptyline on non-cancerous cell (Bartholomä et al., 2002; Lu et al., 2018), the present study investigates the cytotoxic effects of amitriptyline on human renal embryonic (HEK293) cells.

2 Materials and Methods

This experimental laboratory study was approved by the International Committee of Scientists (IAS)'s research ethics committee. HEK293 cells were purchased from the Pasteur Institute. The cells were stored frozen in a nitrogen tank at -196 °C. Amitriptyline was prepared as a pure powder from Iran Daroo Pharmaceutical Company. First, by initial investigation and determination of lethal and non-lethal doses, the drug dose range was determined and finally the desired concentrations were selected for treatment by serial and logarithmic methods. To prepare different concentrations of the drug, 10 mg of the drug was dissolved in 200 µl of DMSO. Then it was slowly increased to 10 ml using cell culture medium. The concentration of

the drug in this solution was 1 mg/ml. After filtering this solution using the 0.2 µm syringe filter, the required concentrations were prepared. HEK293 cells were divided into control group (no treated) and groups treated with 06.39, 12.78, 256/156, 525, 625, 1250 and 2500 µg/ml of amitriptyline. The cells were cultured in plate wells and kept in an incubator for 24 hours (or 48 hours). MTT assay was then used to evaluate the cytotoxicity of the drug (Ahmadi et al., 2017; Norouzi et al., 2020). In brief, after incubation, 10 µl MTT solution was added to each well to achieve a final concentration of 0.45 mg/ml and the cells were incubated 1 to 4 hours at 37 °C. 100 µl solubilization solution was added to each well to dissolve formazan crystals. Finally, the absorbance was read at 570 nm. To calculate the Half-maximal inhibitory concentration (IC50 value), the inhibitor concentration against the percent activity was plotted using the parabolic ($y = ax2 + bx + c$) equation. Data were analyzed using one-way analysis of variance (ANOVA). Differences between groups at the level of $p < 0.05$ were considered significant.

3 Results and Discussions

The results of MTT showed that treatment with 39.06 and 78.12 µg/ml amitriptyline for 24 hours had no significant effect on the viability of HEK293 cells, but concentrations of 156.25 µg/ml and above caused a significant decrease in viability of HEK293 cells (Figure 1).

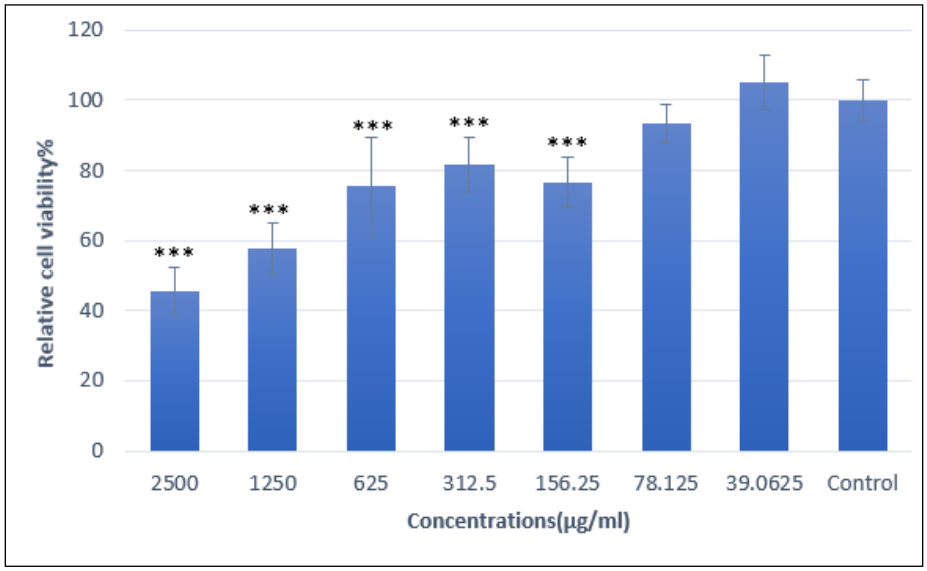

Figure 1: Viability of HEK293 cells treated with amitriptyline for 24 hours. * indicates a significant difference compared to the control group (***: $p < 0.001$).

IC50 of amitriptyline for 24 hours of treatment was calculated 2043 µg/ ml (Figure 2).

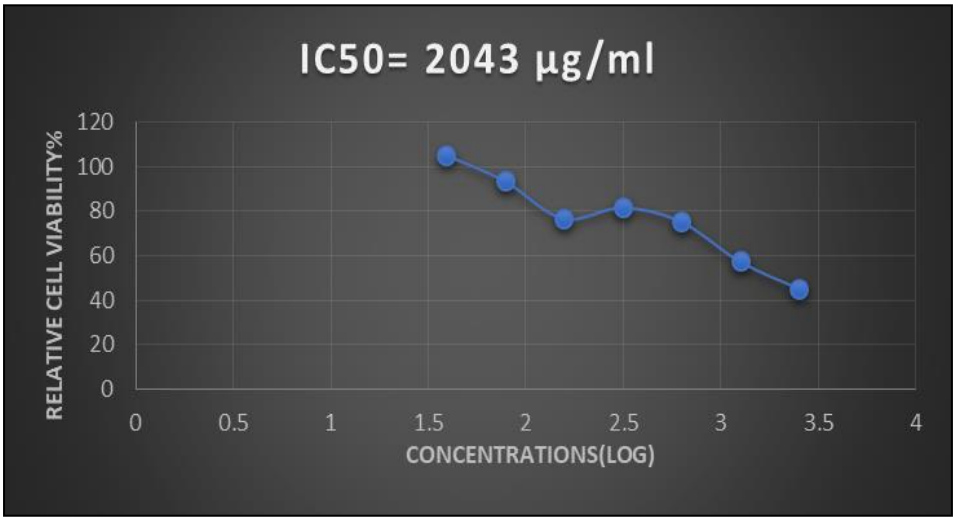

Figure 2: IC50 of amitriptyline calculated from curve constructed by plotting HEK293 cell viability (%) versus amitriptyline 24 hours after treatment.

Treatment of HEK293 cells for 48 hours showed that all concentrations significantly reduced the viability of HEK293 cells (Figure 3).

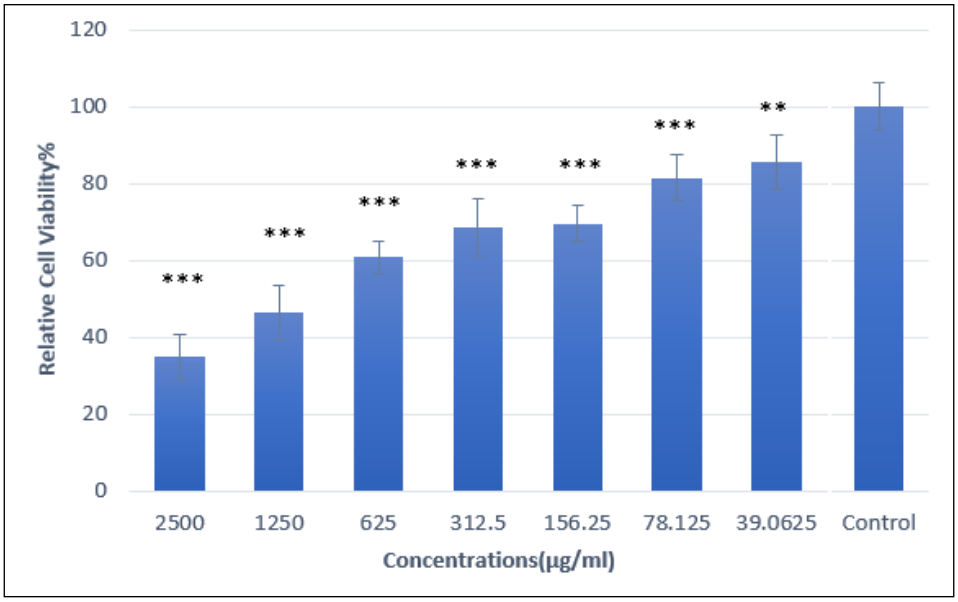

Figure 3: Viability of HEK293 cells treated with different concentrations of amitriptyline for 48 hours. * Shows a significant difference compared to the control group (**: $p < 0.01$, ***: $p < 0.001$).

IC50 of amitriptyline for 24 hours of treatment was calculated was 959 µg/ml (Figure 4).

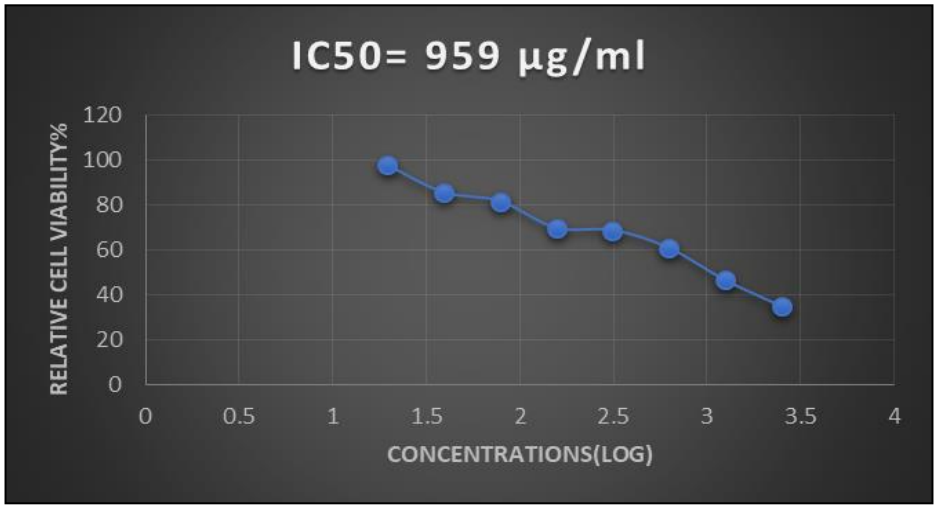

Figure 4: IC50 of amitriptyline calculated from curve constructed by plotting HEK293 cell viability (%) versus amitriptyline 24 hours after treatment.

The cytotoxic effects of antidepressants on various cell lines have been evaluated showing that antidepressant drugs have significant effects on cell viability (Han and Lee, 2009; Sim and North, 2010). The results of the present study showed that amitriptyline can cause cytotoxic effects on normal human renal cells (HEK293) and this effect is dependent on concentration and time. In fact, higher concentrations and longer duration of treatment with amitriptyline have more cytotoxic effects on the cells. Consistent with this study, the findings suggest that antidepressants, including amitriptyline, may have cytotoxic effects on human cells. In this regard, the results of a study on the effects of amitriptyline on human cells showed that this drug has cytotoxic effects by activating the PI3K/ Akt/ mTOR pathway and acetylation of Becline 1 (Kwon et al., 2020). It has also been shown that homomeric Kv7.1 and Kv7.1/KCNE1 channels were inhibited by amitriptyline in HEK293 cells in a concentration-dependent manner with IC50 values of 8.8 ± 2.1 µM and 2.5 ± 0.8 µM, respectively (Villatoro-Gómez et al., 2018). In addition, studies on HEK293 cells have shown that amitriptyline cause non-selective inhibition in Glucuronosyl is human UDP transferases (UGTs) and has cytotoxic effects on some types of cells (Uchaipichat et al., 2006). It has also been reported that some antidepressants, including amitriptyline, have cytotoxic effects on the mitochondrial membrane of cells, one of which is the cessation of cellular respiration resulting in the death of normal human cells by inhibiting the activity of complexes Ị and π in the mitochondrial membrane electron transport chain (Cikánková et al., 2020). Experiments performed with Fura-2AM microfluorometry show that amitriptyline in normal human cells significantly inhibits the entry of Ca^{2+} into macrophages (Krutetskaya et al., 2019). In contrast, some research findings have shown that amitriptyline does not have a significant effect on normal cells (Lu et al., 2018). A study showed that the use of amitriptyline at therapeutic concentrations had no effect on ATP flow in HEK293 P2X4 receptors and did not reduce P2X7 receptor efflux in human renal embryonic P2X4 receptors (Sim and North, 2010). Regarding the mechanism of cytototoxic effects of amitriptyline on HEK293 cells, it should be noted that this drug often affects the expression of proteins and intracellular mechanisms as well as blockage of various cellular channels (Han and Lee, 2009; Nau et al., 2000; Krutetskaya et al., 2019; Kwon et al., 2020) resulting in cell death.

4 Conclusion

Overall, the results of this study showed that the antidepressant drug amitriptyline has cytotoxic effects on cells (HEK293) and these effects depend on the dose and duration of treatment.

Acknowledgments

This research has been carried out in the framework of the plans approved by the International Association of Scientists (IAS) and has been approved by the High Ethics Committee in the research of this association.

Conflict of interests

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

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